THE EFFECT OF QUANTITATIVE AND QUALITATIVE PROTEIN DEFICIENCY ON BLOOD REGENERATION. I. WHITE BLOOD CELLS

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THE LEVEL of dietary protein has been demonstrated by several investigators to be a factor in the regeneration of leukocytes and granulocytes. The studies of Kornberg et al.,[5] Wright and Skeggs[8] and of Daft[2] have shown, that diets of low protein content produce leukopenia and granulocytopenia, and that this abnormality can be effectively corrected by the administration of proteins or of the ten essential amino acids. Wissler[7] has noted, that protein depleted rabbits and rats exhibit a lowered granulocytic response following infection.

The studies outlined in this paper were carried out for the purpose of obtaining additional information concerning the effect of the level of dietary protein on the regeneration of leukocytes and granulocytes in protein depleted rats. Furthermore, the specific effects of various food proteins on the production of leukocytes and granulocytes were studied.

METHODS

For the production of leukopenia, male albino rats within one week after weaning were fed a protein-free basal diet, which consisted of 91 Gm. starch, 5 Gm. olive oil, and 4 Gm. salt mixture; 0.1 mg. thiamine hydrochloride, 0.2 mg. riboflavin, 0.1 mg. pyridoxin, 1.6 mg. calcium pantothenate, 0.25 mg. folic acid, and 100 mg. choline chloride per 100 Gm. ration were incorporated into the diet. Each rat received 100 I.U. vitamin A and 4 I.U. vitamin D twice weekly. After being fed on this diet for two weeks, leukopenia and granulopenia were noted in about 75 per cent of the animals. Leukopenia was considered to be present when the white blood cells numbered 4000 or less cells per cu. mm.; granulocytopenia, when the number of granulocytes amounted to 1200 or less per cu. mm. The granulocytopenia observed was not caused by secondary folic acid deficiency, as in the experiments described by Wright and Skeggs[8] and by Daft,[2] since additional supplementation of the diet with this vitamin did not delay the development of the blood dyscrasia. The hematologic data obtained from 55 normal and 100 protein depleted rats selected at random are listed in table 1.

The leuko- and granulocytopenic rats were used for the determination of the effectiveness of different levels of casein and of various food proteins on the production of white blood cells.

In order to test the effect of different levels of dietary protein the diets listed below were used (grams per 100 grams ration).

	C_3	C_6	C_9	C_{18}	C_{30}
Casein	3	6	9	18	30
Rice starch	88	85	82	73	61
Olive oil	5	5	5	5	5
Salt mixture	4	4	4	4	4

These diets were supplemented with the above-mentioned quantities of vitamins.

In the experiments with qualitative protein deficiency the following protein sources were used: egg powder, dried meat, casein, processed soya bean flour, peanut meal, maize flour, wheat flour (white) and gelatin. Egg powder, dried meat, soya bean flour and peanut meal were fat-extracted. The diets were prepared in the following manner: the various protein sources were incorporated in the protein-free

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basal diet by replacing an appropriate amount of starch, so as to make the protein level of each diet 9 Gm. per 100 Gm. ration.

Total white blood cell and granulocyte counts were made in the usual manner.

After the leuko- and granulocytopenic rats were placed on the experimental diets, white blood cell and granulocyte counts were carried out on the fourth, eighth and fifteenth days respectively. As the numbers of leukocytes and granulocytes after two weeks were almost the same as after one week, the figures of the latter count are omitted.

TABLE 1.—*Number of Leukocytes and Granulocytes in Normal and Protein-Depleted Rats*

		Leukocytes	Granulocytes	
			Number	Per cent of leukocytes
Normal	Mean	7970	2510	32
	σ	2720	1150	10.6
	ϵ	368	155	1.4
Protein-depleted	Mean	2420	690	31
	σ	805	265	10.2
	ϵ	80.5	26.5	1.0

TABLE 2.—*The Effects of Diets Containing Different Levels of Casein, Given to Protein-Depleted Rats, on Changes in Leukocyte and Granulocyte Counts. Means and Standard Errors*

Diet	Manner of feeding	No. of rats	Fourth day			Eighth day		
			Weight, grams	Leukocytes	Granulocytes	Weight, grams	Leukocytes	Granulocytes
				Per cu.mm.			Per cu.mm.	
C_3	ad libitum	20	+1±0.8	−310±179	−340±79	+1±0.8	−1125±150	−510±67
C_6	ad libitum	25	+3±0.5	+450±191	+40±79	+5±1.1	+910±248	+250±98
C_9	ad libitum	23	+7±0.9	+1140±263	+400±30	+13±1.4	+1820±240	+740±137
C_{18}	ad libitum	20	+10±0.6	+2630±300	+1130±146	+19±1.0	+4370±500	+1660±220
C_{18}	Controlled	16	+7±1.0	+1500±330	+510±168	+14±1.6	+2050±302	+1030±267
C_{30}	Controlled	17	+10±0.9	+3180±473	+1280±193	+11±0.9	+4980±244	+1870±244

RESULTS

The effect of quantitative protein deficiency on regeneration of white blood cells. In this series of experiments the effect of various levels of dietary protein on regeneration of leukocytes were studied. Four diets, C_3, C_6, C_9 and C_{18} were offered *ad libitum* to protein depleted rats. The changes obtained in weight and in total white blood cell and granulocyte counts are shown in table 2.

As can be seen from table 2, low protein diets induced a slight increase only and occasionally a further decrease in leukocyte and granulocyte numbers. Diet C_{18}

on the other hand, which contained sufficient quantities of protein, caused in a four day period when given *ad libitum* a considerable increase in white blood cells which reached the normal number after seven days' feeding. In the observed decreases and increases of leukocytes, granulocytes participated to a greater degree than lymphocytes and monocytes; the average percentage of granulocytes in total white blood cells decreased with C_3 from 28 to 15; it remained constant (30) with C_6; with C_9 and C_{18} slight increases were observed (from 25 to 30 and from 30 to 34, respectively). It seems, therefore, that granulocytes exhibit a greater sensitivity to protein intake than lymphocytes and monocytes. Statistical analysis of the changes in total white blood cells and granulocytes, observed after one week, showed that the casein levels of the four diets employed differed one from another to a highly significant degree (table 3).

TABLE 3.—*Statistical Analysis of Changes in Leukocyte and Granulocyte Counts Observed after One Week on Specified Diets. Probability that the Differences are due to Chance*

Diets compared	Leukocytes	Granulocytes
C_3 vs. C_6	0.01	0.01
C_3 vs. C_9	0.01	0.01
C_3 vs. C_{18} ad libitum	0.01	0.01
C_6 vs. C_9	0.01	0.01
C_6 vs. C_{18} ad libitum	0.01	0.01
C_9 vs. C_{18} ad libitum	0.01	0.01
C_{18} ad libitum vs. C_{18} controlled	0.01	0.05
C_3 vs. C_{18} controlled	0.01	0.01
C_{18} controlled vs. C_{30} controlled	0.01	0.01

Since the four above mentioned diets were offered *ad libitum*, the rats receiving the protein low diets ate considerably less than those receiving C_{18}. The food consumption of the rats fed C_3 amounted to 60 per cent only of that of the rats given C_{18}. The observed effect of the diets low in casein may, therefore, be due to protein deficiency or to caloric deficiency or to both protein and caloric deficiency. In order to investigate this question the following series of experiments was conducted. One group of rats received C_{18} with controlled intake, i.e., an amount of food as was consumed by C_3; a second group was fed a protein rich diet, containing 30 per cent casein (C_{30}) also given with controlled intake. These rats received, therefore, the same amount of calories as C_3 and the same quantity of protein as the rats which were allowed to eat C_{18} *ad libitum*. The results obtained and their statistical treatment are shown in tables 2 and 3.

These tables demonstrate that the increase in leukocyte and granulocyte counts obtained with C_{18}, given in restricted amounts is considerably lower than that observed with C_{18} offered *ad libitum*. On the other hand, this diet proved to be statistically significantly superior to C_3, although there was no difference in the caloric intake of these two groups. Furthermore, the rats receiving C_{30} in restricted amounts showed a similar response as those receiving the same quantity of protein

(C_{18} *ad libitum*), and a statistically significantly superior response than those receiving C_{18} in restricted amounts.

The latter observation suggests that the amount of protein eaten, and not its level in diet, is important for the regeneration of white blood cells. The highly significant difference in the response between C_3 and C_{18}, given in restricted amounts, does not contradict this interpretation. In caloric deficiency the organism is forced to divert protein from cell-synthesis to energy production. When the caloric supply is insufficient, it is immaterial to the caloric economy of the animal whether the restricted diet is rich or poor in protein. The decisive role of the protein intake for white cell regeneration is clearly shown by a comparison of the increases reached by C_{18} and C_{30}, both given in restricted amounts. Rats receiving C_{30} exhibit a significantly larger increase of white cells than those fed on the same

TABLE 4.—*The Effect on Increase in Leukocyte and Granulocyte Counts of Diets Containing Various Proteins at 9 per cent Level, Given ad libitum to Protein-Depleted Rats. Means and Standard Errors*

Source of protein	No. of rats	Fourth day			Eighth day		
		Weight, grams	Leukocytes	Granulocytes	Weight, grams	Leukocytes	Granulocytes
			Per cu.mm.			Per cu.mm.	
Egg	22	10±0.4	3320±151	1790±137	20±0.4	4300±188	1880±203
Meat	17	10±1.0	3260±150	1540±114	18±1.1	4120±290	1870±204
Peanut	19	1±0.8	1350±207	750±140	4±1.1	1980±194	890±124
Soya	23	7±0.6	1800±156	900±110	14±0.8	1870±163	850±126
Casein	23	7±0.9	1140±263	400±30	13±1.4	1820±240	740±737
Wheat	19	3±0.7	690±162	580±124	3±0.8	790±182	500±156
Gelatin	19	−1±0.6	620±239	640±120	−2±0.9	610±182	480±105
Maize	19	1±0.7	340±190	230±108	1±1.1	500±207	340±105

amounts of C_{18}. It may, therefore, be concluded, that protein intake plays a decisive role in white blood cell regeneration, and that this effect is due to be masked in a protein-high diet (C_{18}), when given in insufficient amounts.

The effect of qualitative protein deficiency on regeneration of white blood cells. In our second series of experiments the effects of various proteins (egg, meat, peanut, soya, casein, wheat, gelatin, maize—fed *ad libitum* at 9 per cent level) on production of white blood cells in protein depleted rats were compared. The results are shown in table 4.

It follows from table 4, that protein quality as well as quantity determines white blood cell regeneration. Among the food proteins investigated the proteins of egg and meat rank first. Both are similar in this respect, and, given at 9 per cent level, they exhibited an effect similar to that of casein fed at 18 per cent level. Casein, peanut and soy bean protein rank next. It is interesting to note, that peanut protein effects a similar degree of white blood cell regeneration as does soy bean and casein, despite is decidedly inferior effect on growth. Gelatin, wheat and maize proteins are the least effective for white blood cell regeneration as well as in their growth promoting efficiency. The reactions elicited by diets containing

9 per cent of these proteins are similar to those produced by casein at 6 per cent level.

Statistical treatment of the differences in mean increases of total white blood cells and of granulocytes, obtained after one week, revealed the following facts:

Total white blood cells: Egg or meat vs. each of the other proteins tested: differences highly significant (probability of the occurrence of the observed mean difference in a random sample 1:100 or less). Peanut or casein or soya vs. wheat or gelatine or maize: differences highly significant (probability 1:100 or less). The differences within each of the three groups were not found to be significant.

Granulocytes: Egg or meat vs. each of the other proteins: difference highly significant (probability 1:100 or less). Peanut vs. wheat: difference significant (probability 1:20). Peanut vs. gelatin or maize: difference highly significant (probability 1:100 or less). Soya vs. wheat: difference not significant. Soya vs. gelatin: difference significant (probability 1:20). Soya vs. maize: difference significant

TABLE 5.—*Per cent of Granulocytes and of Lymphocytes and Monocytes in Protein-Depleted Rats before and on the Fourth and Eighth Days after Feeding Various Proteins at 9 per cent Level*

Source of protein	Protein depleted		Fourth day		Eighth day	
	Granulocytes	Lymphocytes	Granulocytes	Lymphocytes	Granulocytes	Lymphocytes
Egg	32	68	44	56	40	60
Meat	29	71	38	62	38	62
Peanut	28	72	36	64	35	65
Soya	33	67	38	62	37	63
Casein	25	75	28	72	30	70
Wheat	30	70	43	57	40	60
Gelatin	25	75	40	60	36	64
Maize	29	71	32	68	35	65

(probability 1:100). Casein vs. wheat or gelatin: difference not significant. Casein vs. maize: difference significant (probability 1:50). The difference within each of the three groups were not found to be significant.

It is noteworthy, that all proteins tested produced an increase in granulocytes which was accompanied by a relative decrease in lymphocytes and monocytes. Details are given in table 5. The data shown in table 5 suggest that granulocytes regenerate more quickly after protein feeding than lymphocytes and monocytes. These observations confirm the above mentioned fact that the percentage of granulocytes decreased with C_3, remained constant with C_6 and increased with C_{18}.

DISCUSSION

Our results demonstrate that protein deficient diets invariably impair the regeneration of white blood cells in protein-depleted rats. Normal regeneration will occur only when diets containing quantitatively and qualitatively optimal proteins are administered. A diet, however, containing an optimal level of protein (C_{18}), but given in restricted amounts, will not promote optimal regeneration of

leukocytes. In this masked form of protein deficiency food protein is utilized for energy, and is insufficient therefore for purposes of cell synthesis. A similar phenomenon has already been described by Kosterlitz and Campbell[6] studying the effect of protein deficiency on liver cytoplasm as well as in our studies[3] on the effect of protein deficiency on the bacteriocidal properties and phagocytic activity of peritoneal fluid.

The effect of various food proteins on regeneration of white blood cells corresponds, more or less, to their growth promoting efficiency. Only peanut protein seems to be an exception to this rule. Its relative efficiency on white blood cell production was found to be greater than its growth promoting quality. Since the amino acid composition of each protein determines its nutritive value and growth promoting efficiency (Block and Mitchell[1]), it may be concluded, that, generally speaking, the same amino acid makeup is necessary for both white blood cell production and for growth. Experiments designed to study this question further are in progress.

Summary

1. The effect of diets, varying in quantity or quality of protein, on white blood cell regeneration was studied in leukopenic rats, the leukopenia having been induced by a protein-free diet.

2. Diets containing different amounts of casein (3, 6, 9 and 18 per cent, respectively), were fed *ad libitum*. At the 3 per cent level, a further decrease occurred of white blood cells, whereas the other three diets initiated a regeneration of leukocytes, its degree being more or less in proportion to the casein content.

3. In experiments with diets containing 18 and 30 per cent of casein, the amount of protein eaten and not its level in diet was the decisive factor in the regeneration of leukocytes. The white blood cell regenerating effect of a diet containing an optimal level of protein, may be neutralized when given in restricted amounts.

4. Diets containing nutritionally inferior proteins, fed at 9 per cent level, also impaired normal regeneration of leukocytes. The white blood cell regeneration afforded by the proteins investigated was found to increase in the following order: maize, gelatin, wheat, casein, processed soya, peanut, meat, egg.

5. In white blood cell regeneration promoted by dietary protein, granulocytes were found to react to a greater degree than lymphocytes and monocytes.

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