

Hematological and immunological effects of excess dietary leucine in the young rat¹

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ABSTRACT Young rats were subjected to a dietary leucine overload for several weeks. Although no significant changes in growth, food consumption, and hematological and immune responsiveness occurred when the basic diet was balanced (18% casein), rats which were both overloaded with leucine and subjected to a protein-poor diet (4% casein) displayed a strong impairment of immunological reactions to sheep red cells (rosette and plaque-forming cells in the spleen, serum hemagglutinins, and hemolysins). The effect was significantly more pronounced than in rats on a protein-poor nonsupplemented diet or in pair-fed controls on a balanced diet. The immunodepression was as profound as after complete protein deprivation. It is suggested that a secondary defect in valine and isoleucine utilization may play a role in the harmful effects of excess leucine, because isoleucine and especially valine have been shown to be highly deleterious to lymphopoiesis. *Am. J. Clin. Nutr.* 30: 1645-1654, 1977.

A protein-deprived diet which is protracted for several weeks provokes an important reduction of certain immunological responses in the rat, especially of those responses directed toward heterologous red blood cells. This effect concerns the titers of serum hemagglutinins and hemolysins (1-6) as well as the number of hemolytic plaque-forming cells (PFC) (4-9) and rosette-forming cells (RFC) (3-6, 9), although the latter are much more resistant than PFC in protein-deprived adult rats (3-6).

On the other hand, it has been reported that dietary overload with leucine induced a secondary deficiency in valine and isoleucine (10-12) which may be due to an excessive activation of enzymes of the catabolic pathway of branched chain amino acids (13, 14).

A lack of isoleucine and especially of valine leads to an inhibition of leukopoiesis (granulocytes as well as lymphocytes), with involution of the lymphoid organs, especially the thymus, and a dramatic drop in the blood lymphocyte levels (15-18). Therefore, it was of interest to know whether an overload of leucine also induces lymphopenia and atrophy of the thymus, and whether there is a depression of the immunological potential, which is known to depend primarily on lymphocyte activity.

The experiments reported in this paper indeed revealed an immunodepressive ef-

fect of excess intake of leucine, but only in rats sensitized by a protein-poor diet and not in well-nourished animals.

Materials and methods

Animals and diets

Sixty-one young pathogen-free male Charles River rats which initially weighed about 100 g each were divided into seven groups fed the following diets: I—a balanced 18% casein diet⁴ (eight rats); II—the same diet with L-leucine (Ajinomoto, Tokyo) (nine rats); III—a diet containing only 4% casein⁴ (nine rats); IV—

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⁴ This diet contained (in g/100 g of dry weight): vitamin-free casein, 18; sucrose, 55.4; white dextrin, 10; peanut oil, 10; salt mixture, 5.7; choline chloride, 0.5; inositol, 0.06. It also contained the following vitamins (in milligrams): thiamin, 1.0; riboflavin, 2.0; niacin, 20.0; calcium pantothenate, 3.0; pyridoxine, 2.0; folic acid, 0.1; vitamin B₁₂, 0.005; biotin, 0.02; ascorbic acid, 25.0; DL- α -tocopheryl acetate, 1.0; and retinyl palmitate, 5,000 IU, and ergocalciferol, 1,000 IU. The salt mixture (Orent-Keiles mixture no. 22) (19) consisted of (in percentages): CaCO₃, 18.90; CaHPO₄·2H₂O, 12.60; KCl, 14.90; MgSO₄, 8.77; NaH₂PO₄·H₂O, 20.10; NaCl, 22.20; NaI, 0.0026; Na₂SiO₃·9H₂O, 0.61; FeSO₄·(NH₄)₂SO₄·6H₂O, 1.22; CuSO₄·5H₂O, 0.44; MnSO₄·4H₂O, 0.087; ZnO, 0.18. In the protein-deficient diets casein was partly or completely replaced by sucrose; all the other components were the same.

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the same diet supplemented with L-leucine (nine rats); V—an 18% casein diet given in amounts not exceeding those ingested in group IV (eight rats); and VI and VII—diets completely deprived of protein⁴ administered for 4 weeks (10 rats) or 5 weeks (eight rats). The other diets were maintained for 6 weeks (group I) or 8 weeks (Groups II to V). Drinking water was allowed ad libitum. Leucine was introduced into the diet at a concentration of 3% during the first 18 days and at 7% during the following period in order to accustom the animals to this compound.

The rats were housed in individual compartments at 22 C. Body weights were recorded weekly and the dietary intake was measured daily.

The duration of the diet in groups VI and VII was shortened because of the poor resistance of young rats (30 days of age at the start of the experiments) to complete protein deprivation.

Hematological and immunological examinations

On day -8 before killing, the rats of all groups were immunized with an intraperitoneal injection of sheep red blood cells (SRBC) (2.4×10^9 cells/100 g of body weight) without adjuvant added. The animals were killed by cardiac exsanguination. The blood was used for titration of serum antibodies. Thymus, spleen, and cervical nodes were excised free from any adhering connective tissue and weighed. Cell density in the spleen (number of lymphocytes per milligram of tissue) and the total lymphocytic population of this organ was determined (20), as was the percentage of viable lymphocytes. Viability was measured using a dye (erythrosin) exclusion test, which was performed immediately after addition of the dye to the cell suspension (21).

On the day the animals were killed, a leukocyte count was performed on tail blood samples, with counting of granulocytes and lymphocytes per cubic millimeter, as calculated from their percentage distribution.

The immunological tests consisted of the enumeration of RFC and PFC in the spleen according to the methods of Zaalberg (22) and Cunningham and Szenberg (23), respectively, and of the titration of serum antibodies (hemagglutinins and hemolysins) according to methods used previously (4).

Separation of immunoglobulins IgG was accomplished by treating sera with 2-mercaptoethanol (2-ME) (4, 24). The two antibody titers were expressed as decimal logarithms of geometrical means of the highest dilutions giving an agglutination visible with the naked eye or giving complete hemolysis, respectively. In the protein-deficient groups the quantities of serum were too small to permit the separate titration of IgG hemolysins in each rat.

Results

Body weight and food intake (Tables 1, 2, and 3)

The body weight increased almost 4-fold in 6 weeks in group I (18% casein control rats), and growth was not at all delayed after addition of leucine (group II).

In the protein-deficient (groups III and

IV) (4% casein) the body weight remained at the initial level. Administration of leucine was without any effect. In control rats of group V pair-fed with those of group IV (4% casein + leucine) the increase in body weight was much lower (only +50% of the initial values) than in groups I and II fed the same balanced diet ad libitum without or with extra leucine, but it was significantly higher than in the 4% casein groups III and IV.

The rats which were completely deprived of protein (Groups VI and VII) lost 35% of their initial weight after 4 (VI) or 5 (VII) weeks. Their aspect and behavior (weakness, inactivity, and thinning of the hair, which became dull, ruffled and dirty) indicated a state of cachexia. Indeed, two rats of the group VII died before the end of the experimental period.

The food intake was also the same in the absence and the presence of extra leucine when the diet was well provided with protein (I and II). In group III (4% casein) the food intake was closer to that of protein-free groups (VI and VII) than to that of the 18% casein groups. Contrary to what had been noticed with the latter groups, a 7% leucine supplement still significantly reduced food ingestion in rats on a 4% casein diet (compare group IV to group III, Table 3). In rats on a protein-free diet, food consumption did not exceed 50% of the initial consumption of nondeficient animals (I and II).

Hematological results

Lymphoid organs (Tables 1 and 4). Expressed per 100 g of body weight (which was necessary because of the unequal duration of certain diets and the difference of final body weights), the weights of the spleen and cervical lymph nodes did not differ significantly in rats whose diets were or were not supplemented with leucine, when the protein intake was adequate. Although the thymus was somewhat smaller in supplemented animals, the lack of significant thymic involution militated against the existence of a nonspecific stress effect of excess leucine when given with a balanced diet (Table 1).

Neither were there any changes in the cellular density and total level of splenic lymphocytes, nor in the viability of these cells after overcharge of leucine (Table 4).

TABLE 1
Body weights and weights of lymphoid organs in rats nourished
with balanced or protein-deficient diets—effect of
overload with leucine*

Group (no. of rats)	Diet	Dura- tion (days)	Body weight (g)		Weight of the lymphoid organs (mg)					
			Initial	Terminal	Thymus		Cervical lymph node		Spleen	
					Absolute	Relative	Absolute	Relative	Absolute	Relative
I (8)	18% casein ad libitum	42	105.4 ± 3.0	376.5 ± 5.6	790.6 ± 70.3	210.6 ± 20.2	21.8 ± 5.8	5.9 ± 1.6	812.9 ± 32.1	216.0 ± 8.1
II (9)	18% casein + leucine	56	105.3 ± 1.6	426.9 ± 13.4	695.3 ± 47.8	164.7 ± 13.5	21.1 ± 3.8	5.0 ± 0.9	829.6 ± 55.7	195.6 ± 14.3
III (9)	4% casein	56	105.9 ± 3.1	98.8 ± 9.0	129.7 ± 32.9	114.6 ± 24.5	6.3 ± 0.8	6.4 ± 0.5	163.1 ± 19.0	164.2 ± 11.5
IV (9)	4% casein + leucine	56	107.8 ± 3.9	92.4 ± 2.8	70.6 ± 5.5	76.9 ± 5.9	7.0 ± 1.2	7.6 ± 1.3	166.7 ± 9.0	179.9 ± 5.9
V (8)	18% casein pair-fed with IV	56	108.4 ± 1.3	154.4 ± 4.7	255.8 ± 9.1	166.8 ± 7.8	8.2 ± 3.3	5.1 ± 1.8	317.1 ± 32.1	202.8 ± 14.6
VI (10)	Protein-free	28	106.6 ± 1.8	64.6 ± 0.9	23.3 ± 2.6	36.2 ± 2.2	4.5 ± 0.5	7.0 ± 0.1	100.7 ± 4.5	156.3 ± 4.6
VII (6)	Protein-free	35	105.6 ± 1.4	64.8 ± 2.3	19.7 ± 2.4	30.3 ± 3.7	3.4 ± 0.4	5.3 ± 0.6	81.9 ± 11.3	125.4 ± 16.3

* Values given are means ± SEM. The letters $a = P < 0.05$, $b = P < 0.01$, and $c = P < 0.001$ indicate the degree of significance of differences between 2 means within the same column. The subscripts 1, 2, 3, etc. identify the mean of the group to which the other mean is compared (1 = mean of the group I, 2 = mean of the group 2, etc.).

In group III (4% casein, nonsupplemented) the absolute weights of the lymphoid organs were obviously much lower than in the 18% casein control group I. That was also the case for the relative weights of the spleens and the thymuses as well as for the lymphocyte number and viability in the spleen.

The addition of leucine to a 4% casein diet (group IV) did not significantly change the weight of the thymuses compared to group III. This was due mainly to the high variability in the latter group, with an equal number of low and high values. An important difference was noticed when the weights of the lymphoid organs of the 4% casein groups (III and IV) were compared to those of pair-fed group V. The absolute

weights of the spleens and thymuses and the total number of splenic lymphocytes were all much higher in the pair-fed animals. These differences were greater between IV and V than between III and V for the relative weights of the thymuses, and for the viability of splenic cells. They were significant only for the ratio IV/V. Thus the changes in the lymphoid organs exhibited by the group III and especially the group IV could not be ascribed exclusively to the decrease in the food intake observed in these animals.

In rats completely deprived of protein (VI and VII) the absolute and relative weights of thymuses and spleens were much lower than in rats of group V (Table 1), and after 35 days of protein deprivation the lymphocyte population of the spleen and the viability of these cells were also below those of rats maintained for 56 days on a 4% casein diet with or without added leucine (Table 4).

Blood leukocytes (Table 5). Although important individual variations were observed even in nondeficient rats, a highly significant leukopenia occurred in the five malnourished groups as compared to the controls, with a selective drop in lymphocyte levels. No differences were noticed between rats that were or were not supplemented with leucine.

Immunological results (Tables 6 and 7)

The RFC and PFC levels and the serum antibody titers were not influenced by the addition of leucine to a balanced diet. A 4% casein diet not overloaded with this amino

TABLE 2
Body weight changes after
4 weeks of diet*

Group	Diet	Body weight changes (g) after 28 days
I	18% casein ad lib.	+208.7 ± 7.6 <i>c₃₋₇</i>
II	18% casein + leucine	+205.5 ± 9.3 <i>c₃₋₇</i>
III	4% casein	-10.4 ± 5.4 <i>b₅; c_{1, 2, 6/7}</i>
IV	4% casein + leucine	-21.8 ± 3.9 <i>c_{1, 2, 5, 6/7}</i>
V	18% casein pair-fed with IV	+10.4 ± 2.1 <i>b₃; c_{1, 2, 4, 6/7}</i>
VI + VII	Protein-free	-41.5 ± 1.3 <i>c₁₋₅</i>

* Values given are grams ± SEM. The significant differences are presented as in Table 1.

TABLE 3
Mean daily intake per rat and per week in the
different dietary groups^a

Time periods (weeks)	Group I (18% casein)	Group II (18% casein + leucine)	Group III (4% casein)	Group IV (4% casein + leucine)	Group VI (protein-free, 4 weeks)	Group VII (protein-free, 5 weeks)
1st	16.9 ± 0.6	16.8 ± 0.5	10.9 ± 0.4	10.4 ± 0.3	8.0 ± 0.2	7.5 ± 0.5
2nd	21.7 ± 0.8	21.1 ± 0.4	10.9 ± 0.6	10.2 ± 0.3	7.7 ± 0.4	8.5 ± 0.8
3rd	23.7 ± 0.9	24.2 ± 0.4	10.7 ± 0.5	8.3 ± 0.3	8.0 ± 1.0	7.3 ± 0.6
4th	25.6 ± 0.8	25.9 ± 0.7	10.8 ± 1.0	6.9 ± 0.3	6.4 ± 0.4	7.1 ± 1.2
5th	25.4 ± 0.6	25.8 ± 0.4	10.3 ± 0.6	7.7 ± 0.3		6.8 ± 1.2
6th	25.7 ± 0.6	26.0 ± 0.6	10.7 ± 0.6	7.4 ± 0.5		
7th		25.9 ± 0.6	10.3 ± 0.8	7.3 ± 0.4		
8th		24.1 ± 1.0	9.9 ± 0.8	7.7 ± 0.2		

^a Values given are grams of dry weight ± SEM. The rats of group V pair-fed with group IV consumed completely their daily ration.

TABLE 4
Number and viability of lymphocytes in the spleen as
function of the protein levels in diet—effect
of overload with leucine*

Group (no. of rats)	Diet	Lymphocytes in the spleen			% viable splenic lymphocytes
		No./mg of splenic tissue	No. in the entire organ ($\times 10^3$)		
			Absolute no.	No./100 g of body weight	
I (8)	18% casein ad lib. (42 days)	1,220,860 \pm 62,780	990,789 \pm 59,876	263,168 \pm 15,139	62.45 \pm 1.99
II (9)	18% casein + leucine (56 days)	1,131,020 \pm 57,800	944,050 \pm 88,180	223,908 \pm 23,120	58.26 \pm 1.38
III (9)	4% casein (56 days)	712,578 \pm 101,797	127,684 \pm 26,950	119,616 \pm 19,533	47.51 \pm 2.50
IV (9)	4% casein + leucine (56 days)	623,750 \pm 58,308	106,226 \pm 10,496	113,739 \pm 12,387	44.92 \pm 2.57
V (8)	18% casein pair-fed with IV (56 days)	924,618 \pm 18,346	292,840 \pm 29,710	187,532 \pm 13,879	53.51 \pm 2.87
VI (10)	Protein-free (28 days)	504,464 \pm 48,786	51,955 \pm 6,506	80,499 \pm 10,163	47.64 \pm 3.01
VII (6)	Protein-free (35 days)	325,646 \pm 97,696	31,070 \pm 10,970	47,607 \pm 17,111	31.13 \pm 4.98

* Values given are \pm SEM. The significant differences are presented as in Table 1.

TABLE 5
Numbers of total leukocytes, neutrophils and lymphocytes per cubic millimeter of blood after administration of various diets*

Group	Diet (duration)	Total leukocytes	Neutrophils	Lymphocytes
I	18% casein ad lib. (42 days)	19,562 ± 1,607	2,498 ± 1,178	16,963 ± 839
II	18% casein + leucine (56 days)	19,747 ± 2,176 <i>c₃₋₇</i>	3,747 ± 1,261	15,827 ± 1,352 <i>c₃₋₇</i>
III	4% casein (56 days)	9,433 ± 1,461 <i>b_{3, 6}; c_{4, 5, 7}</i>	1,949 ± 528	7,444 ± 1,165 <i>c₃₋₇</i>
IV	4% casein + leucine (56 days)	8,400 ± 979 <i>b₂; c₁</i>	1,644 ± 241	6,942 ± 537 <i>c_{1, 2}</i>
V	18% casein pair-fed (56 days)	6,000 ± 1,015 <i>c_{1, 2}</i>	818 ± 166	5,090 ± 853 <i>c_{1, 2}</i>
VI	Protein-free (28 days)	10,415 ± 1,198 <i>c_{1, 2}</i>	2,408 ± 641	7,947 ± 696 <i>c_{1, 2}</i>
VII	Protein-free (35 days)	6,975 ± 950 <i>b₂; c₁</i> <i>c_{1, 2}</i>	1,435 ± 241	5,540 ± 785 <i>c_{1, 2}</i> <i>c_{1, 2}</i>

* Values given are ± SEM. The significant differences are presented as in Table 1.

acid (group III) did not induce by itself any significant decrease in the immunological responses studied except for a drop in the total number of RFC and PFC in the spleen as a whole, undoubtedly because of the atrophy of this organ.

On the contrary, the same protein-poor diet supplemented with leucine (group IV) provoked a dramatic fall of the RFC and PFC number, not only per entire spleen but also per 10^6 splenic lymphocytes. Significant decreases in serum antibody titers were also observed as compared to those of 4% casein nonsupplemented rats. This anomaly concerned primarily 2-ME-resistant immunoglobulins (IgG). These Ig's were completely absent 1 week after immunization in all of the rats of group IV (4% casein with extra leucine) which were examined. Separation of IgG could be carried out in seven rats of this group for hemagglutinins but only in three rats for hemolysins because of the low amounts of serum available.

In pair-fed controls (group V) the levels of RFC and PFC were significantly higher than in group IV (4% casein with extra leucine) although they were much lower than in the controls fed ad libitum (group I). However, the serum antibody titers did not differ from those of the latter group except for IgG hemolysins.

Rats completely deprived of protein (groups VI and VII) displayed a highly significant drop in RFC/ 10^6 lymphocytes as

well as per entire spleen when compared to rats of the 18% casein groups (I and II) and to those of the 4% casein group (III) non-supplemented with leucine. There was, however, no significant difference between protein-deprived rats and 4% casein rats supplemented with leucine (group IV). The number of PFC was also sharply decreased in almost all protein deprived animals (groups VI and VII).

Total titers of serum hemagglutinins and hemolysins were significantly decreased as compared to 18% casein controls only in group VII (35 days of protein deprivation), but hemagglutinin levels of the IgG class were low in both protein-deprived groups. IgG hemolysins were completely absent in the three rats of each of the groups VI and VII for which their separation could be performed.

Thus the tests employed in the present research revealed that a protein-poor diet (4% casein) overcharged with leucine given for 56 days was as immunosuppressive as a diet which was completely deprived of protein for 4 or 5 weeks. A protein-poor diet without extra leucine resulted only in a decrease in the total number of splenic immunocytes without any change in their concentration or in the titers of serum antibodies.

Discussion

1) The relatively good tolerance of excess leucine, when given to rats with a diet bal-

TABLE 6
Levels of RFC and PFC in rats submitted to balanced or protein-deficient diet and immunized to SRBC—effect of overload with leucine*

Group	Diet (no. of rats)	Duration (days)	RFC		PFC	
			per 10 ⁶ viable lymphocytes	per entire spleen	per 10 ⁶ viable lymphocytes	per entire spleen
I	18% casein ad lib. (8)	42	6,074 ± 673 C ₄₋₇	3,749,400 ± 530,900 C ₃₋₇	159 ± 23 b ₅ ; C _{4,6}	93,500 ± 9,160 C ₃₋₆
II	18% casein + leucine (9)	56	8,306 ± 2,250 a ₁ ; b ₄₋₆	4,540,400 ± 1,104,900 b _{3,5,7} ; C _{4,6}	241 ± 60 b ₄₋₆	118,550 ± 26,030 C ₃₋₆
III	4% casein (9)	56	5,373 ± 1,230 a ₁ ; b ₄₋₆	461,600 ± 156,200 a _{4,6,7} ; b ₂ ; C ₁	101 ± 32 a _{4,6}	9,000 ± 3,680 a ₄ ; C _{1,2}
IV	4% casein + leucine (9)	56	551 ± 184 b _{2,3,5} ; C ₁	29,890 ± 11,150 a ₃ ; b ₅ ; C _{1,2}	14 ± 3 a ₃ ; b _{2,5} ; C ₁	817 ± 279 a ₄ ; C _{1,2}
V	18% casein pair-fed with IV (8)	56	1,377 ± 182 b ₂₋₄ ; C ₁	240,430 ± 58,070 b _{2,4,6} ; C ₁	54 ± 13 b _{1,2,4}	8,260 ± 2,110 b _{4,6} ; C _{1,2}
VI	Protein-free (10)	28	854 ± 211 b _{2,3} ; C ₁	23,890 ± 7,060 a ₃ ; b ₅ ; C _{1,2}	28 ± 7 a ₃ ; b ₂ ; C ₁	932 ± 385 b ₅ ; C _{1,2}
VII	Protein-free (6)	35	1,517 ± 810 a _{2,3} ; C ₁	82,860 ± 45,410 a ₃ ; b ₂ ; C ₁	64 (2; 23; 0; 232)	1,299 (32; 536; 0; 4628)

* Values given are ± SEM. The significant differences are presented as in Table 1.

anced in its protein content, has already been reported by Harper et al. using 1.5 or 3.0% of this amino acid/100 g of dry food (25), and only temporary retardation of growth was observed with 5 or 7% of leucine when it was added to a low-protein (9% casein) diet (26, 27). As in our experiments, food intake was depressed after a low-protein leucine-supplemented diet (26). The noxiousness of the amino acid was overcome partly by addition of isoleucine (10, 25) and completely by isoleucine and valine given together (27).

The principal new data presented in our paper consist of the demonstration of a marked immunological deficiency after addition of excess leucine to a protein-poor diet in young rats.

Because such overload impairs the utilization of isoleucine and valine (10-12), the immunodepression may result from a secondary deficiency in these two branched chain amino acids. The lower the size of tissue stores of the latter remaining after the reduction of protein intake, the more deleterious is excess leucine for the animals.

Previous work (16-18) has shown that synthetic diets deprived exclusively of valine or isoleucine were particularly harmful for lymphopoiesis, and that the deleterious effect of valine deprivation on blood and thymic lymphocytes was even more pronounced than that of a prolonged protein-free diet. Given the close relationship between lymphocytes and immunity, these hematological data are consistent with the hypothesis of an immunosuppressive effect of valine and isoleucine deficiency induced by excess leucine.

2) In contrast to a complete protein starvation, a diet containing 4% casein without extra leucine provokes only a very moderate disturbance of immunological responses. Thus rats need only a very small intake of protein in order to preserve to a large extent their immunological potential, providing that there is no imbalanced intake of certain amino acids such as those containing branched chains.

3) In the protein-deprived rats of the present experiments (groups VI and VII), RFC's displayed not only an abrupt drop in their total levels per whole spleen but also

TABLE 7

Effect of excess leucine on serum antibody titers in rats fed a balanced or protein-deficient diet and immunized to SRBC*

Group	Diet (no. of rats)	Hemagglutinins (log 10)		Hemolysins (log 10)	
		Total	IgG	Total	IgG
I	18% casein ad libitum (8)	3.424 ± 0.097 <i>b</i> ₇ ; <i>c</i> ₄	2.634 ± 0.136 <i>a</i> _{6,7}	2.634 ± 0.075 <i>c</i> _{4,7}	1.731 ± 0.136 <i>a</i> ₅ ; <i>c</i> _{6,7}
II	18% casein + leucine (9)	3.712 ± 0.071 <i>b</i> _{3,5} ; <i>c</i> _{4,7}	3.010 ± 0.194 <i>b</i> _{6,7}	2.776 ± 0.110 <i>b</i> ₆ ; <i>c</i> _{4,7}	1.919 ± 0.170 <i>b</i> ₅ ; <i>c</i> _{6,7}
III	4% casein (9)	2.876 ± 0.261 <i>b</i> _{2,4}	2.236 ± 0.399 (7 rats)	2.408 ± 0.399 (7 rats)	0.903 ± 0.535 (4 rats)
IV	4% casein + leucine (9)	1.940 ± 0.134 <i>b</i> _{3,7} ; <i>c</i> _{1,2,5}	0 (7 rats)	1.003 ± 0.174 <i>b</i> ₇ ; <i>c</i> ₄	0 (3 rats)
V	18% casein pair-fed with IV (8)	3.311 ± 0.114 <i>b</i> _{2,7} ; <i>c</i> ₄	2.822 ± 0.097 <i>a</i> ₇ ; <i>b</i> ₆	2.860 ± 0.114 <i>b</i> ₆ ; <i>c</i> _{4,7}	1.305 ± 0.100 (6 rats)
VI	Protein-free (10)	2.829 ± 0.420	1.866 ± 0.307 (5 rats)	2.348 ± 0.075 <i>b</i> _{2,5} ; <i>c</i> _{4,7}	0 (3 rats)
VII	Protein-free (6)	2.649 ± 0.175 <i>b</i> _{1,4,5} ; <i>c</i> ₂	1.264 ± 0.525 <i>a</i> _{1,5} ; <i>b</i> ₂	1.505 ± 0.165 <i>b</i> ₃ ; <i>c</i> _{1,2,5,6}	0 (3 rats)

* Values given are log 10 ± SEM. The significant differences are presented as in Table 1.

an important decrease in their concentration per 10⁶ lymphocytes. The latter abnormality had not been observed in previous studies (3-6), which were performed on older rats (weighing initially 200 g instead of 100 g), much more resistant to protein deprivation.

Concerning serum antibodies, one may point to the complete absence of IgG among hemagglutinins and hemolysins 1 week after immunization in rats subjected to a protein-poor diet overcharged with leucine. This phenomenon contrasted with the presence of normal IgG titers after the same time interval in protein-poor nonsupplemented rats. Disappearance (or delayed production) of IgG hemolysins, however, was also observed after complete protein deprivation (groups VI and VII) in six rats in which the parameter could be examined.

In addition, the fall of IgG hemagglutinin titers noticed in the protein-starved young rats in our present study was more pronounced than the decrease in total hemagglutinin levels after 28 days of the diet. An almost exclusive impairment of IgG production (as compared to that of IgM and IgA) has already been reported in rat pups derived from mothers fed a low-protein (8% casein) diet (28). In protein-deprived adult rats, however, no significant difference has been found in previous experiments (6) be-

tween the levels of 2-ME-resistant (IgG) and 2-ME-sensitive serum immunoglobulins 1 week after immunization with SRBC.

In any case, it would be of interest to investigate whether diets lacking valine and isoleucine provoke the same anomalies in Ig production as does a diet poor in protein but overloaded with leucine.

4) Previous work has shown the role played by thymic atrophy due to protein deprivation in the decreased production of antibodies to thymus-dependent antigens such as SRBC (3-6). On the other hand, hematological findings demonstrated that valine deprivation had a particularly harmful effect on the thymus- and peripheral thymus-dependent lymphocytes (16-18).

A selective impairment of this cell class after leucine overload could thus be hypothesized. However, the relative weight of the thymus was much higher after such a diet than after complete protein deprivation, whereas the immunological alterations were at least as important with the former as with the latter diet. It cannot be excluded that peripheral T lymphocytes may be directly injured by the metabolic disturbances caused by extra leucine without an intermediate intervention of thymic atrophy.

As to the almost exclusive impairment of serum IgG production, it could be explained

by an alteration of B cells and B cell-derived plasma cells (through a hypothetical involution of a bursa-like lymphoid organ, the bone marrow itself) (29) and/or a block of the switch from IgM to IgG production controlled by T cells (30).

In actual fact, the question of a selective impairment of the thymus and T lymphocyte activities in the immunodepressive action of excess leucine cannot be resolved without also investigating the influence of this amino acid on immunological cell-mediated reactions (e.g., graft versus host reaction, skin graft rejection, or delayed hypersensitivity) as well as on humoral responses to thymus-independent antigens. It is even possible that a protein-poor, leucine-supplemented diet does not depress but on the contrary enhances the cell-mediated immunity, as has been reported in mice subjected to moderate protein deficiency (8 to 12% casein diets) (31-33).

However, because the humoral immunological anomalies induced by a 4% casein, leucine-overloaded diet resemble the disturbances observed after complete protein deprivation, one may anticipate that at least some cell-mediated responses, such as the graft versus host reaction, would be depressed after the leucine-overloaded diet in the same manner as in protein-deprived rats (18, 34).

5) The relative abundance of leucine in millet (*Sorghum vulgare*) (100 g/100 g of nitrogen (35)) has been claimed to be responsible for endemic pellagra in some parts of India (36), maybe by interfering with the tryptophan-niacin-ribonucleotides pathway (37). Because a number of enzymes of tryptophan catabolism are vitamin B₆-dependent, and some metabolic changes brought about by excess leucine can be reduced by this vitamin (38), an immunoprotective effect of the latter merits testing in leucine-overloaded animals. However, recent investigations (39) failed to confirm a disturbing effect of excess leucine on the urinary excretion of tryptophan and niacin metabolites in human subjects.

6) A rise in blood corticosterone levels has been observed in rats 12 hr after a meal containing 1 g of leucine (40) as well as in rats deprived of valine or isoleucine for 25

days (41). Therefore, secondary hypercorticalism may also intervene in the excess leucine-induced immunodepression, as has already been suggested for the immunological anomalies after protein starvation (42). ■

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