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Dietary Influences on Cell Proliferation in Bone Marrow

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Abstract—*Oral dietary protein–calorie malnutrition was used in rats to study the influence of malnutrition on the distribution of the dividing cells in the bone marrow (stem cells) over the compartments of the cell cycle. A 5-day period of malnutrition induced an increase in the percentage of cells in the G0/G1 phase of the cell cycle while simultaneously inducing a statistically significant decrease in the percentage of cells in S phase. Similar results were obtained after a 14-day period of malnutrition. Nutritional replenishment after 5 and 14 days of dietary deprivation induced a rapid recovery of the percentage of S phase cells and a decrease in the percentage of cells in the G0/G1 phase of the cell cycle. Malnutrition and nutritional replenishment did not influence hemoglobin concentration and platelet numbers in the peripheral blood, but leucocyte numbers increased significantly after short-term replenishment. These results demonstrate a decrease in the percentage of proliferating bone marrow cells after short periods of protein deficiency and general malnutrition. The results also demonstrate that bone marrow cells recover quickly after nutritional replenishment. In malnourished cancer patients, suspected of bone marrow insufficiency and receiving therapy that potentially impairs bone marrow proliferation, a short period of nutritional replenishment preceding treatment could possibly be marrow-protective.*

INTRODUCTION

IT HAS BEEN documented that cancer can induce malnutrition in the host and that various treatment modalities used to treat cancer potentially aggravate the malnourished state. It is also known that malnutrition in cancer patients and experimental cancer-bearing animals is associated with increased host toxicity to chemotherapeutic drugs [1–4]. In patients with cancer undergoing treatment with chemotherapeutic drugs decreased numbers of platelets and leucocytes often necessitate delays and modifications of treatment schedules. It seems probable that this is mediated through effects which these drugs exert on proliferating bone marrow cells. Several authors have described the adverse influence of malnutrition on the cellular proliferation rate [5, 6] and in earlier studies we showed that a decrease in dietary protein content unfavorably influenced the proliferation of bone marrow cells and the dividing cells in the crypts of the small intestinal tract [7]. We also demonstrated that a short period of nutritional deprivation resulted in a marked and statistically significant prolongation of the length of the period of bone marrow depression in rats treated with Methotrexate [8].

Platelets and leucocytes are non-dividing cells and are end-products of a common ancestor, the pluripotent stem cell in the bone marrow. The present study was executed to establish the influence of malnutrition and dietary replenishment on the cell cycle of stem cells in the bone marrow. Rats were subjected to a period of dietary protein deprivation followed by nutritional replenishment and the division of stem cells over the compartments of the cell cycle was determined during this period.

The results indicate that even short periods of malnutrition induce an increase in the percentage of cells that remain in the G0/G1 phase of the cell cycle, while the fraction of cells in the synthesis (S) phase accordingly decreases. However, nutritional replenishment quickly normalizes bone marrow proliferation rates.

MATERIALS AND METHODS

Female WAG/Rij rats, 11–16 weeks old and bred under specific pathogen-free conditions, were used for the experiments. All rats were maintained in temperature-controlled rooms on a 12 h light–12 h

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Table 1. Composition of the two diets used for nutritional manipulation. PD: protein deprived diet, NP: diet with a normal protein content

	PD	NP
Mixed protein*	0.0	27.0
Dextrose (glucose)	75.5	47.3
Corn starch	10.0	10.0
Alpha cellulose	5.0	5.0
KH_2PO_4	0.8	0.8
KCl	0.4	0.4
$CaCO_3$	1.2	1.2
$CaHP_4.2H_2O$	0.7	0.7
MgO	0.2	0.2
Choline chloride 50%	0.3	0.3
Vitamins and trace element mix	1.0	1.0
NaCl-iodized	0.4	0.4
Soy/sunflower oil 50/50	4.5	4.5
Lard	0.0	1.2
	100%	100%

**Mixed protein—79% crude protein*

Casein	26
Lactalbumin	10
Wheat gluten	14
Corn gluten	5
Meat meal	30
Meat protein solubles	5
Fish meal	5
Soy protein 62%	5
	100%

dark schedule. They were housed two rats to a cage and were fed one of the two study diets and water *ad libitum*. Food intake per cage was measured daily. The rats were weighed two times per week and at sacrifice. The effect of the dietary intake was assessed at two levels of protein in the diet: a protein deprived (PD) diet and a diet with a normal protein content of 27% (NP) (both diets from Hope Farms, Woerden, The Netherlands) (Table 1). The PD diet contained 75.5% dextrose, the NP diet 47.3%. The diets were iso-caloric and otherwise identical in composition.

Two experiments were conducted, differing only in the length of the period the rats were nutritionally manipulated, 5 and 14 days respectively. In experiment I, 45 rats, 11–14 weeks old and at an initial weight of 120–145 g, were used. All rats were started on the PD diet. Five randomly chosen rats were sacrificed on days 0, 1, 2, 3, 4 and 5 each. The remaining 15 rats were switched to the NP diet on day 6 and five randomly chosen rats were sacrificed on days 6, 7 and 8 of the experiment.

In experiment II, 20 rats (15 or 16 weeks old and weighing 145–185 g) received the PD diet for a period of 14 days. Five randomly chosen rats were killed on day 14. The remaining 15 rats were switched to the NP diet and five rats were killed on days 15, 16 and 17, respectively.

Before sacrifice blood was obtained via cardiac puncture under ether anesthesia. Thereafter, the rats were killed by cervical dislocation. The hemoglobin concentration (g/100 ml), leucocyte count ($\times 10^3/\mu l$) and platelet count ($\times 10^3/\mu l$) were determined in a Coulter counter (Coulter Electronics, Hialeah, FL, U.S.A.). Bone marrow was harvested from two femora by flushing the bones with phosphate-buffered saline and fixed in ethanol 70%. Cellular DNA was stained with ethidium bromide and Hoechst stain 33218. Histograms of DNA/cell distribution were determined by flow cytometry (FCM) using an ICP 11 flow cytometer (Phywe AG, Göttingen, F.R.G.), and were used to calculate the percentage of S phase cells by planimetry. Area measurements were performed with a Leitz Texture Analysis System image analyzer (Leitz, Wetzlar, F.R.G.). The mean of the outcome of the two femora was used as the percentage of S phase cells in each animal.

The results were analyzed statistically using one- and two-way analysis of variance after logarithmic transformation of the values.

RESULTS

Study I

Mean daily food intake during the experiment is shown in Table 2. Daily consumption of the PD diet was small and the rats steadily lost weight resulting in a mean weight loss after 5 days of 16 g (>10% of initial body weight). The daily food intake of those rats switched to the diet with a normal protein content increased and this was accompanied by a rapid weight gain. The mean total weight gain of the five rats which were nutritionally replenished during 3 days was 18 g and these rats experienced a net overall increase in weight of 2 g. Control rats of the same age on a continuous NP diet had a mean weight gain of 5 g per week, consuming approximately 10 g of food per day.

The percentage of bone marrow cells in the S phase of the cell cycle is also shown in Table 2 and Fig. 1. One day of dietary deprivation induced a statistically significant decrease in this percentage ($P < 0.05$). This decrease continued up to and including day 5. Nutritional replenishment initiated a quick and statistically significant ($P < 0.0001$) increase in the percentage of cells in the S phase of the cell cycle, continued through the following days (Fig. 1). On day 7 values comparable to those in control rats were achieved. The percentage of cells in the G0/G1 phase of the cell cycle changed from 73.9 ± 0.2 to 84.1 ± 0.5 ($P < 0.01$) between days 1 and 5. Following dietary replenishment this percentage decreased to 75.4 ± 0.4 on day 8 ($P < 0.01$) (Table 2).

Table 2. Mean daily food intake (g/day/rat), mean weight change (g) and the percentage of cells in the G0/G1 and S phase of the cell cycle (mean values ± S.E.M., five rats per day). Rats were subjected to 5 days of protein deprivation (PD diet), followed by 3 days of a diet with a regular protein content (NP diet)

Day	0	1	2	3	4	5	6	7	8
Diet	NP	PD	PD	PD	PD	PD	NP	NP	NP
Food intake (g)	—	5	3.4	5.2	6.4	7.4	10	12.5	11.3
Weight change (g) (cumulative)	—	−4	—	−11	—	−16	+10	+15	+18
% G0/G1	73.9 ± 0.2	77.8 ± 1.0	79.0 ± 0.7	79.6 ± 0.6	83.4 ± 1.0	84.1 ± 0.5	79.5 ± 0.4	75.7 ± 1.0	75.4 ± 0.4
% S	19.0 ± 0.3	15.9 ± 0.6	15.5 ± 0.4	15.3 ± 0.8	13.1 ± 0.8	12.4 ± 0.3	16.1 ± 0.2	17.6 ± 0.7	18.3 ± 0.4

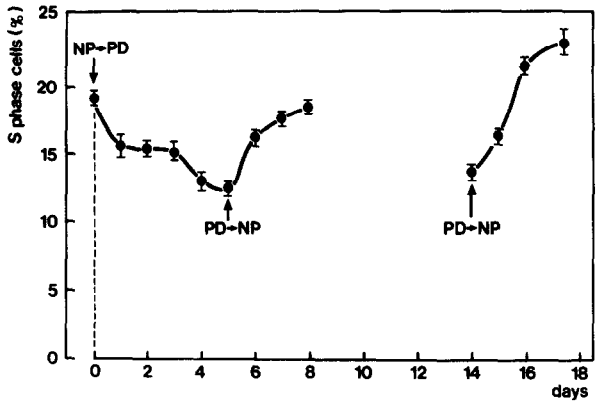

Fig. 1. The percentage of dividing cells in the bone marrow present in the synthesis (S) phase of the cell cycle after dietary protein deprivation and replenishment determined in five rats/day (left). Rats were fed a protein deficient diet (PD) for 5 days. On day 5 the diet was changed to a diet with a regular protein content (NP) (right). Rats consuming a protein deficient diet for 14 days were studied before and after dietary replenishment. The percentage of cells in S phase was measured in five rats before replenishment (days 1–5 and day 14) and daily after replenishment (days 6, 7 and 8, and days 15, 16 and 17). Dietary replenishment induced a rapid rise in the percentage of dividing bone marrow cells present in the S phase of the cell cycle. For experimental details and results, see text.

Hemoglobin concentration and leucocyte and platelet numbers showed no systematic changes between days 1 and 5 and nutritional replenishment did not alter these values (data not shown).

Study II

Table 3 shows the mean daily food intake and weight changes in experiment II. During the 14-day PD diet period mean food consumption was 4.4 g per day, inducing a mean weight loss over this period of 35 g (>20% of initial body weight). After switching the rats to the NP diet mean daily food intake nearly doubled. On day 17 the mean rat weight had increased 22 g, resulting in a net mean weight loss over the study period of 13 g. Control rats of similar age and weight continuously fed the NP diet during this period gained 5 g per week. In Table 3 and Fig. 1 the influence of dietary deprivation on the percentage S phase cells in the bone marrow is shown. As in experiment I the S phase fraction was significantly reduced after dietary deprivation, but the 14-day period of malnutrition did not induce a greater reduction in this value. Nutritional restoration triggered a quick increase in the percentage of S phase cells, resulting in the disappearance of statistically significant differences from pretreatment levels within 24 h. Between days 1 and 14 the percentage of cells in the G0/G1 phase increased from 73.9 ± 0.2 to 81.5 ± 0.8 ($P < 0.01$). Following nutritional replenishment this value decreased to 69.5 ± 1.3 ($P < 0.01$) (Table 3).

Figure 1 shows the percentage of S phase cells following nutritional replenishment after 5- and 14-day periods of protein deprivation. The rate of increase after 14 days of protein deprivation (experiment II) was more rapid ($P = 0.0027$) and resulted in values exceeding both the recovery (experiment I) and normal (control) S phase values ($P < 0.01$). The hematological parameters measured in the peripheral blood in experiments I and II are shown in Table 4. Whereas the hemoglobin concentrations remained virtually unchanged after dietary manipulation (as in experiment I), the number of leucocytes increased sharply after nutritional replenishment, with the value on day 17 being nearly double that of the controls.

DISCUSSION

The majority of patients with cancer experience some form of nutritional disturbance, with symptoms ranging from an altered taste sensitivity and food preference to anorexia and severe weight loss characteristic of cachexia [1–4, 9, 10]. A pre-existing nutritional disturbance can be aggravated by antineoplastic therapies [11–13]. In the present study the effect of dietary deprivation and replenishment on cell cycle parameters of cells in the bone marrow has been studied with a protein-deficient and a normal diet. As indicated in Tables 2 and 3, intake of the PD diet decreased during the periods of dietary manipulation. The effects on the cell cycle cannot, therefore, be attributed solely to protein deprivation, but rather to the combination of protein deprivation and general malnutrition. This combination induced acute and statistically significant changes in the distribution of bone marrow stem cells over the compartments of the cell cycle (Fig. 1). The percentage of cells in the DNA synthetic (S)

Table 3. Mean daily food intake (g/day/rat), *mean total weight change* (g) *and the percentage of cells in the G0/G1 and the S phase of the cell cycle (mean values ± S.E.M., five rats per day) during* 14 *days of protein deprivation (PD diet), followed by* 3 *days of nutritional replenishment with a diet with a regular protein content (NP diet)*

Day	0	1–14	15	16	17
Diet	NP	PD	NP	NP	NP
Food intake (g)	—	4.4	10	8	9.3
Weight change (g)	—	−35	+10	+15	+22
% G0/G1	73.9 ± 0.2	81.5 ± 0.8	78.8 ± 0.6	72.5 ± 0.8	69.5 ± 1.3
% S	19.0 ± 0.3	13.5 ± 0.6	16.2 ± 0.5	21.4 ± 0.5	23.1 ± 0.8

Table 4. Mean values of hemoglobin concentration (g/100 ml), *leucocytes* (× 10^3/μl) *and platelets* (× 10^3/μl) *in the peripheral blood after* 5 *and* 14 *days of protein deficiency in the diet (PD diet, days* 1–5 *and* 1–14) *and after nutritional replenishment with a diet with a normal protein content (NP diet, days* 15–17) *(five rats per day)*

Day	Diet	Hb	Leucocytes	Platelets
0	NP	9.1	7.6	830
5	PD	10.1	7.5	949
14	PD	9.6	8.4	745
15	NP	9.1	8.3	606
16	NP	8.9	10.5	556
17	NP	8.5	14.8	646

phase of the cell cycle decreased throughout days 1–5, but extending this period to 14 days did not induce a continuing decrease, probably indicating that a plateau had been reached (Fig. 1). Concomitantly, the percentage of cells in the G0/G1-phase of the cell cycle increased. A clear-cut explanation for these observations cannot be given from these experiments alone because of limitations in FCM as practised in this study. Other authors, using different systems, have demonstrated that protein–calorie malnutrition causes a prolongation of the cellular proliferation cycle. Deo *et al.*, describing the influence of protein deficiency on cell population kinetics in regenerating livers of rats after partial hepatectomy, found a marked depression of the rate of cellular proliferation, the result of an increased cell cycle length [5]. Ortiz and Betancourt studied cell proliferation in bone marrow cells of young rats and demonstrated a clear elongation in the total cell cycle time in malnourished animals, probably due to a lengthening of the mitotic period [6].

Nutritional replenishment induced a quick increase in the percentage of S phase cells, with nutritional restoration after 14 days of protein–calorie deprivation resulting in a more pronounced response in regard to final (maximal) S phase values and a significantly steeper rate of increase ($P < 0.001$ and $P < 0.0027$ respectively, Fig. 1). This suggests the existence of a relationship between the length of the period of malnutrition and the magnitude of recovery. With the methods used in this study, we were unable to detect changes in cellular distribution over the compartments of the cell cycle between days 5 and 14, but it is possible that after 14 days of malnutrition and protein deprivation a larger proportion of cells is blocked in G1 or hovering on the boundary of the late G1, early S phase than after 5 days of dietary manipulation. Additionally, it is also possible that a dietary stimulus after a prolonged period of dietary deprivation will induce recruitment of dormant (G0) cells. In experiment II the supra-normal S phase values after replenishment exceeding normal values are accompanied by increased numbers of leucocytes in the peripheral blood and this would seem to indicate a stimulated proliferative response.

No consistent reductions were found in the other peripheral blood parameters following 5 and 14 days of dietary deprivation (Table 4). This is not unexpected in view of the longer turn-over times of erythrocytes and platelets, but leucocytes have a much shorter life-span and would be expected to decrease gradually in numbers. It is possible that a reduction in leucocyte production has been compensated by leucocyte recruitment from stores outside the bone marrow. Because it seems probable that the increased cellular proliferation rate in the bone marrow will affect all hematopoietic progenitors, the absence of an increase in hemoglobin concentration and platelet numbers must be explained by longer transit times from progenitor cells to end products of the lineage. Alternatively, it is possible that dietary manipulation selectively influences hematopoietic progenitors.

The observation that malnutrition decreases the DNA synthesis fraction of stem cells in the bone marrow can offer an explanation for the frequently observed secondary anemia of malignancy and the decreased marrow production of platelets in cancer patients [14]. Why acute or chronic states of malnutrition in cancer patients are seldom accompanied by granulocytopenia is not known, but it seems possible that immunogenic stimuli can obscure the effects of malnutrition.

In this study we have not addressed the role of (human) hematopoietic colony-stimulating factors

(CSFs) (for review article see [15]). It is possible that the observed effects of malnutrition on the distribution of stem cells over the compartments of the cell cycle are mediated via hematopoietic growth factors and that administration of CSFs will correct hematopoietic dysfunction without nutritional replenishment. This can be of interest for those patients for whom nutritional replenishment is impossible or desired results are not obtainable with nutritional manipulation.

This study demonstrates that rats which are subjected to a relatively short period of protein deprivation and general malnutrition showed severe signs of a decreased proliferation rate of bone marrow cells. Similar effects might be present in humans although it is probable that in cancer patients the nutritional problem is lack of eating rather than eating no protein at all. Nevertheless, these results could indicate that malnourished patients will be more vulnerable to the toxic side-effects of chemotherapeutic drugs on the bone marrow. This study also shows that a short period of nutritional restoration normalizes the distribution of cells over the compartments of the cell cycle in combination with transient supra-normal S phase values and a proliferative burst.

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REFERENCES

1. Theologides A. Cancer cachexia. *Cancer* 1979, **43**, 2004–2012.
2. DeWijs WD, Begg C, Lavin PT *et al.* Prognostic effect of weight loss prior to chemotherapy in cancer patients. *Am J Med* 1980, **69**, 491–498.
3. Costa G, Lane WW, Vincent RG *et al.* Weight loss and cachexia in lung cancer. *Nutr Cancer* 1981, **2**, 98–103.
4. Freeman M, Fankmann C, Beck J, Valdivieso M. Prognostic nutrition factors in lung cancer patients. *J Parent Ent Nutr* 1982, **6**, 122–127.
5. Deo MG, Mathur M, Ramalingaswami V. Cell regeneration in protein deficiency. *Nature* 1967, **216**, 499–500.
6. Ortiz R, Betancourt M. Cell proliferation in bone marrow cells of severely malnourished animals. *J Nutr* 1984, **114**, 472–476.
7. Dunki Jacobs PB, de Graaf PW, Ruevekamp M *et al.* The influence of dietary protein content on cell cycle parameters of tumor and host tissue. Impact of protein deprivation and replenishment. *Clin Nutr* 1988, **7**, 1–6.
8. Dunki Jacobs PB, Ruevekamp M, Varrosieau FJ *et al.* Alterations in serum levels, antitumor activity and toxicity of Methotrexate in rats after a short period of nutritional depletion. *Eur J Cancer Clin Oncol* 1989, **25**, 415–422.
9. Heber D, Byuerley LO, Chi J *et al.* Pathophysiology of malnutrition in the adult cancer patient. *Cancer* 1986, **58**, 1867–1873.
10. Bernstein IL. Etiology of anorexia in cancer. *Cancer* 1986, **58**, 1881–1886.
11. Chlebowski RT. Critical evaluation of the role of nutritional support with chemotherapy. *Cancer* 1985, **55**, 268–272.
12. Nixon DW. The value of parenteral nutrition support. Chemotherapy and radiation treatment. *Cancer* 1986, **66**, 1177–1195.
13. Torosian MH, Daly JM. Nutritional support in the cancer-bearing host. Effects on host and tumor. *Cancer* 1986, **68**, 1915–1929.
14. Williams WJ, Beutler E, Ersier AJ, Litchtman MA, eds. *Hematology*, 3rd edn, New York, McGraw-Hill, 1983, 162–177.
15. Clark SC, Kamen R. The human hematopoietic colony-stimulating factors. *Science* 1987, **236**, 1229–1237.