EFFECT OF CALORIE RESTRICTION ON THE DEVELOPMENT OF VIRUS INDUCED LEUKAEMIA IN MICE

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Abstract The present study reports for the first time the influence of dietary restriction to mother of suckling animals in reducing incidence of Virally induced leukaemia. A delay in the carcinogenic process was also noted in the offsprings maintained on dietary restriction during suckling.

Introduction Dietary restriction, more specifically, the restriction of the total calories in an animals diet has been known for several years to reduce the incidence of both naturally occuring and induced tumors in several organs (1-6).

The present study is an attempt for the first time to determine the effect of calorie restriction during suckling on the development of virus induced leukaemia in mice.

Materials and Methods Animals:Mice of Strain A/Rb. Experimental Groups : Group I (Normal Diet): The mother was given food and water ad libitum and caged with 6-7 new borns. On an average, the total intake of a mother was found to be 7.0 gm of food pellet per day. Group II (Calorie restricted) : The amount of pellet diet given to mother was cut down by 50% of its normal requirement by providing only 3.5 gm of pellet per mouse, instead of the normal 7 gm. Further calorie restriction was induced by the method of Widdowson and McCance (7) by increasing the litter size to 16 per mother.

The young animals were separated from their mother when they were four weeks old and thereafter maintained on normal diet during the experimental period in both groups.

Diet : Food pellets were purchased from Lipton India Ltd. The composition of food pellet is as follows :

Crude protein (% min) 20.0, Ether extract (% min) 4.0, Crude fibre (% max) 4.0, Ash (% max) 8.0, Ca (% min) 1.0, P (% min) 0.6, N2-free extract (%) 55.0.

Total metabolized energy of the pellet estimated is 3600 K Cal/Kg.

Virus Inoculation : Cell free extracts of affected organs of Moloney's Virus induced lymphoblastic leukaemia in mice, were prepared by homogenization of spleen, lymph nodes, mesenteric glands and liver in normal saline followed by centrifugation at 15,000 g for 30 minutes at 4°C. New born mice in both groups were inoculated with 0.1 ml of the extract on the second day of their post natal life.

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Development of leukaemia was assessed by (1) serial peripheral blood leukocytes count (2) external palpation of thymus, spleen and axillary lymph node for detecting enlargement in these organs which was confirmed by autopsy and histological examination. Body weight and weight of affected organs were recorded from the 15th day post inoculation. The latent period during the development of the disease was estimated from the observations which continued through 250 days.

Results Our initial observation revealed that mortality was higher in the calorie restricted group during the first month (Table I). However, this was reduced once the restricted group switched over to full diet.

Calorie restriction was found to significantly retard the Virus induced leukaemogenesis by extending the latent period as evident from the fall in incidence of disease development in group II in comparison in group I (Table II).

A gradual increase in total leukocyte count in peripheral blood was observed which is more marked in the group receiving full diet (Table III).

Appearance of blast cells were apparent after the 10th week post inoculation in both groups, but the percent of lymphoblastoid cells were very low in the calorie restricted group .

Body weight of animals in calorie restricted and full fed groups were recorded during the ongoing process of virally induced leukaemogenesis (Fig.1). The full fed group maintained higher body weight all through in comparison to the calorie restricted group. Observation on the weight of spleen and thymus reveal increase in spleen and thymus weight in Group I in comparison to Group II (Figs.2 and 3).

The incidence and survival of the virus induced leukaemia has been shown in Tables IV and V. The incidence of leukaemia was found to be sharply reduced in the calorie restricted group. A considerable improvement in the survival (31%) was also recorded.

Discussion It appears from the present study that restriction of diet to the mother during suckling has a pronounced retarding effect on the development of leukaemia following virus inoculation. Spleen and thymus are known to

be the chief sites of replication of virus particles (8). In case of induction of lymphoblastic leukaemia by Moloney's Virus used in the present study, there is a gradual increase in size and weight of these organs following virus inoculation. Histological examination reveal massive infiltration of these organs with immature lymphoid cells as well as lymphocytes, which obliterates the normal architecture of the tissue in later stages (after the 10th week). A gradual increase in total blood leukocytes was noted from the 4th week onwards and presence of lymphoblastoid cells is found after 10 weeks following virus inoculation. While the early signs of manifestation of the disease was noted during 7-8th week postinoculation in the full fed group (Group-I), dietary restriction of the lactating mother and consequently to the suckling new

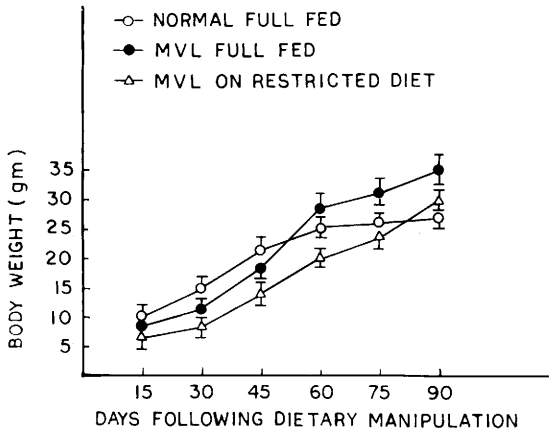

Figure 1. Body weight of normal and leukaemic mice from 15th to 90th day of their postnatal life. The animals were reared with their mothers who were maintained on normal and calorie-restricted diet. Values are mean ± SE (vertical bars) of 12 animals.

Table I. Mortality Rates at Different Experimental Stages[a]

Experimental group	% Mortality		
	30th day	60th day	90th day
Group I (normal diet)	32	20	20
Group II (restricted diet)	42	16	10

[a] Mortality rate of leukaemic mice from 30th to 90th day of their postnatal life. The animals were reared with their mothers who were maintained on normal and calorie-restricted diets.

Table II. A Comparison of the Latent Period during Development of Moloney Lymphoblastic Leukaemia and Its Incidence in the Two Dietary Groups

Group	Latent period (days)		Incidence (%)
	Range	Mean ± SE	
Group I (normal diet)	70–122	99.8 ± 4.0 (32)[a]	62
Group II (restricted diet)	82–191	144.2 ± 8.9 (39)[a]	25

[a] Values are the mean ± SE of the number of mice indicated in parentheses; P 0.001.

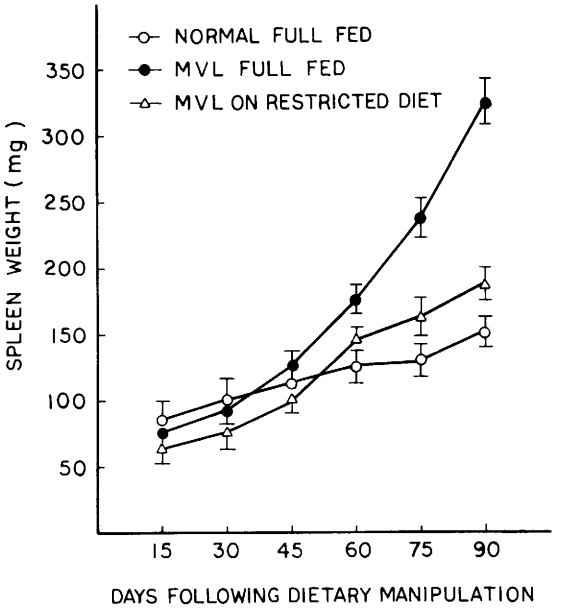

Figure 2. Weight of spleen in normal and leukaemic mice under different dietary schedules which include normal and dietary restriction during their suckling stage. Values are mean ± SE (vertical bars) of eight animals.

Table III. Total Leukocyte Count in Peripheral Blood[a]

	Total leukocyte count × $10^3/mm^3$			
	4th week	6th week	8th week	10th week
Group I (normal diet)	8.3 ± 0.912 (5)[b]	10.5 ± 0.910 (5)[b]	14.75 ± 0.820 (7)[b]	17.20 ± 1.10 (9)[b]
Group II (restricted diet)	7.12 ± 0.525 (5)	7.50 ± 0.825 (%)	9.75 ± 0.620 (7)	11.50 ± 0.980 (7)
Nontumor mice	6.60 ± 0.612 (7)	6.92 ± 0.595 (6)	6.52 ± 0.495 (6)	7.25 ± 0.820 (5)

[a] Peripheral leukocyte count in normal and leukaemic mice under different dietary schedules which include normal diet and dietary restriction during their suckling stage.
[b] Values are the mean ± SD of the number of mice indicated in parentheses.

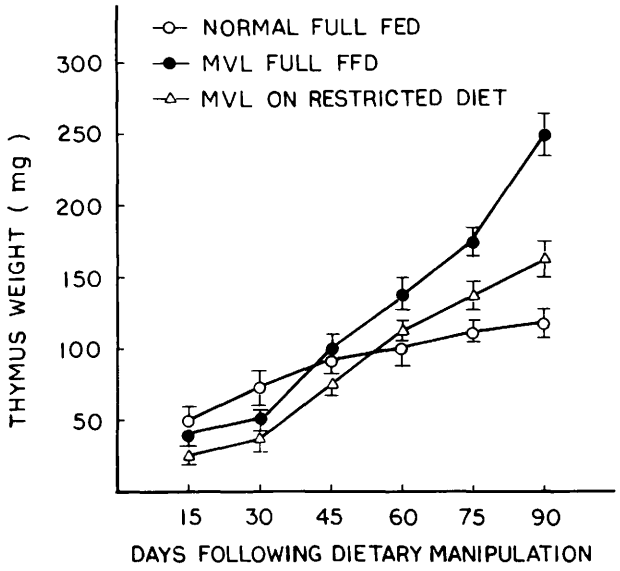

Figure 3. Weight of thymus at different intervals postinoculation with leukaemia virus during growth following dietary modulation at the suckling stage. Values are mean ± SE (vertical bars) of eight animals.

Table IV. Incidence of Moloney Virus-Induced Leukaemia in the Two Dietary Groups

Group	No. of animals inoculated	No. of animal developing leukaemia	% of incidence
Group I (normal diet)	32	20	62.5
Group II (restricted diet)	39	10	25.6

borns produced a prolongation of the latent period thereby delaying the onset of the disease. Cellular proliferation in spleen and thymus was also retarded as revealed by organ weight and histological examination. Besides prolonging the latent period, calorie restriction was also found to reduce the incidence of leukaemia considerably along with an increase in the life span of the leukaemic mice.

While there have been reports on the role of restricted diet in the prevention of carcinogenesis (2,5) the present report furnishes new light on the influence of dietary manipulation of the mother in checking viral carcinogenesis in the offsprings. The full implication of this

Table V. Survival of Leukaemic Mice in the Two Dietary Groups

Groups	Survival (days)		% Increase in life-span[b]
	Range	Median survival[a]	
Group I (normal diet)	110–180	145	—
Group II (restricted diet)	130–250	190	31.03

[a] Median survival: $\frac{\text{First death} + \text{Last death}}{2}$.

[b] % Increase in life-span: $\frac{\text{Median survival of group I}}{\text{Median survival of group II}} - 1 \times 100$.

observation in terms of human health is as yet too early to be appreciated. Hence further studies are being conducted in detail in this line in murine model.

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