Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 5918–5922, August 1989
Immunology

Decrease by chronic energy intake restriction of cellular proliferation in the intestinal epithelium and lymphoid organs in autoimmunity-prone mice

(calorie restriction/cell cycle/autoimmune disease)

MOTOHIRO OGURA*†, HIROKO OGURA*†, SUSUMU IKEHARA‡, MY LIEN DAO*, AND ROBERT A. GOOD*§

*Department of Pediatrics, All Children's Hospital, University of South Florida, Saint Petersburg, FL 33701; and ‡Department of Pathology, Kansai Medical University, Osaka 570, Japan

Contributed by Robert A. Good, May 2, 1989

ABSTRACT **In previous studies we demonstrated that chronic energy intake restriction (CEIR) by a diet relatively low in fat, relatively high in carbohydrate, and reduced 40% in total calories extends life span and delays development of autoimmune disease in autoimmunity-prone mice. To investigate a possible cellular basis for this dramatic action of CEIR, we analyzed the rate of incorporation of [^{3}H]thymidine by cells of the intestinal epithelium, thymus, spleen, and mesenteric lymph nodes in ad libitum-fed mice vs. CEIR mice of three autoimmunity-prone strains. In New Zealand Black (NZB), MRL/MP-*lpr/lpr* (MRL/*lpr*), and BXSB mice, CEIR slowed the rate of uptake of [^{3}H]thymidine and, by inference, the rate of cellular proliferation among epithelial cells along the entire length of the gastrointestinal tract. Furthermore, CEIR decreased the apparent proliferative rate of lymphoid cells of the thymus, spleen, and mesenteric lymph nodes. This action by CEIR on the proliferative rate of cells of these rapidly replicating cell populations may point to an important mechanism by which calorie restriction inhibits the development of autoimmune disease and extends longevity in autoimmunity-prone mice.**

Chronic energy intake restriction (CEIR) prolongs life span and span of health in a number of long-lived rodents, including inbred mice (1–4). The influences of CEIR, or undernutrition without malnutrition, have been even more dramatic when genetically short-lived, autoimmunity-prone strains of mice have been investigated (5–10). Indeed, it has been possible regularly to double life span in mice of each of the autoimmunity-prone strains studied simply by restricting the total amount of food consumed (5–10). Prolongation of life in these strains is accompanied by the inhibition of development of chronic diseases associated with aging, as has also been observed in long-lived strains of inbred mice and in long-lived rats (1–4). By following a lead enunciated by Gabrielsen and Olsen (11, 12) that the pathogenesis of autoimmunity in NZB mice might involve a problem of DNA disposal, we investigated the influence of diet in autoimmunity-prone strains of mice on the rates of proliferation and, by inference, perhaps on the rates of cell turnover in certain rapidly replicating tissues in the body. Previously, we studied the short-lived autoimmunity-prone (NZB × NZW)F_1 (B/W) mouse (where NZB and NZW are New Zealand Black and White, respectively) and showed that uptake of [^{3}H]thymidine by nuclei of cells of the intestinal mucosa, thymus, spleen, and mesenteric lymph nodes (MLNs) is greatly reduced by CEIR (ref. 13 and unpublished observations). In the present study we have investigated the incorporation of [^{3}H]thymidine at numerous levels of the intestine and in thymus and other lymphoid tissues in mice at different ages from three additional autoimmune-prone short-lived strains, the NZB, BXSB, and MRL/Mp-*lpr/lpr* (MRL/*lpr*) strains. We show that CEIR greatly limits [^{3}H]thymidine incorporation in the nuclei of cells at all levels of the gut (stomach, duodenum, ileum, upper colon, and lower colon) and in each of the lymphatic tissues studied.

MATERIALS AND METHODS

Animals. Inbred 6-week-old NZB female, MRL/*lpr* female, and BXSB male mice were obtained from The Jackson Laboratory and maintained in the Animal Research Center at the University of South Florida. The mice were housed individually and fed as specified. Animal rooms were operated on a 12-hr light and 12-hr dark cycle, and temperature (20°C) and humidity (60%) were held constant. Long-lived C57BL/6 mice were housed in groups and fed a commercial laboratory diet ad libitum. The number of mice in each group studied is recorded in Table 1.

Diets. Diets were prepared weekly and stored at 4°C. The composition of the high-carbohydrate, low-fat diets A and B fed to the autoimmune-prone mice and the ratios of diet and energy intake for each has been described (9). The full-fed mice (diet A) were begun on ad libitum feedings at weaning (≈4 weeks of age) and were fed this diet for 1–2 weeks to establish an average daily intake. The animals placed on the restricted diet (diet B) were then fed 60.2 g of diet B per 100 g of diet A consumed by the ad libitum-fed mice. Diet B contained increased amounts of vitamins, minerals, and essential fatty acids so that it provided 100% of the amounts of these ingredients delivered by diet A. Protein intake expressed per animal per day was decreased 40% for the mice fed diet B. However, protein intake expressed as percent dietary energy was 30.18% for both diets. The level of protein intake in relation to metabolic mass [body weight $(kg)^{0.75}$] was essentially the same for both ad libitum-fed and CEIR mice due to the smaller size of the latter.

The CEIR mice were fed twice weekly, and their food intake was adjusted weekly to represent 60% of the prior week's consumption of the mice on diet A. One week before sacrifice, CEIR mice were fed diet B daily to eliminate influences attributable to food restriction terminally.

Microautoradiographic Studies. Autoradiographic measurements were performed on all mice, essentially by the method of Deschner (14) and Deschner and Lipkin (15). One

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: B/W mice, (NZB × NZW)F_1 mice; NZB or NZW mice, New Zealand Black or White mice; MRL/*lpr* mice, MRL/Mp-*lpr/lpr* mice; CEIR, chronic energy (calorie) intake restriction; MLN, mesenteric lymph node.

†Current address: Division of Toxicology, Tokushima Research Institute, Tokushima 7701-01, Japan.
§To whom reprint requests should be addressed.

Table 1. Influence of dietary energy (calorie) intake on body and organ weights of autoimmunity-prone mice

Strain	Age, months	Group	*n*	Body weight, g	Spleen weight		MLN weight	
					mg	% of body weight	mg	% of body weight
NZB	5	A	15	35.9 ± 5.6	162 ± 40	0.5	62 ± 26	0.2
		B	15	25.7 ± 1.6	67 ± 19	0.3	51 ± 11	0.2
	10	A	18	39.6 ± 4.5	279 ± 122	0.7	100 ± 33	0.3
		B	18	25.7 ± 1.8	62 ± 31	0.2	66 ± 15	0.3
MRL/*lpr*	3	A	13	38.9 ± 1.9	335 ± 80	0.9	618 ± 165	1.6
		B	11	21.8 ± 2.2	76 ± 34	0.3	92 ± 27	0.4
	5	A	16	41.8 ± 7.1	694 ± 196	1.7	2146 ± 269	5.1
		B	16	28.7 ± 2.7	151 ± 18	0.5	365 ± 163	1.3
BXSB	3	A	13	23.0 ± 1.2	218 ± 101	0.9	71 ± 22	0.3
		B	13	15.6 ± 0.9	71 ± 21	0.5	22 ± 6	0.1
	5	A	16	25.5 ± 1.5	514 ± 256	2.0	109 ± 48	0.4
		B	16	18.0 ± 0.5	66 ± 23	0.4	32 ± 15	0.2

Values are mean ± SD. Group A, ad libitum-fed mice; group B, CEIR mice; *n*, number of animals.

hour prior to sacrifice, the mice were injected intraperitoneally with 25 μCi of [^{3}H]thymidine (New England Nuclear; specific activity, 20.0 Ci/mmol; 1 Ci = 37 GBq) in 0.2 ml of phosphate-buffered saline (0.1 M sodium phosphate, pH 7.2/0.9% NaCl) (GIBCO). Mice were killed by cervical dislocation, and specimens from the thymus, spleen, MLN, stomach, duodenum, terminal ileum, rectum, and distal colon were removed. Tissues were fixed in 10% (vol/vol) neutral formal solution for histology and microautoradiography. The specimens were dehydrated, embedded in paraffin, and sectioned at 3 μm. Sections were deparaffinized, dipped in Kodak NTB2 liquid emulsion, and exposed in the dark room for 10 days. Slides were developed with Kodak D-19 solution and stained with hematoxylin and eosin.

Counting Methods. A cell was considered labeled if it had at least four grains over the nucleus. The number of grains deposited over the nucleus occupying a square area of 100 μm^2 was counted under oil immersion using a Zeiss universal binocular microscope. Ten positions in each of the areas of the tissues being studied were recorded, and the average was calculated as follows: (labeled cells in each position/total cells in each position) × 100 = % cells labeled. The rate of proliferation of cells from each site studied was quantified by measuring the average number of [^{3}H]thymidine-labeled cells per 2000 cells of each fraction examined.

Statistics. Statistical analyses were perfomed using Student's *t* test; *P* values <0.05 were considered significant.

RESULTS

Body and Organ Weight. Throughout the experiment, the mean body weight of the CEIR mice was approximately 70% that of the ad libitum-fed mice. Mean spleen and MLN weights are shown in Table 1. In the ad libitum-fed mice spleen weights increased rapidly, whereas in CEIR mice spleen size remained constant relative to body weight. MLN weights of ad libitum-fed NZB mice did not differ greatly as a function of diet, but MLN weights in ad libitum-fed MRL/*lpr* or BXSB mice increased rapidly. In CEIR mice of these strains, MLN weights were not greatly increased.

Proliferation of Epithelial Cells of the Intestine (as Revealed by [^{3}H]Thymidine Incorporation and Nuclear Staining by [^{3}H]Thymidine) in Mice Fed the Two Diets. As measured by microautoradiography, epithelial cell proliferation in the intestinal mucosa differed dramatically according to dietary energy intake (Fig. 1). The mice subjected to CEIR showed a dramatic reduction of epithelial cell proliferation at almost every level of the intestinal mucosa studied. We conclude that energy intake restriction markedly suppresses epithelial cell proliferation. This decrease was statistically significant in both age groups studied. For example, the highest proportion of [^{3}H]thymidine-labeled cells (52.3 ± 0.6%) was found among the predominant epithelial cell population (the middle layer) of the duodenum in ad libitum-fed MRL/*lpr* mice at age 5 months. By contrast, only 9.4 ± 1.2% of the epithelial cells from the CEIR mice of this strain were [^{3}H]thymidine-labeled ($P < 0.001$). It is clear from the data recorded in Fig. 1 that CEIR resulted in a decreased rate of cellular proliferation at almost every level of the intestine in each of the autoimmunity-prone strains studied.

Lymphoid Cell Proliferation. As measured by autoradiography, lymphoid cell proliferation in the spleen, thymus, and MLNs also differed dramatically according to different energy intakes in each of the three autoimmunity-prone strains of mice investigated (Fig. 2). CEIR mice showed a markedly lower level of cell proliferation in each of these lymphoid organs as compared to mice of the same age and strain fed the same diet ad libitum.

Spleen. The ad libitum-fed autoimmune-prone mice showed a greater percentage of proliferating splenic cells than did the CEIR mice, as recorded in Fig. 2. In comparison, young autoimmune-resistant C57BL/6 mice fed a commercial laboratory diet and included for comparison showed rates of cellular proliferation comparable to those that were exhibited by the autoimmune-prone mice fed the CEIR diet. Particularly at age 5 months, the ad libitum-fed MRL/*lpr* mice showed a greater percentage of proliferating splenic cells (42.3 ± 1.7%) than did CEIR MRL/*lpr* mice (17.2 ± 0.9%) or mice of a long-lived strain (C57BL/6) fed a commercial laboratory diet (11.2 ± 0.6%).

Thymus. The CEIR mice also exhibited a dramatically lower rate ($P < 0.01$) of proliferation of thymic cells compared to the rate observed in mice fed the same diet ad libitum. In these experiments, the rates of [^{3}H]thymidine incorporation by thymus cells taken from C57BL/6 mice fell between the rates observed in thymus cells taken from the experimental groups of autoimmune-prone mice consuming the two diets.

MLN. Significant differences in the rate of [^{3}H]thymidine incorporation in cells of MLNs was also observed among the groups of autoimmune-prone mice fed diets A or B. The most dramatic influence of CEIR on proliferation of MLN cells was observed in mice of the MRL/*lpr* strain. At age 5 months, the ad libitum-fed MRL/*lpr* mice showed more vigorously proliferative MLN cells (56.8 ± 1.2%) compared to MLN cells taken from MRL/*lpr* mice restricted in calorie intake (19.3 ± 1.2%) or in the long-lived C57BL/6 mice (7.1 ± 0.8%).

DISCUSSION

CEIR increases both life span and health span in autoimmunity-prone mice as well as in long-lived, autoimmunity-resistant mice and rats (1–10). The beneficial effects of CEIR

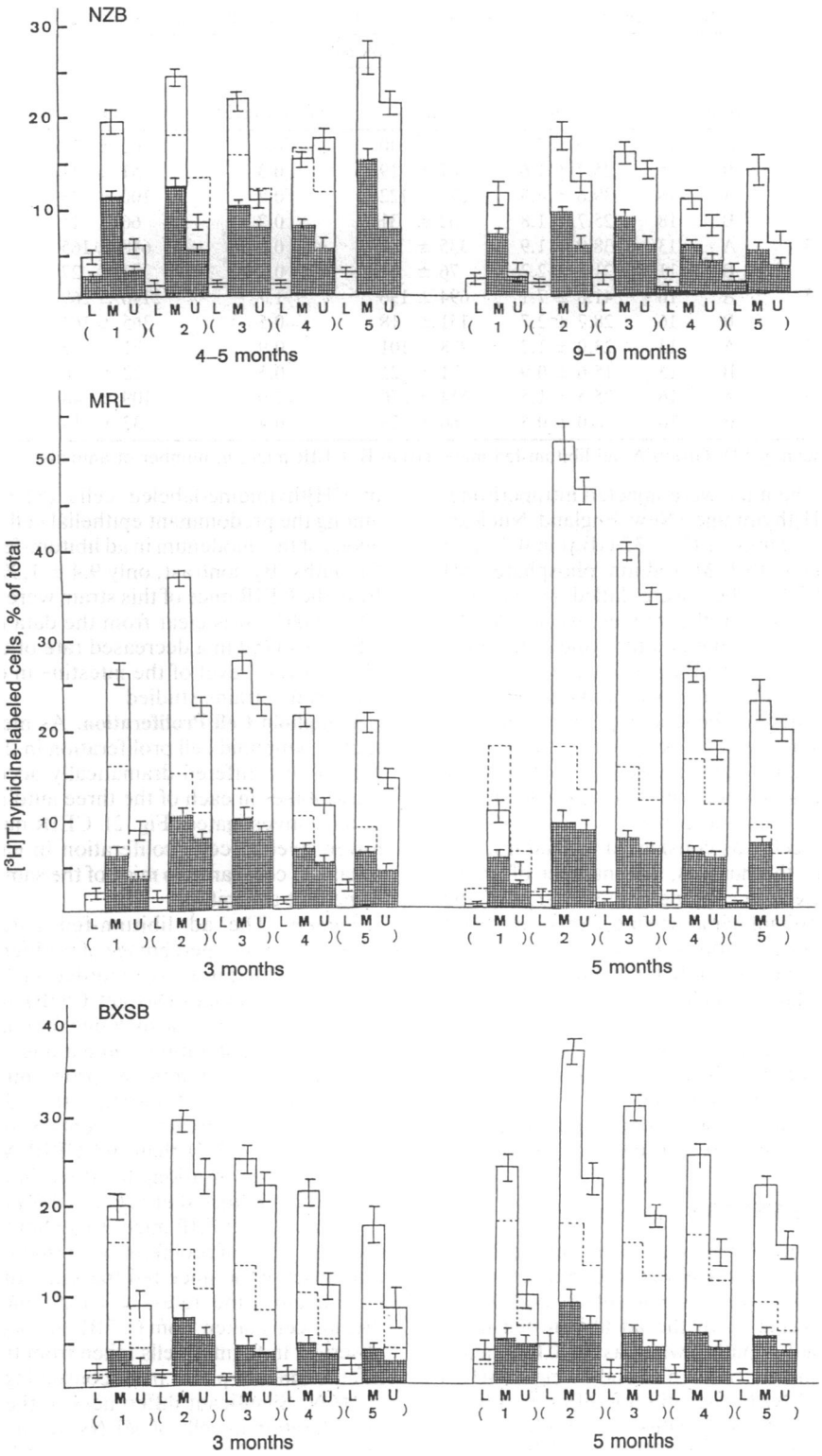

FIG. 1. Percentage of [^{3}H]thymidine-labeled cells at five levels (1–5 in parentheses) of the gut in ad libitum-fed (open bars) or CEIR (cross-hatched bars) mice of three autoimmunity-prone strains at the indicated ages, as well as in long-lived C57BL/6 mice studied for comparison (dotted line) that were fed a commercial diet. L, lower epithelium; M, middle (major) epithelium; U, upper epithelium; 1, stomach; 2, duodenum; 3, ileum; 4, upper colon; 5, lower colon.

have been demonstrated even when dietary manipulation is not imposed until after midlife in long-lived mouse strains or until after the appearance of autoimmune disease in short-lived strains (16–18).

Although a dramatic influence of food restriction has been recognized for more than 50 years, laboratory investigations have only recently begun to point to mechanisms that might explain the cellular and molecular bases of this phenomenon. A number of possible explanations for the influence of calorie restriction on immune function and life span have been brought forth. In work with long-lived mouse strains, Walford and coworkers (19) have reported that CEIR alters intestinal lipid absorption, resulting in an increased capacity to absorb fat-soluble vitamin A. Evidence also suggests that

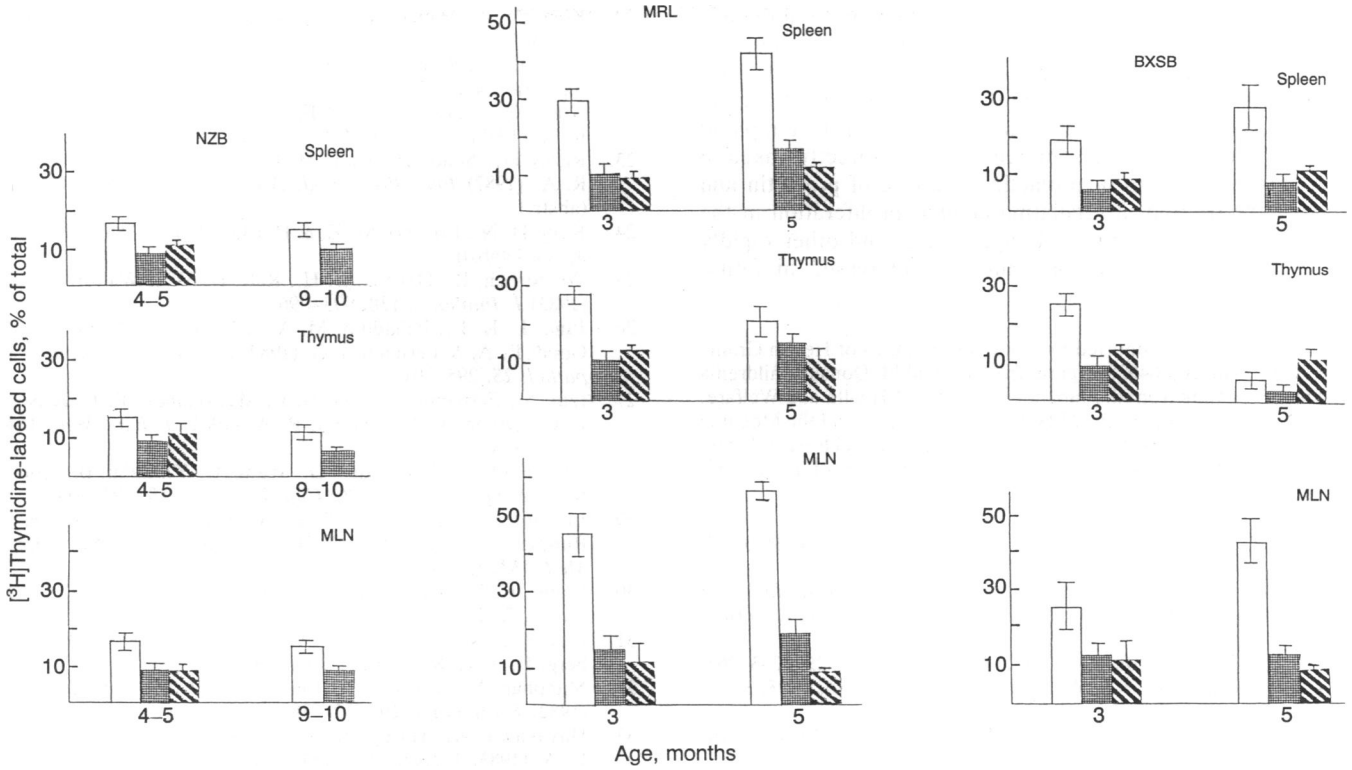

FIG. 2. Percentage of [^{3}H]thymidine-labeled cells among cells of the spleen, thymus, and MLN tissues taken from ad libitum-fed (open bars) or CEIR (cross-hatched bars) mice of three autoimmunity-prone strains at ages 3 months and 5 months. Long-lived C57BL/6 mice (hatched bars) fed a commercial laboratory diet were used for comparison.

CEIR may regulate enzymes that might be associated with oxidative injury and its role in the aging process. Koizumi *et al.* (20) found that CEIR may selectively augment levels of a radical-scavenging liver enzyme, catalase. Semsei and Richardson (21) reported that CEIR produced increased levels of catalase as well as superoxide dismutase in mice. In studies conducted in our laboratory (22), CEIR also yielded increased levels of catalase and cyanide-sensitive superoxide dismutase. However, the increase in catalase levels we observed did not appear to be directly correlated with the increase in longevity. Since different experimental designs and different mouse strains were used in these investigations, further studies of the influence of CEIR on expression of these enzymes seem warranted.

It has also been suggested that a decline in gene expression accompanies senescence. Studies in our laboratory have shown that CEIR significantly increases expression of intestinal alkaline phosphatase (ref. 23 and unpublished observations), and others (24) have observed that calorie restriction influences expression of mRNA for calcitonin, phospho*enol*pyruvate carboxykinase, and Na,K-ATPase in a complex manner. These observations suggest that CEIR exerts a selective and intricate influence on gene expression.

CEIR also profoundly affects immune function. Walford and coworkers (25) found that calorie restriction concurrently lowers natural killer cell activity but increases responsiveness of natural killer cells to stimulation by poly(I·C). Jung *et al.* (26) demonstrated that CEIR dramatically stems the progressive deficiency of interleukin 2 production that accompanies aging in B/W mice and also increases responsiveness of interleukin 2 production by thymic cells in this short-lived strain. CEIR also decreases formation of potentially injurious antigen–antibody complexes and reduces deposition of these complexes along with complement in the glomerular capillaries (27, 28).

Another interesting effect of calorie restriction on the immune system involves the interrelationship of energy intake and the growth of a subpopulation of B lymphocytes, the Ly-1 B lymphocytes, that is closely associated with autoimmune disease in autoimmunity-prone mice (29–34). In man, a homologous subset of B cells has been identified (Leu1$^+$ B lymphocytes) and closely linked with production of autoantibodies and rheumatoid factors (34, 35). We have shown that CEIR decreases the absolute and relative numbers of Ly-1$^+$ B lymphocytes among cells of the spleen, thymus, MLN, bone marrow, peritoneal exudate, and peripheral blood of mice of the three autoimmunity-prone strains investigated in the present report as well as in short-lived B/W mice (ref. 36 and unpublished observations).

In the present study we have shown that CEIR lowers [^{3}H]thymidine incorporation in the thymus, spleen, and MLNs. Moreover, CEIR decreased [^{3}H]thymidine uptake by epithelial cells along the entire length of the gastrointestinal tract. We infer that calorie restriction reduces the rate of proliferation in this major, rapidly replicating cell population of the body. These findings correlate well with the observations of Prescott (37), who showed that restriction of calories and nutrients *in vitro* inhibits replication of mammalian skin cells, and with the findings of Albanes *et al.* (38) that calorie restriction decreases the rate of cell division as well as the total number of dividing cells in the colonic mucosa of rats.

The mechanisms that may link this decrease in the rate of cellular proliferation by CEIR to the prevention of disease and extension of life span have not yet been determined. In probing the association between energy intake and mammary adenocarcinoma, a development first reported by Tannenbaum (39), we have found that in C3H mice energy restriction greatly inhibits the development of breast tumors (40–43), reduces the development of minimal alveolar lesions or hyperplastic nodules (41), inhibits replication of A and B particles of the murine mammary tumor virus (41), reduces levels of circulating murine mammary tumor virus antibodies (43), and decreases circulating prolactin levels (41). Indeed, prolactin may be a key element in the role of CEIR as an

immune regulator. This hormone is known to influence replication of lymphoid cells (44) and hepatic cells (45), and it has been found to be a potent immunomodulator (45, 46). It is possible that the capacity of CEIR to slow dramatically the rate of cellular proliferation among cells of the lymphoid organs and the intestinal epithelium can be directly linked to the powerful action of prolactin. The role of prolactin and calorie restriction in regulating cellular proliferation in the intestine, thymus, spleen, lymph nodes, and other rapidly replicating cell populations must be addressed in future investigations.

This work was supported by National Institutes of Health Grants AG 05633 and AG 04628; a grant from Ronald McDonald Children's Charities; grants from the Japanese Ministry of Health and Welfare, The Naito Foundation, the Mitsubishi Foundation, and the Mochida Memorial Foundation for Medical and Pharmaceutical Research; and Grant-in-Aid 65480147 from the Japanese Ministry of Education, Science and Culture.

1. McCay, C. M., Crowell, M. F. & Maynard, L. A. (1935) *J. Nutr.* **10,** 63–79.
2. Weindruch, R. & Walford, R. L. (1988) *The Retardation of Aging and Disease by Dietary Restriction* (Thomas, Springfield, IL).
3. Ross, M. H. & Bras, G. (1974) *Nature (London)* **250,** 263–265.
4. Weindruch, R. H. & Walford, R. L. (1982) *Science* **215,** 1415–1418.
5. Fernandes, G., Yunis, E. J. & Good, R. A. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1279–1283.
6. Fernandes, G., Friend, P., Yunis, E. J. & Good, R. A. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 1500–1504.
7. Fernandes, G., Yunis, E. J., Miranda, M., Smith, J. & Good, R. A. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2888–2892.
8. Fernandes, G. & Good, R. A. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6144–6148.
9. Kubo, C., Day, N. K. & Good, R. A. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5831–5835.
10. Johnson, B. C., Gajjar, A., Kubo, C. & Good, R. A. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5659–5662.
11. Gabrielsen, A. E. & Olsen, C. T. (1977) *Immunology* **33,** 449–452.
12. Gabrielsen, A. E. & Olsen, C. T. (1980) *Clin. Immunol. Immunopathol.* **17,** 257–264.
13. Ogura, M., Ogura, H., Pahwa, R., Johnson, B. C. & Good, R. A. (1987) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **46,** 1514 (abstr.).
14. Deschner, E. E. (1974) *Cancer* **34,** 824–828.
15. Deschner, E. E. & Lipkin, M. (1975) *Cancer* **35,** 413–418.
16. Friend, P. S., Fernandes, G., Good, R. A., Michael, A. F. & Yunis, E. J. (1978) *Lab. Invest.* **38,** 629–632.
17. Kubo, C., Johnson, B. C., Day, N. K. & Good, R. A. (1984) *J. Nutr.* **114,** 1884–1889.
18. Kubo, C., Johnson, B. C., Gajjar, A. & Good, R. A. (1987) *J. Nutr.* **117,** 1129–1135.
19. Hollander, D., Dadufulza, V., Weindruch R. & Walford, R. L. (1983) *Age* **9,** 57–60.
20. Koizumi, A., Weindruch, R. & Walford, R. L. (1987) *J. Nutr.* **117,** 361–367.
21. Semsei, I. & Richardson, A. (1986) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **45,** 217 (abstr.).
22. Kubo, C., Johnson, B. C., Misra, H. P., Dao, M. L. & Good, R. A. (1987) *Nutr. Rep. Int.* **35,** 1185–1194.
23. Risley, J., Shao, R., Dao, M. L., Johnson, B. C. & Good, R. A. (1987) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **46,** 567 (abstr.).
24. Kalu, D. N., Lozano, M. E. & Hardin, R. R. (1989) *FASEB J.* **3,** A461 (abstr.).
25. Weindruch, R., Devens, B. H., Raff, H. V. & Walford, R. L. (1983) *J. Immunol.* **130,** 993–996.
26. Jung, L. K. L., Palladino, M. A., Calvano, S., Mark, D. A., Good, R. A. & Fernandes, G. (1982) *Clin. Immunol. Immunopathol.* **25,** 295–301.
27. Izui, S., Fernandes, G., Hara, I., McConahey, P. J., Jensen, F. C., Dixon, F. J. & Good, R. A. (1981) *J. Exp. Med.* **154,** 1116–1124.
28. Safai-Kutti, S., Fernandes, G., Wang, C. Y., Safai, B., Good, R. A. & Day, N. K. (1980) *Clin. Immunol.* **15,** 293–300.
29. Ledbetter, J. A., Evans, R. L., Lipinski, M., Cunningham-Rundles, C., Good, R. A. & Herzenberg, L. A. (1981) *J. Exp. Med.* **153,** 310–323.
30. Wang, C. Y., Good, R. A., Ammirati, P., Dymbort, G. & Evans, R. L. (1980) *J. Exp. Med.* **151,** 1539–1544.
31. Lanier, L. W., Warnder, N. W., Ledbetter, J. A. & Herzenberg, L. A. (1981) *J. Exp. Med.* **153,** 998–1003.
32. Manohar, V., Brown, E., Leiserson, W. M. & Chused, T. (1982) *J. Immunol.* **29,** 532–538.
33. Hayakawa, K., Hardy, R. R., Parks, D. R. & Herzenberg, L. A. (1983) *J. Exp. Med.* **157,** 202–218.
34. Hayakawa, K. & Hardy, R. R. (188) *Annu. Rev. Immunol.* **6,** 197–218.
35. Casali, P., Burastero, S. E., Nakamura, M., Inghirami, G. & Notkins, A. L. (1987) *Science* **236,** 77–81.
36. Ogura, M., Ogura, H., Ikehara, S. & Good, R. A. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 4225–4229.
37. Prescott, D. M. (1982) *Ann. N.Y. Acad. Sci.* **397,** 101–109.
38. Albanes D., Salbe, A. D., Winick, M., Levander, O. A. & Taylor, P. R. (1989) *FASEB J.* **3,** A462 (abstr.).
39. Tannenbaum, A. (1945) *Cancer Res.* **5,** 609–615.
40. Fernandes, G., Yunis, E. J. & Good, R. A. (1976) *Nature (London)* **263,** 504–507.
41. Sarkar, N. H., Fernandes, G., Telang, N. T., Kourides, I. A. & Good, R. A. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7758–7762.
42. Engelman, R. W., Day, N. K., Bauer-Sardina, I., Smith, M., Dao, M. L. & Good, R. A. (1989) *FASEB J.* **3,** A751 (abstr.).
43. Day, N. K., Fernandes, G., Witkin, S. S., Thomas, E. S., Sarkar, N. & Good, R. A. (1980) *Int. J. Cancer Res.* **26,** 813–818.
44. Russell, D. H., Buckley, A. R., Montgomery, D. W., Larson, N. A., Gout, P. W., Beer, C. T., Putnam, C. W., Zukoski, C. F. & Kibler, R. (1987) *J. Immunol.* **138,** 267–284.
45. Bernton, E. W., Meltzer, M. S. & Holaday, J. W. (1988) *Science* **239,** 401–404.
46. Buckley, A. R., Crowe, P. D. & Russell, D. H. (1988) *Proc. Natl. Acad. Sci. USA* **81,** 8649–8653.