Tr. J. of Veterinary and Animal Sciences
23 (1999) 417–429

The Effects of Feeding Gelatin Containing Diet and Following Complete Feeding on The Counts of The Peripheral White Blood Cells of The Male Female Wistar Albino Rats

Muharrem BALKAYA
Adnan Menderes University, Faculty of Veterinary Medicine, Department of Physiology, Aydın-TURKEY

Received: 02.02.1998

Abstract: The effects of a long term qualitative and quantitative protein deficiency and following feeding a complete, standard commercial diet for mice and rats on the peripheral white blood cells of circa four months old female and male Wistar rats were investigated. In doing so, the animals was given first for 60 days a diet consisting of 10 or 20% gelatine and thereafter, for refeeding for 14 days, a complete chow diet for rats and mice. The concentrations of leukocytes were determined directly in the hemocytometry by the method *ad modum* Randolph (17), as it is lightly modified (16), and the results tested merely with a three-way analysis of variance with repetitions of measures in factor time where the duration of the experiment, the gelatine content of the diet and the sex of animals have been examined as possible influencing factors.

With feeding of gelatine as protein source in the diet, the number of the neutrophils increased ($p < 0,001$), whereas the number of mononuclear leukocytes and eosinophils felt dramatically ($p < 0,01$ and $p < 0,0001$, respectively). There were no significant alterations on the number of total leukocytes. The followed refeeding with a complete rat and mouse chow diet led to an increase in the number of total leukocytes ($p < 0,0001$), eosinophils ($p < 0,0001$), neutrophils ($p < 0,001$) and mono nuclear leukocytes ($p < 0,01$).

Also the differential blood picture showed significant alterations during both the protein deficiency and standard commercial rodent diet feeding periods.

Key Words: *Qualitative* and *quantitative protein deficiency, amino acid deficiency, gelatine, tryptophan, peripheral blood cells, leukocytes, rats.*

Jelatin İçeren Diyetle Besleme ve İzleyen Komple Beslemenin Erkek ve Dişi Wistar Ratlarının Perifer Kan Lökositleri Üzerine Etkileri

Özet: Uzun süreli bir protein yetersizliği ve izleyen komple, standard bir kommersiyal fare ve sıçan yeminin yaklaşık 4 aylık erkek ve dişi sıçanların periferal beyaz kan hücreleri üzerine etkileri araştırıldı. Bu amaçla hayvanlara ilk 60 gün için %10 veya %20 jelatin içeren bir yem ve arkasından 14 gün süreyle yeniden (normal) beslenme için fare ve sıçanlara özgü komple bir yetiştirme yemi verildi. Lökositlerin konsantrasyonları Randolph (17) tarafından tanımlanan metodun biraz modifiye edilmesi ile (16) direkt olarak hemositometride saptandı ve sonuçlar, zaman faktöründe tekrarlayan ölçümler için üç-yol variyans analizi ile test edildi. Değerlendirmede deneyim süresi, yemin jelatin oranı ve hayvanların cinsiyeti olası etkileyici faktörler olarak incelendiler.

Diyette protein kaynağı olarak jelatin yedirilmesi ile nötrofillerin sayısı artarken ($P<0.001$), mononükleer lökositler ve eozinofillerin sayısında dramatik bir azalma görülmüştür ($P<0.01$ ve $P<0.001$, sırasıyla). Total lökosit konsantrasyonlarında istatistiksel açıdan önemli bir değişiklik görülmemiştir. Komple fare ve sıçan yetiştirme yemi ile izleyen yemleme total lökositler ($P<0.0001$), eozinofiller ($P<0.0001$), nötrofiller ($P<0.001$) ve mononukleer lökositlerin ($P<0.01$) sayılarında bir artışa neden olmuştur. Lökosit formülü de protein yetersizliği ve izleyen standard kommersiyal kemirici hayvanlar diyeti ile besleme sırasında istatistiksel açıdan önemli değişiklikler göstermiştir.

Anahtar Sözcükler: Kalitatif ve kanitatif protein yetersizliği, Amino asit yetersizliği, jelatin, triptofan, perifer kan hücreleri, lökositler, sıçan.

Introduction

It has long been known that on protein and/or energy malnutrition the morbidity and mortality of both humans and animals are increased. This association has consequently given the rise to the question, in which extent the composition of the diet affects the specific and non-specific immune reactions as well as the hematopoiesis, and to numerous clinical and experimental investigations about the dependence of immunological and haematological reactions on the nutrition. Earlier research findings about the interactions among nutrition and hematopoietic system as well as the immune system were reviewed under others by Aschkenasy (1), Chandra and Newberne (2), Chandra (3), Wilkinson (4), Stinnett

(5) and Myrvik (6). Recently, Chevalier and Aschkenasy (7) have reported that a deficiency or surplus of a certain essential amino acid or an imbalance among essential amino acids can affect the immune system as a consequence of a simultaneous peripheral leukopenia and lymphopenia.

In experimental investigations a diet has mostly been used which leads to a protein or protein-calorie malnutrition. The corresponding dietary proteins were, in general, plants derived like wheat gluten (8-11), whose protein values are limited because of their sparse certain amino acid contents. Partly amino acid mixtures have also been used, in which one or more of essential amino acid(s) were absent (12,13). However, investigations, which used proteins from animal origin, for example gelatine, have been rarely carried out, so that their effects on the white blood cells still remain unknown. Evidence about the effects of gelatine on the concentration of eosinophils in blood is carried out solely by a short-time study at humans (14).

In the present study the effects of long-term feeding of a gelatine-diet in dependence of its gelatine content and followed feeding a complete standard commercial diet for mice and rats on the peripheral white blood cells of female and male Wistar rats will be determined.

Materials And Methods

Animals

A total of 28 female and male Wistar rats (14 females and 14 males), breaded since over 35 years conventionally as closed colony, were used as experimental animals. At the beginning of the investigation the animals were circa 4 months old and their average body weights were 430 g and 240 g for the males and females, respectively. The animals of both sexes were randomly divided into 2 experimental groups; one male and one female group received a semi-synthetic diet containing 10% gelatine and the others a diet containing 20% gelatine as dietary protein source for 60 days, and than all animals were refeed with a complete standard commercial diet for mice and rats for the following 14 days.

Housing And Nutrition Of Animals

During the experiment the animals were held single in nitrogen balance cages *ad modum* Rufeger (15). The food and water intake of animals were *ad libitum*. In the first part of the experiment, a diet containing 10% or 20% gelatine was given (Table 1), in the second part, on the other hand, all animals were given a standard commercial rodent diet for rats and mice Nr. 1314 (Fa. Altrromin, D-4937 Lage), which contains 23% protein (16). This diet was also used for breeding.

Procedures

The total experimental period was 76 days, which consisted of two parts. In the first part, which lasted 60 days, the effects of semi-synthetic gelatine containing diet on the white blood cells was investigated in 14 female and 14 male rats, which were also divided into two equal groups. A group of both sexes received the diet containing 10% gelatine and the other group the diet containing 20% gelatine as dietary protein. Subsequently, in the second part of the experiment all animals were fed with the complete standard commercial rodent diet for rats and mice for 14 days.

The blood samples were taken on the first, 7^{th}, 21^{st}, 35^{th}, 56^{th}, 67^{th} and 74^{th} days of the experiment, and that is, in each case in a half of animals in each group on two subsequent days, respectively. EDTA added to the blood to prevent the coagulation. The number of different leukocytes were determined by the means of the method described by Randolph (17) with a slight modification (16), directly in the Bürker's hemocytometry. The numbers of total white blood cells, mononuclear cells (MNL), neutrophils and eosinophils were separately determined by this method.

Statistical Processing

The data collection and processing were carried out on the EDV-System Cyber 860 of the Justus-Liebig University in Gießen under usage of BMDP (18). The changes of body weight and white blood cells during the feeding of gelatine containing diets and subsequent a standard commercial rodent diet have been studied in each case by means of a three-way analysis of variance with repeated measures in factor time under usage of the program BMDP2V, wherein the duration of experiment, the gelatine content of the diet and the sex of animals have been examined as influencing parameters.

While the usage of parametrical test methods requires under others that all data are usually assumed to come from populations with a Gaussian distribution, before carrying out of variance analysis -according to the recommendations of Sachs (19), the variance equality of groups were tested with LEVENE-Test under usage of program BMDP7D. Thus, the necessity have arisen to transform the number of neutrophils logarithmically and the number of eosinophils squire rooted whereas the count of total white blood cells and mononuclear leukocytes were nearly normal distributed.

Results

At the beginning of the experiment, the average body weight of male and female rats were 430 g and 239 g, respectively. The males lost 25% and the females 28% of their body weight by feeding the gelatine containing diet for ca. 8 weeks regardless of the gelatine content of the diet (Table 2), also independent of, if the diet contained 10% or 20% gelatine. This statistically significant loss of the body weight ($p < 0,0001$) could recompensated during the feeding with standard commercial rodent diet for 14 days approximately in all experimental animals.

The changes of white blood cells during nutrition with gelatine containing diet and during the followed refeeding with a complete diet, are represented in the tables 3 to 6 and in figures 1 to 8.

Food components	Diet containing 10% gelatine	Diet containing 20% gelatine
Gelatine	10	20
Reis starch1	63	53
Saccharose	10	10
Mineral premix2	6	6
Soja oil, raffine	5	5
Cellulose pulver3	4	4
Vitamin premix 4	2	2
Sum	100	100

Table 1. The content of food gelatine (Dry mass in % of total mass).

[1] DAB-Quality

[2] Mineral premix (60 g per kg food): $CaCO_3$ 14 g; $CaHPO_4$ 14 g; K_2HPO_4 (sicc.) 10 g; NaCl 8 g; Na_2HPO_4 (sicc.) 7 g; $MgSO_4$. 7 H_2O_5 g; Fe(II)-Gluconat . 2 H_2O 1480 mg; $MnSO_4$. 4 H_2O 450 mg; $ZnCO_3$ 40 mg; $CuSO_4$. 5 H_2O 19 mg; NaF 10 mg; KJ 0,5 mg; Na_2MoO_4 . 2 H_2O 3,5 mg.

3 Cellulose pulver Nr. 123 of Fa. Schleicher und Schüll, D-3354 Dassel.

4 Vitamin premix (20 g per kg of food): Vitamin A 15000 IE; Vitamin D_3 500 IE; Vitamin E 150 mg; Vitamin K_3 10 mg; Vitamin B_1 20 mg; Vitamin B_2 20 mg; Vitamin B_6 15 mg; Vitamin B_{12} 0,03 mg; Nicotinic acide 50 mg; Pantothenic acide 50 mg; Folic acide 10 mg; Biotin 0,2 mg; Cholin 1000 mg; p-amino -benzoic acide 100 mg; Inosit 100 mg; Vitamin C 20 mg; Reis - starch ad 20 g.

Diet	Time [d]	Animals			
		Male Animals		Female Animals	
		$\bar{X}$	Minimum-Maximum	$\bar{X}$	Minimum-Maximum
GCD-10%	0	435	486-370	239	250-227
"	7	402	438-351	227	236-214
"	21	370	390-338	211	221-191
"	35	347	365-318	197	210-178
"	56	325	344-300	177	187-162
CDRM	67	377	402-348	219	232-201
"	74	407	433-366	235	250-217
GCD-20%	0	426	458-398	238	256-226
"	7	398	425-373	223	247-218
"	21	378	408-350	204	224-196
"	35	349	376-320	185	209-177
"	56	322	331-294	167	185-160
CDRM	67	375	392-346	218	235-212
"	74	404	420-400	236	251-230

Table 2. Living body weight developments of rats during the feeding with a diet containing 10% or 20% gelatine (GCD-10% and GCD-20%) and followed with a complate standart diet for rats and mice (CDRM).

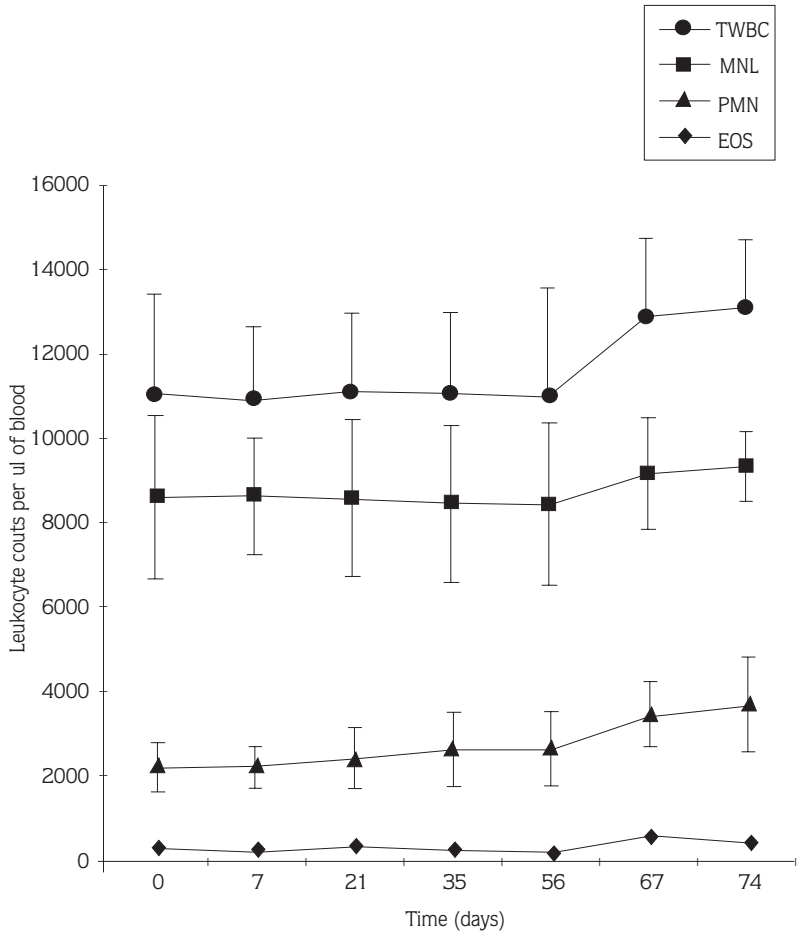

Figure 1. Concentrations of leukocytes from male rats by feeding with a 10% gelatine containing diet (0-60 days) and fallowing refeeding with a complate diet (61. 74 days).

Diet	Time	WBC		MNL		PMN		EOS	
	[d]	$\bar{X}$	± s	$\bar{X}$	± s	$\bar{X}$	± s	$\bar{X}$	± s
Male animals (n =7)									
GCD-10%	0	11015	2391	8589	1963	2190	570	236	41
"	7	10894	1707	8579	1377	2162	492	154	40
"	21	11024	1856	8498	1851	2318	686	208	75
"	35	10994	1879	8355	1861	2516	875	122	30
"	56	10910	2533	8334	1927	2520	875	56	35
CDRM	67	12756	1844	9028	1294	3317	752	411	57
"	74	12968	1581	9182	827	3531	1118	255	78
Female animals (n =7)									
GCD-10%	0	9587	1393	7444	844	1947	569	197	77
"	7	8405	866	6465	900	1762	348	177	41
"	21	8921	1427	6240	1296	2512	560	170	60
"	35	8557	1359	6229	911	2296	759	132	48
"	56	9629	1871	6641	1019	2897	1373	90	32
CDRM	67	13558	2836	8303	1634	4819	1768	436	129
"	74	11680	2866	7854	1775	3363	1510	263	120

Table 3. The count of leukocytes of rats which were fed first with a diet containing 10% gelatine (GCD-10%) and followed with a complate standart diet for rats and mice (CDRM). [WBC: Leukocytes, MNL: Mononuclear leukocytes, PMN: Neutrophils, EOS: Eosinophils] [Counts per µl of blood].

Diet	Time	WBC	MNL	PMN	EOS				
	[d]	$\bar{X}$	± s	$\bar{X}$	± s	$\bar{X}$	± s	$\bar{X}$	± s
Male animals (n =7)									
GCD-20%	0	11069	1122	8512	893	2005	244	252	40
"	7	10063	1510	8677	1117	2215	547	172	40
"	21	10351	2617	7472	1157	1732	1504	146	77
"	35	9251	1781	7204	1423	2114	680	74	45
"	56	9263	1755	8788	1423	2013	495	46	28
CDRM	67	13156	1411	8788	931	3864	697	503	114
"	74	11323	680	8089	725	2958	338	274	82
Female animals (n =7)									
GCD-20%	0	10001	1636	7567	1123	2231	600	203	65
"	7	8543	878	6624	883	1782	249	136	26
"	21	10147	1303	6763	635	3251	1400	133	42
"	35	9359	2047	6727	1114	2564	1363	68	43
"	56	9793	1036	6961	932	2788	639	45	34
CDRM	67	10949	2167	6614	1187	3879	1292	457	190
"	74	9895	1526	6587	936	3057	751	251	58

Table 4. The count of leukocytes of rats which were fed first with a diet containing 20% gelatine (GCD-20%) and followed with a complate standart diet for rats and mice (CDRM). [WBC: Leukocytes, MNL: Mononuclear leukocytes, PMN: Neutrophils, EOS: Eosinophils] [Counts per µl of blood]

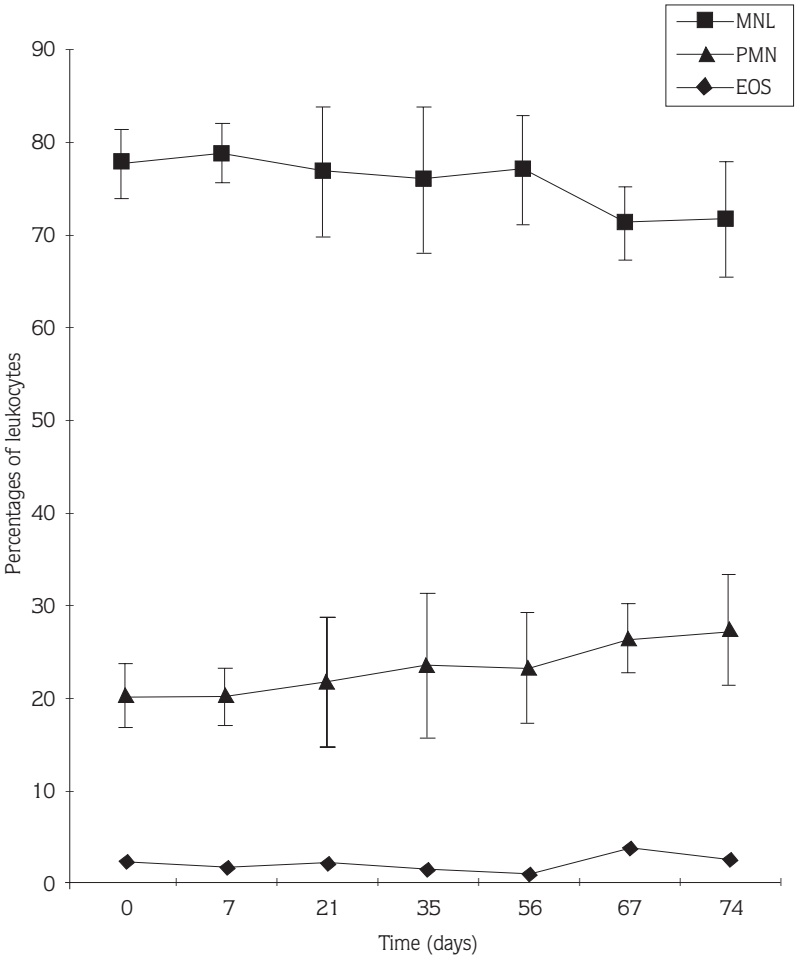

Figure 2. Relative percentages of leukocyes from male rats by feeding with a 10% gelatine containing diet (0-60 days) and fallowing refeeding with a complate diet (61-74 days).

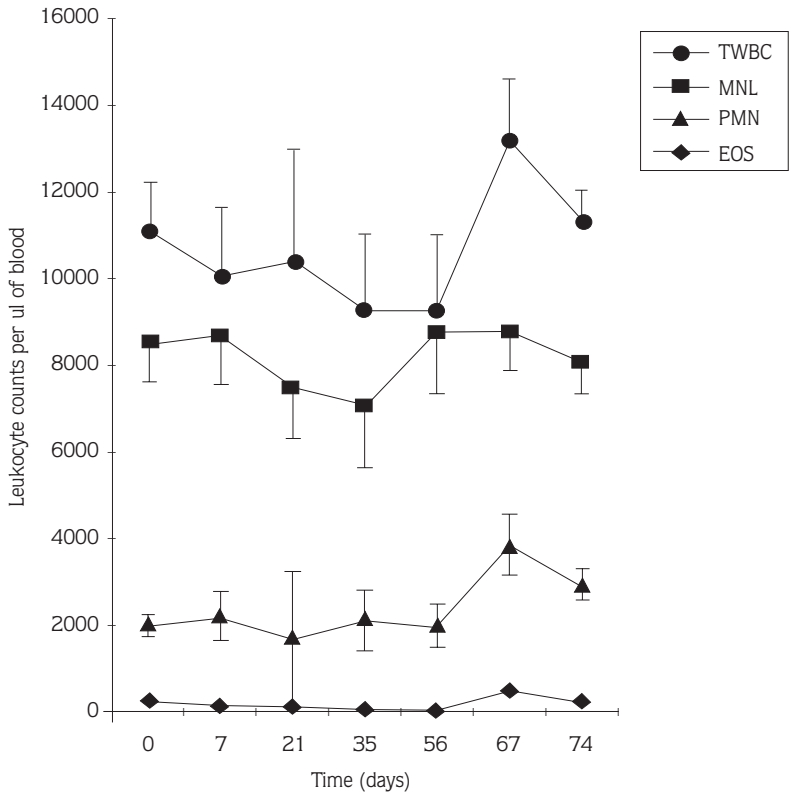

Figure 3. Concentrations of blood leukocytes from male rats by feeding with a 20% gelatine containing diet (0-60 days) and fallowing reffeding with a complate diet (61-74 days).

Diet	Time	MNL		PMN		EOS	
	[d]	$\bar{X}$	± s	$\bar{X}$	± s	$\bar{X}$	± s
Male animals (n =7)							
GCD-10%	0	77,70	3,58	20,09	3,35	2,21	0,47
"	7	78,73	3,15	19,84	3,00	1,43	0,38
"	21	76,66	6,99	21,43	7,03	1,91	0,64
"	35	75,74	7,77	23,14	7,82	1,11	0,21
"	56	76,70	5,87	22,81	5,92	0,48	0,24
CDRM	67	70,92	3,84	25,85	3,68	3,23	0,23
"	74	71,21	6,09	26,84	5,81	1,95	0,45
Female animals (n =7)							
GCD-10%	0	77,94	3,40	20,04	3,10	2,02	0,71
"	7	76,76	4,54	21,10	4,39	2,14	0,53
"	21	69,69	5,95	28,43	5,91	1,88	0,42
"	35	73,32	6,00	26,17	5,66	1,52	0,46
"	56	69,97	9,14	29,06	9,28	0,97	0,44
CDRM	67	61,82	7,77	34,96	7,89	3,22	0,65
"	74	67,78	5,97	29,86	6,49	2,37	1,06

Table 5. The relative percentages of white blood cells of rats which were fed first with a diet containing 10% gelatine (GCD-10%) and followed with a complate standart diet for rats and mice (CDRM). [WBC: Leukocytes, MNL: Mononuclear leukocytes, PMN: Neutrophils, EOS: Eosinophils].

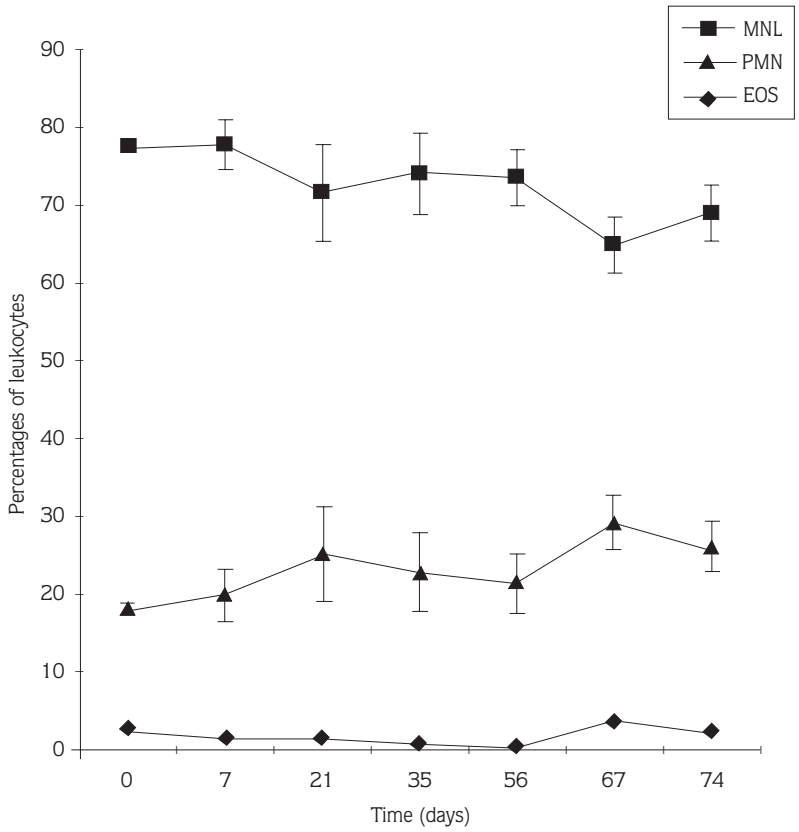

Figure 4. Relative percentages of leukocytes from male rats by feeding with a 20% gelatine containing diet (0-60 days) and by refeeding with a complate diet (61-74 days).

Diet	Time	MNL		PMN		EOS	
	[d]	$\bar{X}$	± s	$\bar{X}$	± s	$\bar{X}$	± s
Male animals (n =7)							
GCD-10%	0	77,70	0,92	18,10	0,74	2,29	0,42
"	7	77,82	3,20	19,91	3,35	1,56	0,31
"	21	71,73	6,24	25,22	6,08	1,42	0,76
"	35	74,23	5,10	22,87	5,04	0,83	0,55
"	56	73,75	3,75	21,66	3,64	0,52	0,33
CDRM	67	65,09	3,51	29,29	3,52	3,82	0,79
"	74	69,20	3,57	26,19	3,30	2,41	0,68
Female animals (n =7)							
GCD-10%	0	75,81	2,53	22,11	2,96	2,08	0,78
"	7	77,35	3,41	21,04	3,50	1,61	0,31
"	21	65,44	8,88	31,23	9,10	1,33	0,45
"	35	72,86	7,76	26,39	8,06	0,74	0,38
"	56	71,10	5,42	28,47	5,43	0,44	0,33
CDRM	67	60,99	7,52	34,87	6,85	4,14	1,37
"	74	66,75	4,07	30,65	4,06	2,59	0,78

Table 6. The relative percentages of white blood cells of rats which were fed first with a diet containing 10% gelatine (GCD-10%) and followed with a complate standart diet for rats and mice (CDRM). [WBC: Leuko–cytes, MNL: Mono-nuclear leukocytes, PMN: Neutrophils, EOS: Eosino-phils].

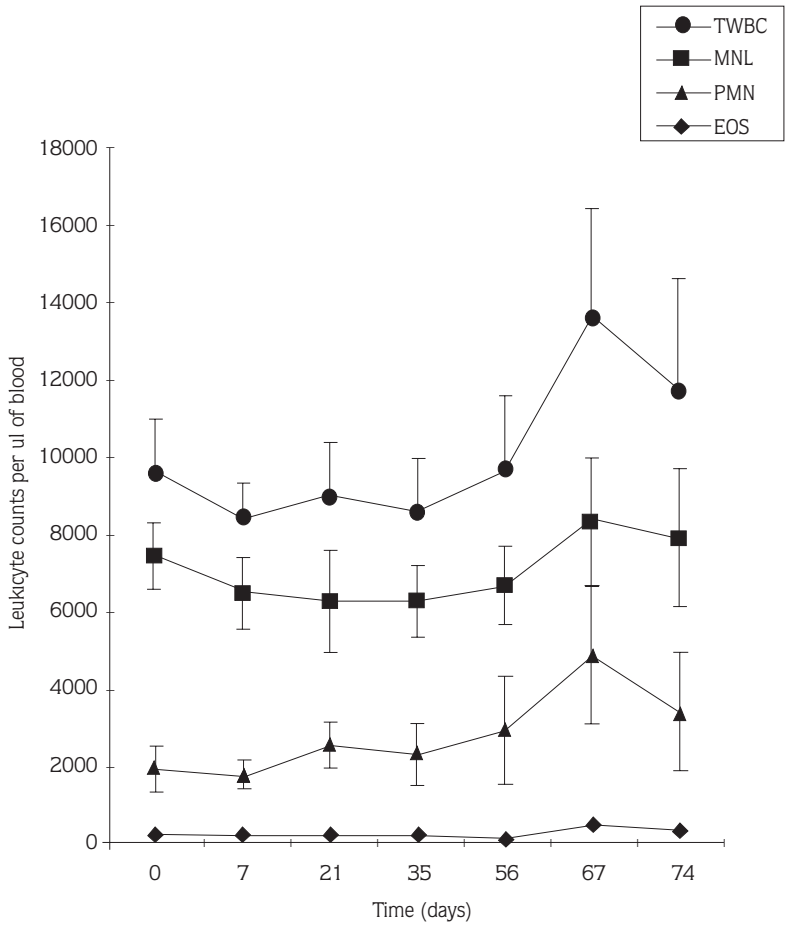

Figure 5. Concentrations of blood leuko–cytes from female rats by feeding with a 10% gelatine containing diet (0-60 days) and by reffeding with a complate diet (61-74).

The results of three-way analysis of variance are summarized in the tables Table 7 and 8. As a result, three-way analysis of variance showed that by nutrition with gelatine containing diet the effects of factors duration of experiment, gelatine content of diet and sex of animals on the concentration of different types of leukocytes in peripheral blood was statistically significant, in general. The count of eosinophils and mononuclear

Table 7. The effect of gelatine-containing diet (GCD), duration of experiment (T) and sex (S) of animals on the body weight and whith blood cells of rats [Sample size in each case is 7; p values from a three-way analysis of variance with repeated measures in factor time] [WBC: Leukocytes, MNL: Mononuclear leukocytes, PMN: Neutrophils, EOS: Eosinophils] [The counts were transformed logaritmically (*) or squire rooted (#).

Dependent variable	Independent variables			Interactions			
	GCD	T	S	GCD/T	GCD/S	T/S	GCD/T/S
BW	n.s.	< 0,0001	< 0,0001	n. s.	n. s.	< 0,0001	n. s.
			Counts per ml of blood				
WBC	n. s.	n. s.	< 0,05	n. s.	n. s.	(< 0,10)	n. s.
MNL	n. s.	< 0,01	< 0,01	n. s.	n. s.	n. s.	n. s.
PMN (*)	n. s.	< 0,001	n. s.	n. s.	n. s.	(< 0,10)	n. s.
EOS (#)	< 0,05	< 0,0001	n. s.	< 0,05	n. s.	n. s.	n. s.
			Proportions in %				
MNL	n. s.	< 0,0001	< 0,05	(< 0,10)	n. s.	n.s.	n. s.
PMN (*)	n. s.	< 0,0001	< 0,05	n. s.	n. s.	(< 0,10)	n. s.
EOS (#)	< 0,05	< 0,0001	n. s.	(< 0,10)	n. s.	(< 0,10)	n. s.

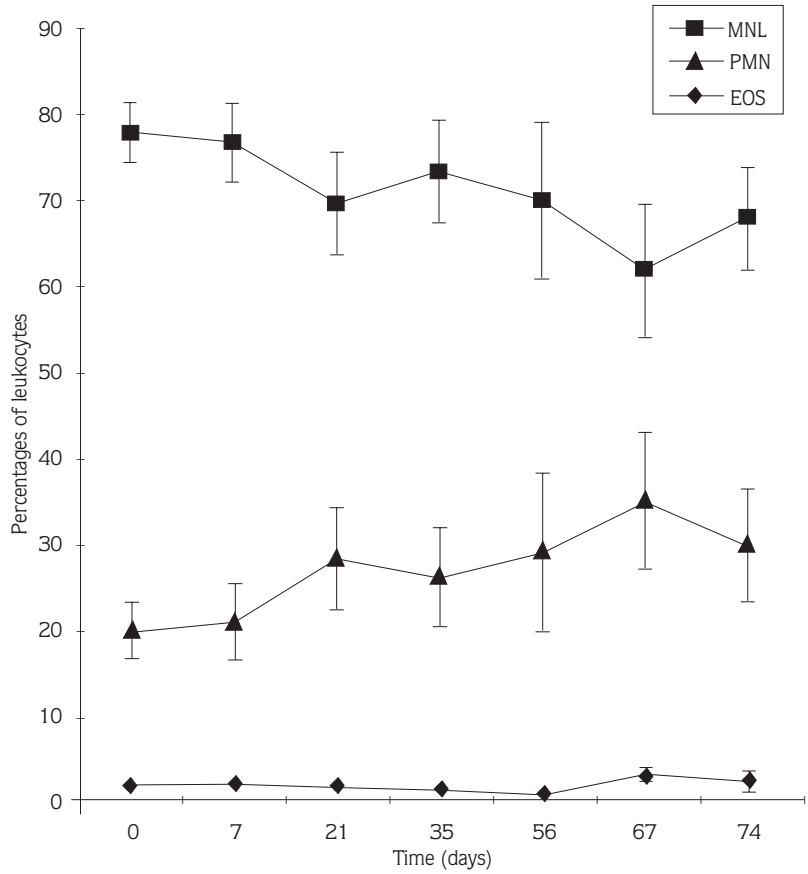

Figure 6. Relative percentages of blood leukocytes from rats by feeding with a 10% gelatine containing diet (0-60 days) and fallowing refeeding with a complate diet (60-74 days).

leukocytes felt significantly ($p < 0,0001$ and $p < 0,01$, respectively), while the count of neutrophils increased ($p < 0,001$). Because the increase of neutrophils nearly compensated the decrease of mononuclear cells and eosinophils, the changes in concentrations of total leukocytes was not significant.

The gelatine content of the diet had only a significant effect on the concentration of eosinophils. Compared with the animals fed the diet containing 10% gelatine, the animals with 20% gelatine in the diet had approximately 20% less eosinophils in peripheral blood ($p < 0,05$). Also the sex of animals had a significant effect on the concentration of eosinophils in blood. So the males had significantly more total leukocytes than the females ($p < 0,01$) which was attributable to especially significantly higher count of mononuclear leukocytes ($p < 0,01$) in this animals. The difference of the count of eosinophils between males and females was, in contrast, not significant. Between three factors tested, also the duration of experiment, gelatine content of the diet and sex of the animals, no significant interactions occurred. Solely, in the count of eosinophils there was a twofold significant interaction between the factors the duration of experiment and gelatine content of the diet. The decrease of the count of eosinophils by 10% gelatine in the diet was 65%, in contrast by 20% gelatine it was 80% ($p < 0,05$).

During the refeeding with standard commercial rodent diet, the count of total leukocytes significantly increased ($p < 0,0001$). This is so far remarkable, as the preceded protein malnutrition had no significant effect on this variable. Also the increase of mononuclear leukocytes and eosinophils were in each case significant ($p < 0,0001$), after that they -in contrast to the count of total leukocytes- were decreased strongly during the preceded protein malnutrition. Merely, the count of neutrophils continued further the trend in protein malnutrition and increased with $p < 0,001$ significantly. In contrast, the effects of factors: gelatine content of preceded diet and sex of animals were solely accidental. Among the factors duration of experiment, gelatine content of the diet and sex of animals, there were a significant threefold interactions in the number of total leukocytes, mononuclear leukocytes and neutrophils ($p < 0,01$, $p < 0,05$ and $p < 0,05$, respectively) during this experimental period.

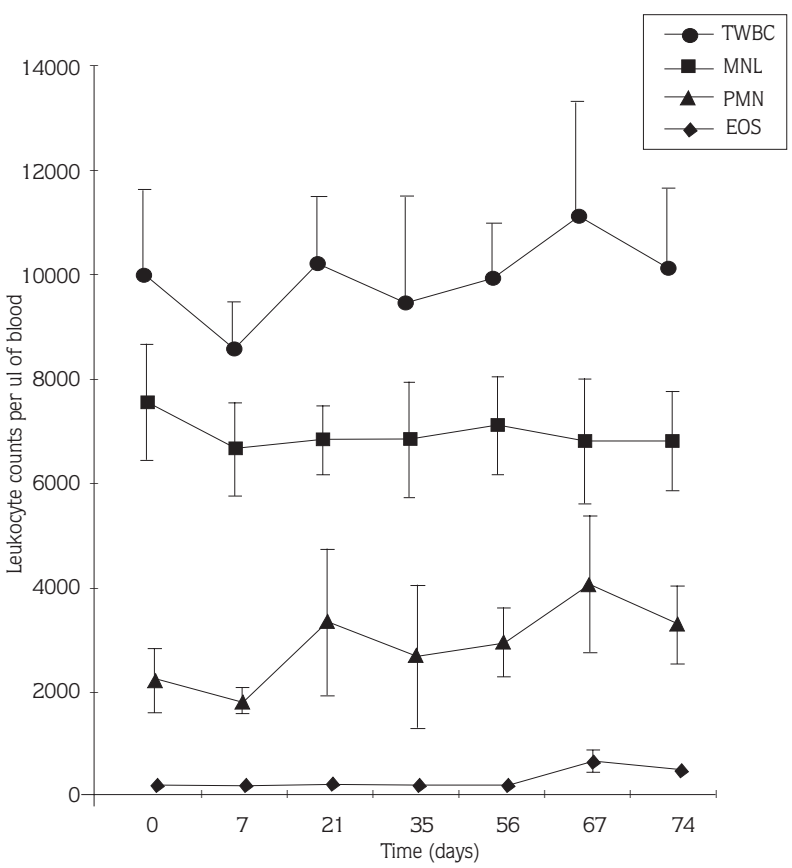

Figure 7. Concentrations of leukocytes from female rats by feeding with a 20% gelatine contaning diet (0-60 days) and by refeeding with a complate diet (61-74 days).

Table 8. The effect of refeeding with a complate standart diet for rats and mice (CDRM) after a proceeding qualitative and quantitative protein malnutrition, the duration of experiment (T) and sex (S) of animals on the body weight and whith blood cells of rats [Sample size in each case is 7; p values from a tree-way analysis of variance with repeated measures in factor time] [WBC: Leukocytes, MNL: Mononuclear leukocytes, PMN: Neutrophils, EOS: Eosinophils] [The counts were transformed logarithmically (*) or squire rooted (#).

Dependent variable	Independent variables			Interactions			
	CDRM	T	S	CDRM/T	CDRM/S	T/S	CDRM/T/S
BW	n.s.	< 0,0001	< 0,0001	(< 0,10)	n. s.	< 0,0001	(< 0,10)
			Counts per ml of blood				
WBC	(< 0,10)	< 0,0001	n. s.	n. s.	n. s.	n. s.	< 0,01
MNL	(< 0,10)	< 0,01	n. s.	n. s.	n. s.	n. s.	< 0,05
PMN (*)	n. s.	< 0,0001	n. s.	n. s.	n. s.	n. s.	< 0,05
EOS (#)	n. s.	< 0,0001	n. s.	n. s.	n. s.	n. s.	n. s.
			Proportions in %				
MNL	n. s.	< 0,0001	< 0,01	n. s.	n. s.	n.s.	n. s.
PMN (*)	n. s.	< 0,0001	< 0,01	n. s.	n. s.	(< 0,10)	n. s.
EOS (#)	(< 0,10)	< 0,0001	n. s.	< 0,05	n. s.	(< 0,10)	n. s.

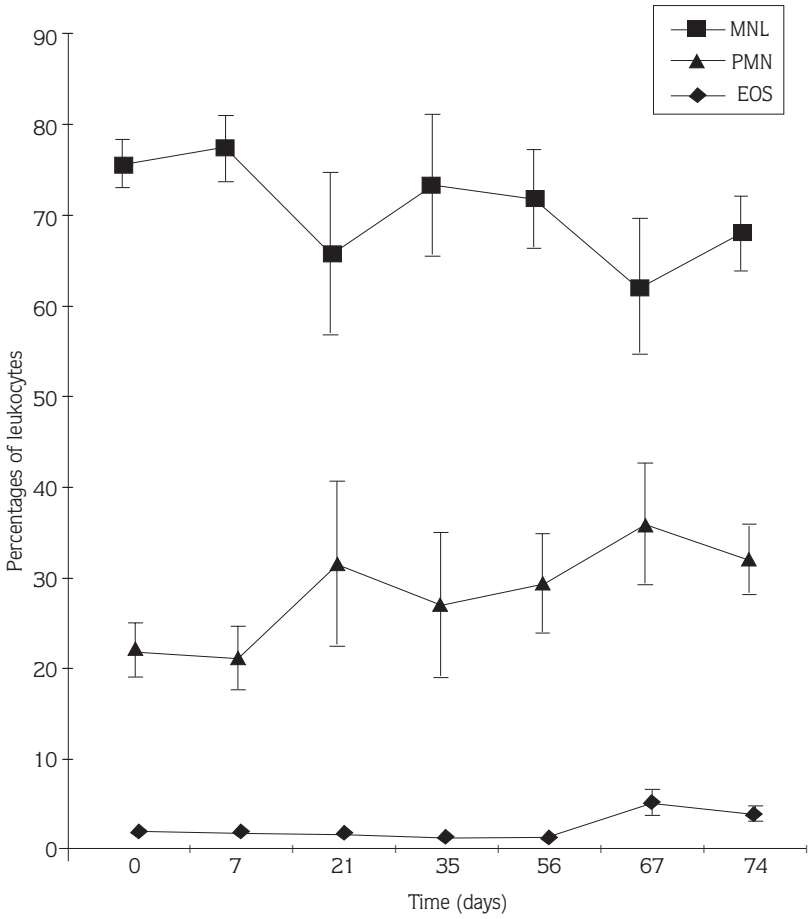

Figure 8. Relative percentages of blood leukocytes from rats by feeding with a 20% gelatine containing diet (0-60 days) and fallowing refeeding with a complate diet (61-74 days)

In differential blood picture the nutrition with gelatine diet lead in general to similar alterations like in the concentrations of different types of leukocytes. The proportions of eosinophils and mononuclear leukocytes decreased while the proportion of neutrophils increased. This alterations were in each case significant ($p < 0,0001$). The different gelatine content of the diet had a significant effect merely on the proportion of eosinophils, which was lower on animals with 20% gelatine in the diet than on animals with 10% ($p < 0,05$). Furthermore, sex dependent differences were also occurred. The proportion of mononuclear leukocytes of males was greater ($p < 0,05$), in contrast to that of neutro-phils which was significantly smaller than of females ($p < 0,05$). Among the tested factors no significant interaction occurred.

Also, by the feeding with standard commercial rodent diet a similar picture occurred in differential blood composition like in the concentrations of different leukocytes. The proportion of eosinophils increased -in contrast to preceded protein malnutrition- significantly ($p < 0,01$), while the proportion of neutrophils and mononuclear leukocytes continued the trend seen in protein malnutrition. Thus, the proportion of neutrophils increased further and of mononuclear leukocytes continued to decrease; both alterations were statistically significant ($p < 0,001$). The gelatine content of the preceded diet had significant effect on the proportion of mononuclear leukocytes and that of neutrophils ($p < 0,01$), but not -in contrast to the nutrition with gelatine diet - on the proportion of eosinophils. Merely, by the proportion of eosinophi-ls, a significant two-fold interaction occurred between the factors of duration of experiment and gelatine content of the diet ($p < 0,05$).

Discussion

This study represented the alterations of the number and proportions of white blood cells from female and male rats in the course of nutrition with a gelatine as a source of protein in the diet (also a severe protein

malnutrition) and followed refeeding with a conventional complete standard commercial rodent diet for mice and rats.

By feeding a gelatine containing diet as the source of dietary protein the body weight of animals decreased profoundly.

If the effects of protein malnutrition and following feeding of a complete standard commercial rodent diet on the numbers and proportions of white blood cells are considered, it can be seen that the strongest alterations occurred in the eosinophils.

The poor quality of gelatine is due to its almost complete absence of the amino acid tryptophan and very sparse amount of tyrosine (20).

Vartiainen and Apajalahti (14) have determined the concentrations of eosinophils on the men six hours after the consumption of gelatine or casein (0,5 g/kg body weight in each case) or tyrosine (0,3 or 0,6 g/kg body weight). They found that, the consumption of gelatine had no significant effect, while the consumption of casein and -depending on dose- the of tyrosine caused a reduction in the count of eosinophils around more than 30%.

Aschkenasy (13) observed on rats, that if their diet included all essential amino acids except tryptophan, the count of eosinophils in blood reduced to 33% of its initial level within seven weeks. This strong decrease is widely confirmed by the present study with feeding gelatine containing diet.

Köhl-Oppitz (21) could found, that both the decrease of body weight and the concentration of eosinophils in blood of rats would always lower with the increasing wheat gluten in the diet from 0% over 3% to 10%. In contrast, in the present study the body weight of animals decreased almost equally by feeding 10% and 20% gelatine in diet, while the number of eosinophils by the group with 20% gelatine in the diet was substantially lower than the group with 10% ($p < 0,05$). These different effects of wheat gluten and gelatine are to be attributable to the different protein quality of these dietary proteins as well as to the differences in feed consumption of experimental animals resulting from this. Then, it is well known that feed consumption in the case of a marginal amino acid or protein deficiency is generally maintained or increased. By using of wheat gluten as dietary protein source, whose protein quality is limited by lysine and threonine and lying distinct over that of gelatine, the lack of this limiting amino acids can be compensated by enhanced feed consumption partly, while this can not be the case by feeding with gelatine diet, because the gelatine almost completely fails to supply under others the essential amino acid tryptophan.

The results from Aschkenasy (22,23) indicate that tryptophan, an essential amino acid, is for the regulation of the number of eosinophils and lymphocytes of the rat is, in fact, very important, but not for the number of neutrophils. Then, this author could determine, that a refeeding for ten days with tryptophan deficient diet following an almost complete N-free nutrition led to no normalisation of the number of lymphocytes and to a further reduction of the number of eosinophils, while the number of neutrophils in spite of tryptophan deficiency, increased again.

Consequently, the observed increase of the concentration of neutrophils and decrease of the concentration of mononuclear leukocytes consisting to a great extent of lymphocytes and eosinophils in this study with gelatine containing cost are in good agreement with the results observed by Aschkenasy (22,23).

The increase of concentration of white blood cells during the refeeding with complete diet following an almost complete N-free nutrition was also found out by Aschkenasy and Jobard (24) as well as by Aschkenasy (13,22-25). Therefore, it should be concluded, that the hemopoietic system is affected by a long maintained, excessive protein deficiency, but this affection is solely reversible.

The mechanisms of alterations of leukocytes in blood picture under physiopathological conditions are still unclear. For the alterations of concentrations of different leukocytes in protein deficiency a variety of factors could come into question. While Aschkenasy (1) and Köhl-Oppitz (21) put the importance of endocriniums in the foreground, Balkaya (16) attributed these alterations to the interactions of regulation systems, especially to the central nervous system, endocriniums and immune system as neuro-endocrine-immune network, and thereby as an effector, a great importance had attributed to the immune system and to cytokines, e.g., IL1, IL2 and prostaglandins. The eosinopenia were attributed to the imbalance between different lymphocyte subpopulations. The possible reasons of these type of leukocyte changes and changes in different aspects of the immune system will be discussed in detail elsewhere (26).

References

1. Aschkenasy A., 1971: Nutrition et hématopoïesis. C.N. Roy. Soc., Paris.
2. Chandra R.K., Newberne P.M., 1977: Nutrition, immunity and infection: Mechanisms of interactions. Plenum Press, New York.
3. Chandra R.K., 1980: Immunology and nutritional disorders. Edward Arnold Ltd, London.
4. Wilkinson A.W., 1981: The immunology of infant feeding. Plenum Press, New York and London.
5. Stinnett J.D., 1985: Nutrition and the immune response. CRC Press, Inc., Boca Raton, Florida.
6. Chevalier, P.; Aschkenasy, A., 1977: Hematological and immunological effects of excess dietary leucine in the young rat. Am. J. Clin. Nutr. 30: 1645-1654.
7. Myrvik Q.N., 1988: Nutrition and immunology. In: Modern nutrition in health and disease (Eds., M. E. SHILS, V.R. YOUNG). Seventh Edition, Chapter 35,pp:585-645. Lea & Febiger, Philedelphia.
8. Gray, I., 1964: Effect of protein nutrition on leukocyte mobilisation. Proc. Soc. exp. Biol. Med. 116: 414-416.
9. Kenney, M. A.; Magee, J. L.; Piedad-Pascual, F., 1970: Dietary amino acids and immune response in rats. J. Nutr. 100: 1063-1072.
10. Mokady, S.; Lotan, R.; Horenstein, L., 1979: The effect of dietary wheat gluten in protein malnutrition on the immune response of growing rats. Nutr. Rep. Intern. 20: 615-624.
11. Lotan, R.; Mokady, S.; Horenstein, L., 1980: The effect of lysine and threonine supplementation on the immune response of growing rats fed wheat gluten diets. Nutr. Rep. Intern. 22: 313-318.
12. Sebrell, W. H. Jr.; McDaniel, E. G., 1952: Amino acids in the production of blood constituents in rats. J. Nutr. 47: 477-486.
13. Aschkenasy, A., 1965: Effects des carances en divers acides aminés sur la production des éosinophiles et sur la répeti-tion de ces cellules dans le sang et dans la muqueuse gastro-intestinale. Rôle des corticosurrénales dans la génèse de l'éosinopénie carantielle. Rev. Franç. Études Clin. et Biol. 10: 299-307.
14. Vartiainen, I.; Apajalahti, J., 1953: Effect of ingested protein and tyrosine on circulating eosinophils. JCEM 13: 1502-1506.
15. Rufeger, H., 1967/1968: Ein neuer N-Bilanzkäfig für Ratten. Z. Tierphys. Tierern. Futtermittelkd. 23: 217-223.
16. Balkaya, M., 1991: Der Einfluß des Proteinmangels auf das weiße Blutbild von Wistarraten und Hähne der Rasse Weißes Leghorn. Inaugural-Dissertation, Fachbereich Veterinär-medizin, Justus-Liebig-Universität Gießen.
17. Rarndolph, T. G., 1944: Blood studies in allergy. I. The direct counting chamber determination of eosinophils by propylene glycol aqueous stains. J. Allergy 15: 89-96
18. Dixon, W. J. (Hrsg.), 1985: BMDP Statistical Software Manual. Univer-sity of California Press: Berkeley, Los Angeles, London.
19. Sachs, L., 1984: Angewandte Statistik: Anwendung statistischer Methoden. 6. Auflage, Springer Verlag: Berlin, Heidelberg, New York, Tokyo.
20. Block, R.J.; Bolling, D., 1951: The amino acid composition of proteins and foods. 2nd Ed., Springfield, _llinois, Charls C Thomas, Publisher, pp. 486-490.
21. Köhl-Oppitz, G., 1989: Endogene und exogene Einflüsse auf die mit Anilinblau bestimmte Eosinophilinkonzentration im Blut von Ratten. Inaugural-Dissertation, Fachbereich Veterinärmedizin, Justus-Liebig-Universität Gießen.
22. Aschkenasy, A., 1966a: Effets de divers mélanges d'acides aminés sur la régénération des leucocyts chez des rats carancés en proteinés. I. Neutrophiles et éosinophiles. C. R. Soc. Biol. 160: 933-937.
23. Aschkenasy, A., 1966b: Effets de divers mélanges d'acides aminés sur la régénération des leucocyts chez des rats carancés en proteinés. II. Lymphocyts. C. R. Soc. Biol. 160: 1787-1792.
24. Aschkenasy, A., 1969: Restoration de la leucopoïèse aprés une carance prolongée en protéines chez des rats intact et des rats privés de thymus, de rate ou des deux organes à la fois. C. R. Soc. Biol. 163: 295-300.
25. Aschkenasy, A.; Jobard, P., 1961: Répartition tissulaire des granulocyts éosinophiles, chez le rat rendu éosinopénique par une carance prolongée en protéins. Nouv. Rev. Fr. Héma-tol. 1: 202-212.
26. Balkaya M., 1988: The possible mechanisms of changes in leukopoietic system and immune system in protein and/or calorie malnutritions as well as in deficiencies of some other nutrients: Hypotheses about nutrition and leukocyte-immune system interactions (In preparation).