STUDIES ON THE EFFECT OF VITAMIN A DEFICIENCY ON HUMORAL IMMUNITY

Pages with reference to book, From 224 To 227

Misbah-ul-Islam Khan Sherwani (Department of Biochemistry, Nishter Medical College, Multan.)
N.A. Chaudhry (Department of Biochemistry, Punjab Medical College, Faisalabad.)
A.S.Hashmi, A.D. Anjum (Department of Biochemistry, University of Agriculture, Faisalabad.)

Abstract

Twenty four albino rats of similar age and weight were used in the study. Twelve rats were given vitamin A-supplemented diet while the other twelve were kept on vitamin A-free diet. Rats were weighed at weekly interval and their plasma vitamin A, total serum proteins and protein fractions were also estimated.

The results revealed a significant decrease in weights of rats on vitamin A-free diet as compared to other group. Vitamin A level in vitamin A-supplemented rats ranged between 38-47 ug% while in vitamin A-free rats the level declined to 10.9 ug per cent.

Total proteins were found to be significantly lower in rats on vitamin A-free diet as compared to rats on vitamin-A supplemented diet. Among protein fractions, albumin and beta globulin differed nonsignificantly in both groups. Alpha-1, alpha-2 and gamma globulins showed significantly low levels in rats on vitamin A-free diet as compared to rats on vitamin A-supple-mented diet.

The study has confirmed that vitamin A is involved in growth promotion and helps in maintaining the immune status of an individual (JPMA 30:224, 1980).

Introduction

Vitamin A plays a crucial role in the health of man and animals. It is well known that vitamin A is involved in the maintenance of epithelium (Wolback and Howe, 1925) and the complex defence system. Its deficiency usually leads to impairment of the immune response (Krishnan et al., 1974). Hof and Conrkers (1977) studied the influence of latent vitamin A deficiency on productoin of humoral antibodies against sheep erythrocytes in mouse and found that the ability to produce antibodies was not hampered. However, Trimothy et al (1976) reported a depression of alpha one macroglobulin in the serum of vitamin A deficient rats.

Chaudhry (1977) observed significantly low levels of vitamin A in patients with carcinoma of the oral cavity and oropharynx as compared to controls of age, sex and socio-economically matched group. Furthermore, he observed that the fall in vitamin A level was more in advanced stages than in the earlier stage of the carcinoma. It is an established fact that cellular immunity is depressed in almost all carcinomas and in some of these carcinomas, a low level of vitamin \ has also been observed. These concomitant findings needed further studies to establish if it was a chance finding or vitamin A has some role in the depression of immunity.

Materials and Methods

Twenty four albino rats of the same age (35 days) and weight were procured and randomly divided into six experimental units, each consisting of four rats. Three randomly selected units, consisting of 12 rats, were given vitamin A-free diet (Table I)

Table I: Percentage Composition of Diet

Ingredients	*Percentage*
Casein	12.50G
Maize Starch	42.50G
Glucose	20.00G
Potato Starch	10.00G
Ground nut oil	5.00G
Vitamin Mineral Mixture (1:1)*	10.00G

*SALT MIXTURE

Calcium citrate		308.2
$Ca(H_2PO_4)2\ H_2O$		112.8
K_2HPO_4		218.7
KCL		124.7
NaCl		77.0
$CaCO^{\varepsilon}$		68.5
$MgCO_3Mg(OH)_2\ 3H_2O$		35.1
$MgSO_4$ (anbydrous)		38.3
Ferric ammonium citerate	91.41	
$CuSO_4\ 5H_2O$	5.98	
NaF	0.76	
$MnSO_4\ 2H_2O$	1.07	
$KAI\ (SO_4)\ 2.12\ H_2O$	0.54	
KI	;.24	
	100.00	1000.00

*VITAMIN MIXTURE

Thiamin hydrochloride	0.060
Riboflavin	0.200
Pyridoxine hydrochloride	0.040
Calcium pentothenate	1.200
Nicotinic acid	4.000
Inositol	4.000
P-aminobenzoic acid	12.000
Biotin	0.040
Folic acid	0.040
Cyanocobalamine	0.001
Choline chloride	12.000
Maize starch	966.419
	1000.000

for a period of two months. The other 12 rats were given 100 I.U. of vitamin A per oz daily to each rat. Weights were recorded initially and then at weekly intervals.

Blood samples were collected by cardiac puncture from one rat per unit once a week. The rat was replaced in its cage after each sampling. Plasma vitamin A and carotene levels were estimated by Carr-

Pricc method of Kaser and Sketol (1943).
When the rats given vitamin A-free ration attained deficient levels i.e., 75% less than normal, the total proteins were estimated following modified Biuret method (Kingsley, 1942). Serum fractional proteins were also determined using Beckman Microzone electrophoresis apparatus.
The data was analysed statistically using t-test and regression (Steel and Torrie, 1960).

Results

The plasma vitamin A levels, weights, total serum proteins and fractional serum proteins in rats fed vitamin A-free and vitamin A-supple-mented diets have been summarized in table II-IV and Figs. 1-4.

Table II: Vitamin A Levels of Rats at Weekly Intervals

Period (weeks)	*Vitamin A level (ug/100 ml of plasma)*		*t-calculated*
	Vitamin A supplemented	*Vitamin A deficient*	
0	47.33±0.67	47.33±1.01	0.41
1	45.33±1.45	28.67±0.33	3.93**
2	44.00±1.55	24.00±0.57	7.81**
3	42.00±0.57	20.00±1.55	8.59**
4	43.33±2.18	17.67±0.45	6.79**
5	39.33±2.18	14.67±0.88	5.28**
6	36.00±0.95	14.00±1.15	7.26**
7	42.00±1.15	10.87±0.32	13.16**
8	38.00±0.57	11.67±0.44	18.28**

t-tab. = 2.074

** = Highly significant ($P < 0.001$)

Table III: Weights of Rats at Weekly Intervals

Period (weeks)	*Weights of rats (grams)*		*t-calculated*
	Vitamin A supplemented	*Vitamin A deficient*	
0	179.42±5.40	178.82±5.89	0.08
1	187.42±4.50	177.33±5.80	1.39
2	193.67±4.90	174.42±5.94	2.55*
3	197.50±4.99	156.17±6.04	5.38**
4	202.00±4.80	170.25±5.72	4.31**
5	207.00±4.86	169.17±5.70	5.35**
6	217.58±3.75	167.92±5.70	7.40**
7	226.33±4.47	167.33±5.50	8.40**
8	237.50±4.70	166.08±5.60	10.00**

t-tab. = 2.074

* = Significant ($P < 0.001$)

** = Highly significant ($P < 0.001$)

Table IV: Total and Fractional Serum Proteins of Rats (grams per cent) after Eight Weeks

	Vitamin A supplemented rats	*Vitamin A deficient rats*	*t-calculated*
Total proteins	4.97±0.095	4.20±0.18	3.87**
Albumin	2.40±0.12	2.32±0.12	0.56
Alpha-1 globulin	0.57±0.04	0.34±0.02	4.69**
Alpha-2 globulin	0.45±0.05	0.31±0.02	2.14*
Beta globulin	0.98±0.67	0.81±0.08	1.46
Gamma globulin	0.57±0.02	0.33±0.07	3.38**

t-tab. = 2.074

* = Significant ($P < 0.05$)

** = Highly significant ($P < .01$)

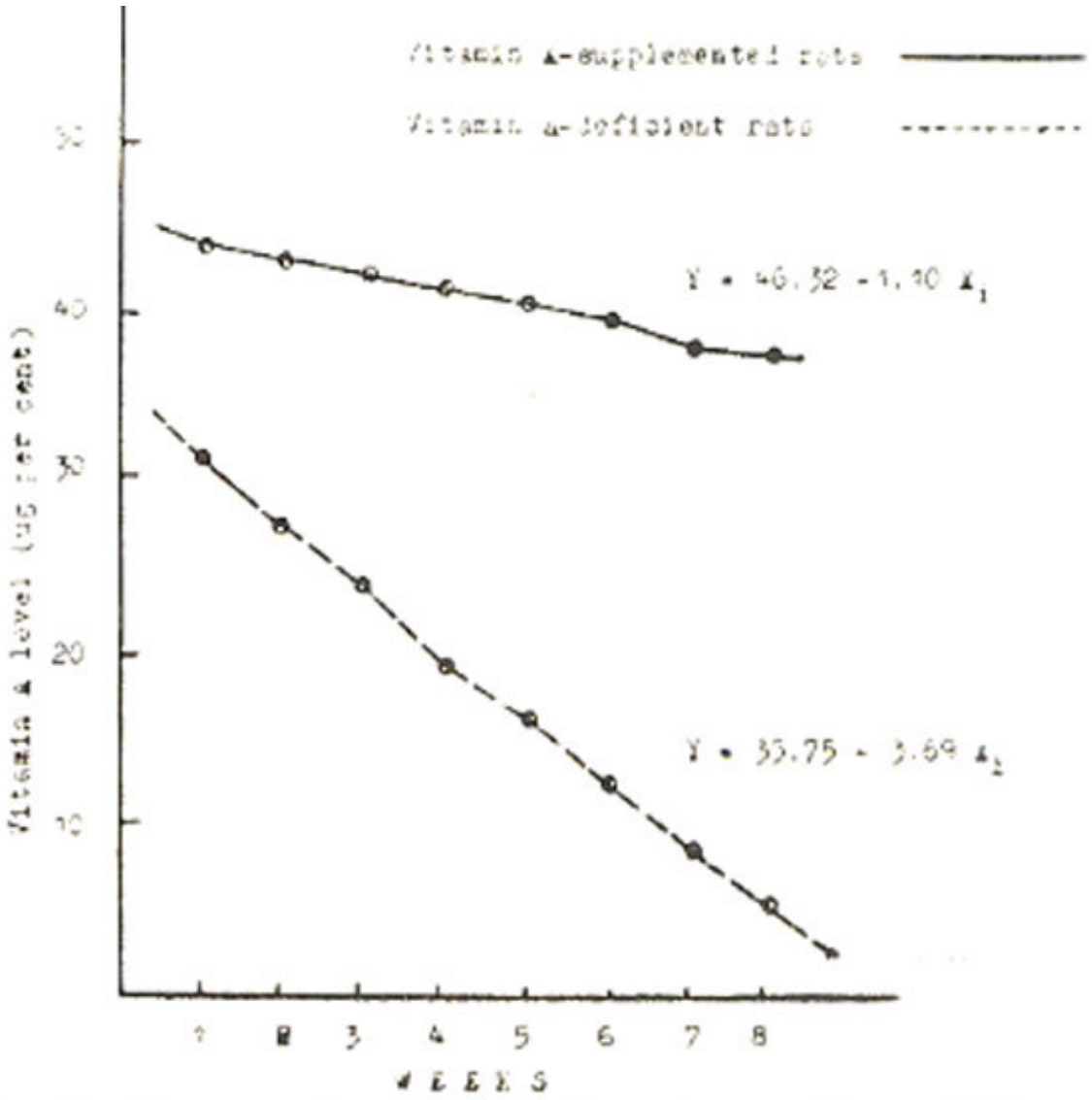

Fig. 1: Regression Line showing effect of duration on vitamin A levels in plasma of rats.

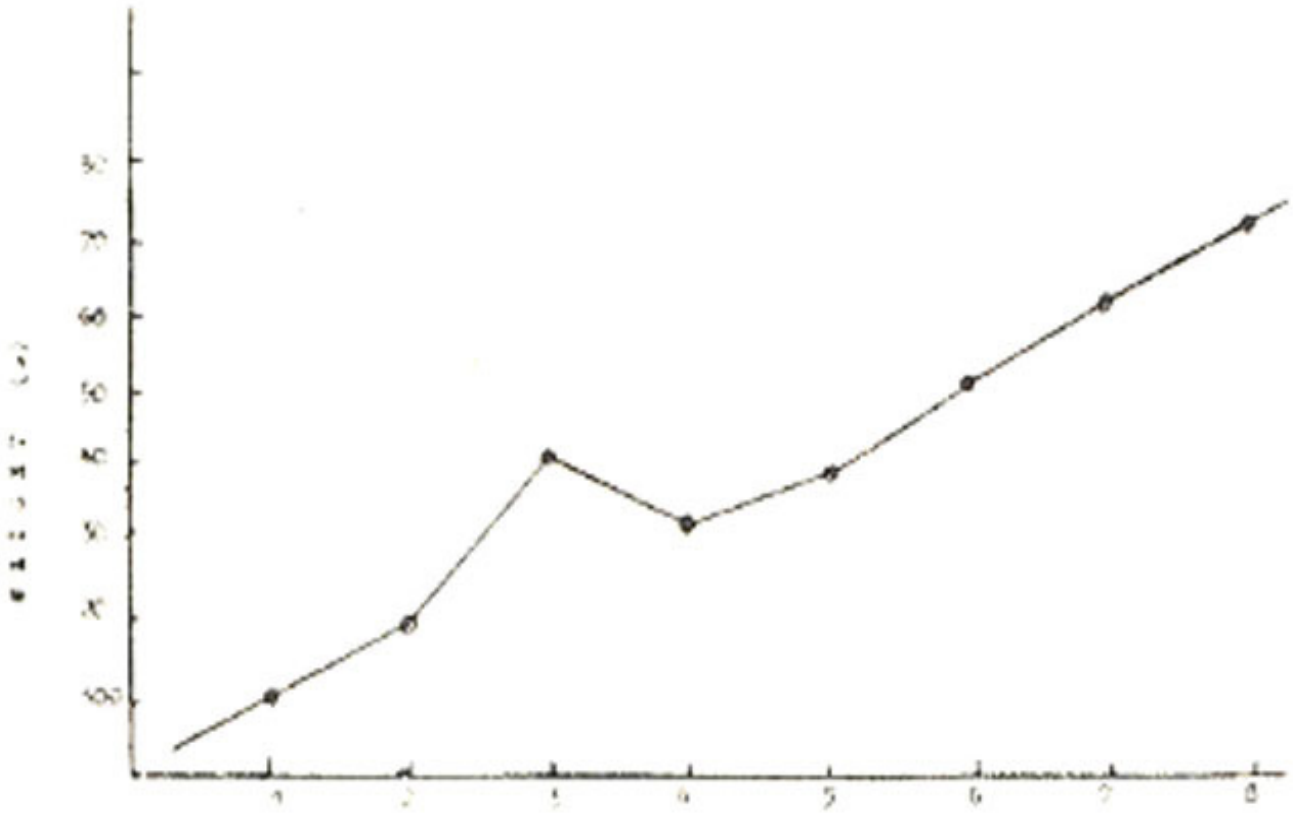

Fig. 2: Weekly difference in weight of rats.

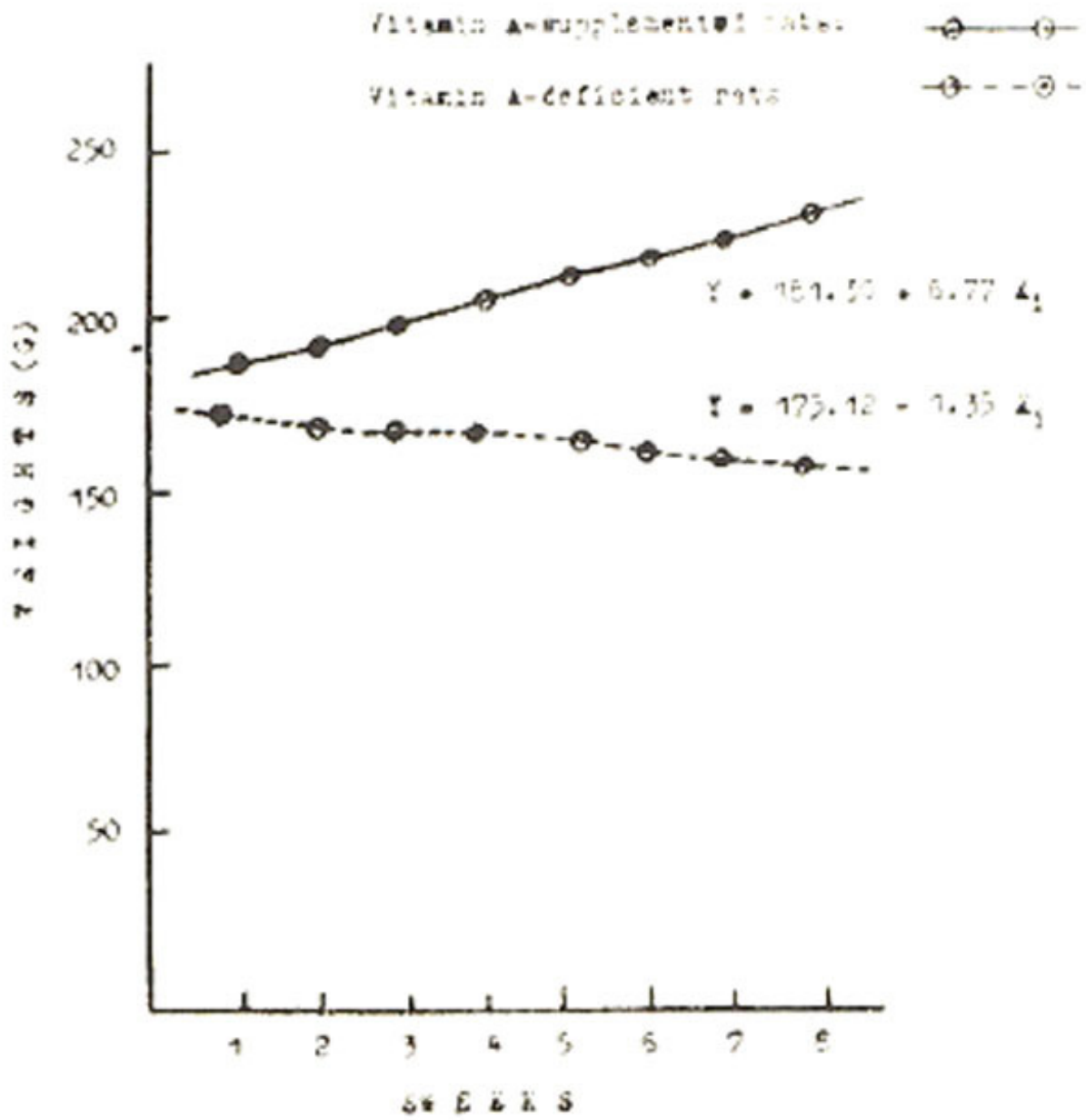

Fig. 3: Regression line showing effects of duration on the weights of rats.

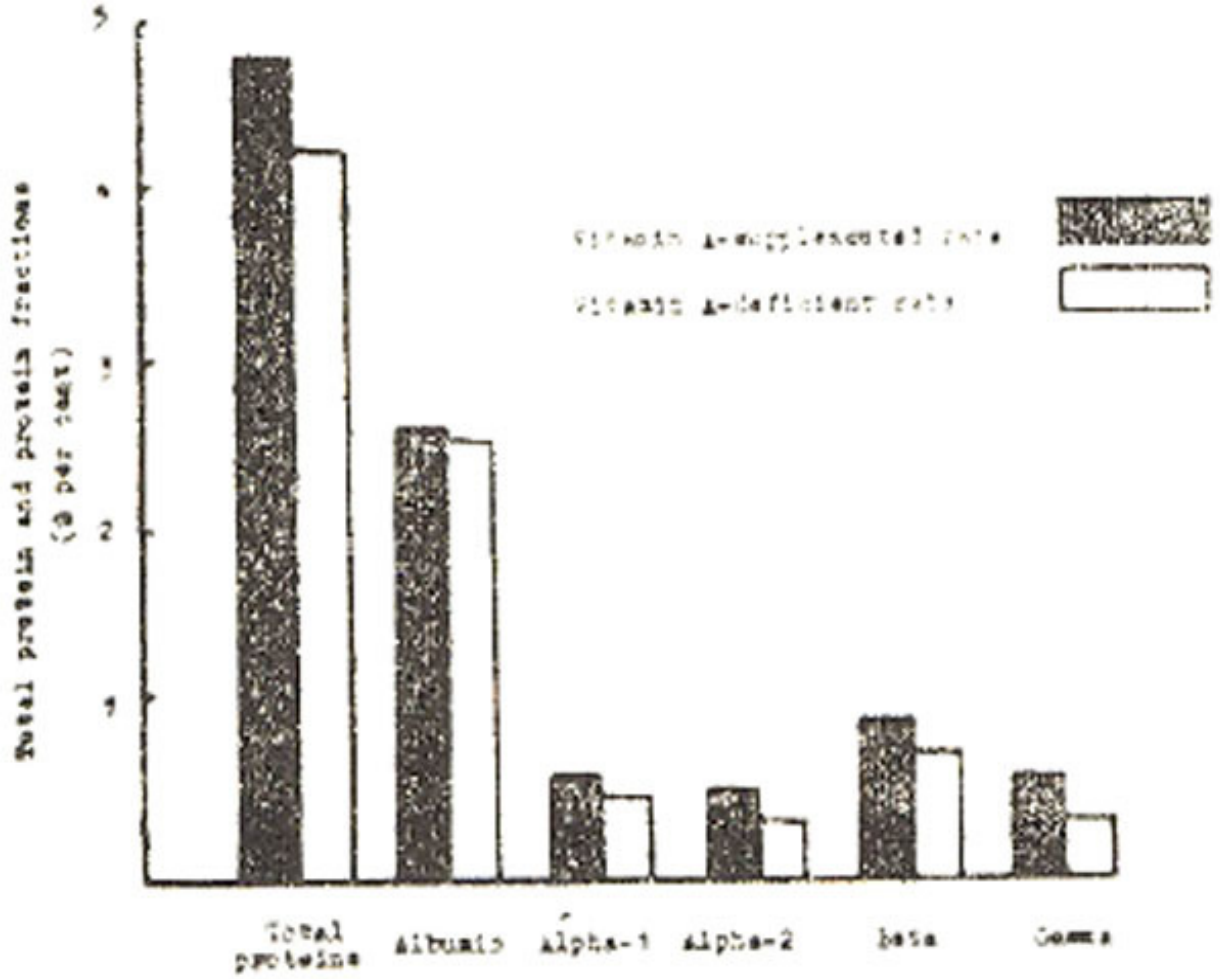

Fig. 4: Difference in total and fractional serum proteins of rats.

Discussion

Table II presents a significantly low level of vitamin A in rats fed vitamin A-free diet when compared with vitamin A-supplemented rats. Regression values indicate an average weekly change of 1.10 ug per cent in vitamin A-supplemented rats and 3.69 ug per cent in Vitamin A-deficient rats (Fig. I).
Humoral immunity was assessed in both groups after eight weeks. This period was selected due to two reasons: firstly, the fall of vitamin A level in vitamin A-deficient rats became persistent after two months. Secondly, the deficient level, after this period, was in accordance with the criteria of Varley (1976), who reported deficient level as 75 per cent less than normal.
Table III shows a constant increase in the weight of rats fed vitamin-A-supplemented diet and gradual decrease in the weight of vitamin A deficient rats. The difference between the two groups was statistically significant. The weekly differences in weights of two groups showed a persistent increase (Fig. 2). The maximum difference was noted between 7th and 8th week. Regression values indicated an

average weekly change of 6.77 G in vitamin A-supplemented rats and 1.35 G in vitamin A-deficient rats (Fig. 3). The retarded growth in vitamin A-deficient rats is consistent with the findings of other workers (McCollum and Simmond, 1917; Deluca et al., 1969).

Table IV shows a significant difference in total serum proteins of the two groups. The total proteins were significantly lower in vitamin A deficient rats as compared to vitaminA-sup-plemented rats. Albumin was found to be almost equal in both the groups whereas the beta globulin showed very small difference. However, clear cut differences were observed in alpha-and gamma globulins. Alpha-2 and gamma globulin were significantly lowered in vitamin A-deficient rats when compared to the vitamin A-supplemented rats. The defferences in total and fractional serum proteins of the two groups arc shown in Fig. 4.

The decrease in alpha and gamma globulins is in accordance with the findings of Trimothy et al (1976) who reported a 30 per cent depression of alpha-1 macroglobulins in vitamin A-deficient rats. The changes in proteins are also in agreement with Bang et al (1973) and Tangerdy and Cheryl (1975).

The study has confirmed that vitamin A is involved in growth promotion and also helps in maintaining immune status by affecting the globulins, in particular, the alpha and gamma fractions.

References

1. Bang, B. G., Foard, M. A. and Bang, F. B. (1973) The effect of vitamin A deficiency and Newcastle disease on lymphoid cell systems in chicks. Proc. Soc. Exp. Biol. Med., 143:1140.
2. Chaudhry, N.A. (1977) A study of carcinoma of the oral cavity and oropharynx, its relationship with herpes simplex virus and immune status. Karachi, JPMC-Dept. of Pathology, 1977.
3. Deluca, L.M., Little, E.P. and Wolf, G. (1969) Vitamin A and protein synthesis by rat intestinal mucosa. J. Biol. Chem., 244:701.
4. Hof, H.P., Emmerling, H., Finger, C. Wirsing, E. Karle and W. Reiners. (1977) Influence of latent vitamin A deficiency of mouse on the production of humoral antibodies against sheep erythrocytes and on resistance against infection with Listeria monocytogenes. Med. Microbiol. Parasitol., 237:310.
5. Kaser, M. and Sketol, J.A. (1943) A critical study of Carr-price reaction for the determination of beta-carotene and vitamin A in the biological materials. J. Lab. Clin. Med. Res., 13:37.
6. Kingsley, G.R. (1942) The direct biuret method for the determination of serum protein as applied photometric and visual colorimetry. J. Lab. Clin. Med., 27:840.
7. Krishnan, S., Bhuyan, U.N., Talwar, G.P. and Ramaling-aswami, V. (1974). Effect of vitamin A and protein-calorie undernutrition on immune responses. Immunology, 27:383.
8. McCollum, E.V. and Simmond, N. (1977) A biological analysis of pellagra producing diet. The minimum requirements of the two unidentified dietary factor for maintenance as contrasted with growth. J. Biol. Chem., 32:181.
9. Steel, R.G.D., and Torrie, J.H. (1960) Principles and procedures of statistics. New York, McCraw Hill, 1960.
10. Trangerdy, R.P. ,and Cheryl, F.N. (1975) Vitamin E or vitamin A protects chicken against E. coli infection. Poult. Sci., 54:1992.
11. Timothy, C.K., Salvatore, K., Molica, J. and Gorge, W. (1976) Plasma glycoprotein depressed in eitamin A. deficiency in the rat at macroglobulin. J. Nutr., 106:1659.
12. Varley, H. (1976) Practical clinical biochemistry. 4th ed. vol. 2. London, Heinemann, 1976.
13. Wolback, S.E. and Howe, P.R. (1925) Tissue changes following deprivation of fat soluble A vitamin. J. Exp. Med., 43:753.