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Effect of Protein on Utilization of Vitamin A in the Chick.* (26426)

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Conflicting observations have been reported on the effect of dietary protein level upon vit. A requirement. Baumann *et al.* (1) observed that rat diets low in protein reduced the storage of vit. A in the liver and increased rate at which stored vitamin A was depleted. Arnich and Pederson(2) found the concentration of vit. A per gram of liver to be almost doubled in rats receiving diets low in protein as compared to faster-growing rats receiving adequate protein; the total vit. A per liver, however, being approximately equivalent. Dye *et al.*(3) observed little effect upon utilization of vit. A by rats, regardless of dietary protein level.

On the other hand, Mayer and Krehl(4) observed in rats fed diets deficient in vit. A, that as dietary protein increased, there was a concomitant increase in severity of vit. A deficiency symptoms. Bohman *et al.*(5) observed a reduced level of vit. A and carotene in the liver and blood plasma when extra protein supplement was fed to wintering beef calves. Olsen *et al.*(6), using Columbian Rock progeny of hens maintained on diets low in vit. A, observed that as dietary protein in a practical chick diet increased from 16.9% to 24.6%, liver storage of vit. A in the chicks decreased about 40%.

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In view of the lack of agreement on the effects of dietary protein levels upon vit. A requirements, experiments were undertaken in our laboratory to study this relationship in the chick, and to investigate blood uric acid levels of chicks fed various amounts of protein and vit. A, in an effort to extend the findings of Elvehjem and Neu(7) and Stoewsand and Scott(8) as to factors concerned in producing elevated blood uric acid levels, and to determine if these levels can be used as a criterion of assessment of vit. A deficiency in the chick.

Methods. Male White Plymouth Rock chicks were randomly distributed among the various lots at one-day of age, using duplicate lots of 10 chicks per treatment in Experiment 1 and duplicate lots of 14 chicks per treatment in Exp. 2 and 3. Chicks in Exp. 1 were fed *ad libitum*. At the end of one week, 12 chicks per lot in Exp. 2 and 10 chicks per lot in Exp. 3 were selected according to uniformity of weights within each lot, and continued on treatment for the subsequent 3 weeks. To achieve approximately equivalent growth, the feed consumption of the chicks in Exp. 2 and 3 was limited during this 3 week period by equalized paired feeding by groups to that of the chicks receiving the highest protein treatment within each level of vit. A supplementation.

The diets are presented in Table I. Protein levels were 71.5% (high); 47.5% (mod-

TABLE I. Basal Diets.

Ingredients	Protein levels: High	Moderately high	Moderate	Low
	%			
Cerelose	—	30.23	60.55	71.05
Isolated soybean protein (ADM)	83.94	54.50	25.09	14.59
Cellulose	3.00	3.00	3.00	3.00
Stripped lard	3.00	3.00	3.00	3.00
Mineral mix*	6.13	6.13	6.13	6.13
Vitamin mix†	1.00	1.00	1.00	1.00
DL-Methionine	1.20	1.20	.70	.70
Cystine	1.00	.30	—	—
Glycine	.50	.40	.30	.30
Choline Cl	.22	.22	.22	.22
	μg/kg			
Vitamin B_{12}	50	35	20	20
Santoquin	.0125	.0125	.0125	.0125
Protein, %	71.5	47.5	21.9	12.9
Metabolizable energy/g	3.30	3.30	3.30	3.30

* g/100 g of diet: $CaHPO_4$, 2.15; $CaCO_3$, 1.49; KH_2PO_4, .867; NaCl, .8; $MgSO_4$, .25; $FeSO_4 \cdot 7H_2O$, .0333; $MnSO_4 \cdot H_2O$, .0333; KI, .00026; KCl, .5; $CuSO_4 \cdot 5H_2O$, .00167; $ZnCl_2$, .0114; $CoCl_2 \cdot H_2O$, .00017; $Na_2MoO_4 \cdot H_2O$, .00083.

† mg/kg of diet: biotin, .2; menadione sodium bisulfite, 1.0; pyridoxine, 4.5; folic acid, 4.0; thiamine, 10.0; riboflavin, 10.0; Ca pantothenate, 20.0; niacin, 50.0; inositol, 250.0. Units/kg: vit. D_3, 975; d-α-tocopheryl acetate, 66.

erately high); 21.9% (moderate) and 12.9% (low). Each of these protein levels was fed with graded levels of vit. A as indicated in the individual experiments. Liver vit. A was determined according to the procedure of Ames *et al.*(9); blood uric acid by the method of Brown(10); serum vit. A by the method of Garbers *et al.*(11). Excreta vit. A was determined after eluting the ether extract of the non-saponifiable material through an alumina column according to a method described by Thompson *et al.*(12).

Results and Discussion. Experiment 1. The experimental plan and results are presented in Table II. These results show that the vit. A requirement of the chicks increased as protein content of the diet was increased. This was evidenced by increased mortality and decreased vit. A liver stores in chicks receiving similar amounts of vit. A, but increasing amounts of protein. Results of the blood uric acid analyses are presented in Table III. A high blood uric acid level was observed in the chicks fed the high protein diets regardless of vit. A content of the diet. On the moderate protein diet without added vit. A, 75% of the chicks showed the ataxic symptoms characteristic of vit. A deficiency, yet no increase occurred in the uric acid content of the blood. Thus it appears that blood uric acid in the young chick does not necessarily increase in acute vit. A deficiency.

Experiment 2. The experimental plan and results are presented in Table IV. Since Popper and Greenberg(13) reported that vit. A is bound to certain fatty materials within

TABLE II. Effect of Dietary Protein and Vit. A Level upon Chick Growth and Liver Storage of Vit. A (Exp. 1).

Treatment: Protein* level	Vit. A level, USP units/kg	4 wk wt, g	USP units liver vit. A: Per g	Per liver
High	0	All dead	0	0
Moderate	0	" "	0	0
Low	0	257 (6/20)†	0	0
High	440	235 (4/20)	0	0
Moderate	440	483 (18/20)	0	0
Low	440	308 (20/20)	0	0
High	5280	350 (20/20)	2	23
Moderate	5280	536 (20/20)	35	680
Low	5280	327 (20/20)	51	617

* Percentage protein in each diet is presented in Table I.

† Figures in parentheses show No. of chicks alive at 4 wk of age over No. of chicks at start. Most of the chicks that died showed severe ataxia of vit. A deficiency prior to death.

TABLE III. Blood Uric Acid of Chicks Receiving Various Levels of Dietary Protein and Vit. A.

Treatment: Protein level	Vit. A level, USP units/kg	Age, days	Blood uric acid, mg %
High	0	19	11.7 ± 2.2*(70%)†
Moderate	0	19	4.4 ± 1.0 (75%)
Low	0	19	2.2 ± .4
High	440	24	16.0 ± 1.5 (33%)
Moderate	440	24	5.2 ± .6
Low	440	24	2.4 ± .2
High	5280	29	16.3 ± 1.0
Moderate	5280	29	3.8 ± .6
Low	5280	29	3.1 ± .3

* Stand. error of mean.

† No. of chicks showing symptoms and mortality due to vit. A deficiency.

TABLE IV. Effect of Dietary Protein upon Blood Ascorbic Acid, Liver Lipids and Liver Storage of Vit. A (Exp. 2).

Treatment					
Protein level	Vit. A level, USP units/kg	4 wk wt, g	Total liver vit. A, USP units/liver	Total liver lipids, %	Blood ascorbic acid, mg %
High	440	249 (19/24)*	0	—	1.45
Moderately high	440	289 (23/24)	0	—	1.92
Moderate	440	293 (24/24)	0	—	2.37
High	5280	331 (24/24)	41	4.6	1.67
Moderately high	5280	356 (24/24)	203	4.8	1.49
Moderate	5280	342 (24/24)	431	4.3	2.05

* No. of surviving chicks over No. at start.

the liver, measurements were made of the total liver lipids in the chicks receiving the 3 levels of protein. Furthermore, in view of the report by Mayer and Krehl(4) that vit. A deficiency in the rat decrease the blood, adrenal and liver vit. C reserves, it was considered desirable to determine whole blood ascorbic acid levels in chicks from each treatment. Leucocyte or whole blood vitamin C is considered by Butler and Cushman(14) to be a suitable measure of the state of vit. C nutrition. The results of these determinations also are presented in Table IV.

No changes were observed in total liver lipids of chicks fed 5,280 USP units of vit. A per kg of diet regardless of protein level, in spite of the fact that chicks fed the high protein diet showed liver stores of vit. A which were only 10% of the amount of vit. A in the livers of chicks fed the moderate protein diet. The amount of vit. A in the diet did not influence blood ascorbic acid levels appreciably. It appears, therefore, that in the chick, vit. A deficiency does not cause decreased ascorbic acid blood levels. The slight decreases in blood ascorbic acid levels as the dietary protein was increased, probably are a reflection of the need for ascorbic acid in the metabolism of certain amino acids, particularly phenylalanine and tyrosine(Sealock *et al.*, 15).

Experiment 3. In this experiment 2 extremely high levels of vit. A were fed to chicks by the equalized paired feeding technique, in order to conduct a study on the effects of protein upon apparent absorbability of vit. A. The experimental plan and results are shown in Table V.

These results showed that total liver vit. A and blood serum vit. A decreased as dietary protein increased. Vit. A content of the excreta (urine and feces combined) was accurately measurable only in the chicks fed 100,000 USP units of vit. A per kg of diet. The livers of the chicks fed the high protein diet contained 60% less, and 24-hour vit. A

TABLE V. Effect of Various Dietary Protein Levels upon Vit. A Storage and Excretion.

Treatment			Vitamin A				
					Excreta		
Protein level	Vit. A level, USP units/kg	4 wk wt, g	USP units per liver	USP units per cc blood serum	USP units per g (D.M. basis)	USP units per 24 hr (D.M. basis)	Apparent absorbability, %
High	440	189 (5/20)*	—	—	—	—	—
Mod. high	440	314 (15/20)	—	—	—	—	—
Moderate	440	288	—	—	—	—	—
High	50,000	275	6,723	2.30	—	—	—
Mod. high	50,000	284	11,249	2.64	—	—	—
Moderate	50,000	285	15,158	3.20	—	—	—
High	100,000	293	25,092	2.65	68	18,156	71
Mod. high	100,000	301	40,674	2.85	64	9,280	83
Moderate	100,000	300	63,369	3.71	75	6,000	89

* No. of chicks surviving over No. started. No mortality in the other lots.

excretion of these chicks was 200% greater than in the chicks fed the moderate protein diet. Since little if any intact vit. A is excreted in the urine(16), it appears, therefore, most likely that the higher than normal levels of protein interfere with absorbability of vit. A. Regardless of protein level, the amount of vit. A per gram of dry matter excreted was equivalent. However, the chicks receiving the high protein diets showed a very high total excretion, together with a markedly increased dry matter excretion. Therefore, total vit. A excreted by the chicks receiving the highest protein diet was much greater than that of chicks receiving the moderate protein diet.

During this experiment, a leg weakness was observed in many of the chicks receiving the high protein diets. At termination of the experiment tibias from chicks receiving the 2 highest vit. A levels were analyzed for bone ash. The results of these analyses indicated that the high protein levels interfered with calcification. Bone ash values were 36%, 40% and 43% for chicks receiving the high, moderately high and moderate protein diets, respectively. The reduced bone ash content of the tibias of chicks fed the high protein diets may indicate an interrelationship between dietary protein and vit. D or mineral metabolism in the chick.

Summary. 1. At a constant level of dietary vit. A, as the dietary protein was increased, vit. A content of the liver and blood serum of the chick decreased. Also in chicks receiving purified diets containing marginal amounts of vit. A and high protein levels, symptoms of vit. A deficiency occurred sooner and mortality was increased as compared to chicks fed diets containing moderate or low amounts of protein. 2. The decreased vit. A liver storage, resulting when the dietary protein was increased, could not be correlated with changes in liver lipids, nor vit. A blood levels. 3. The ataxia, mortality and other symptoms in young vit. A-deficient chicks was neither preceded nor necessarily accompanied by an increased blood uric acid level. A high protein diet produces high blood uric acid levels regardless of amount of vit. A in the diet. 4. Regardless of dietary vit. A level, the tibia bone ash of the chicks fed the high protein diets decreased as compared to that of chicks receiving a moderate level of protein in the diet.

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