Storage and transport of vitamin A in relation to protein intake

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Several studies have indicated a close relationship between vitamin A and protein metabolism.

McLaren (1959) found that the onset of vitamin A deficiency in rats could be delayed by reducing their protein intake. Rechcigl, Berger, Loosli & Williams (1962) observed that when rats were given different levels of dietary protein for a 3-week period their total liver stores of vitamin A were inversely proportional to their protein intake, indicating that more vitamin A was utilized by the organism as the dietary protein intake was increased. Esh & Bhattacharya (1960), however, demonstrated that prolonged inadequate or suboptimal protein feeding lowers liver storage of vitamin A in rats. Friend, Heard, Platt, Stewart & Turner (1961) found reduced serum vitamin A levels in pigs when their serum protein levels fell, regardless of the animals' liver stores of the vitamin.

The purpose of the experiments now described was to investigate the effect of dietary vitamin A on serum and liver proteins in rats, and to observe the effect of the sudden onset of negative nitrogen balance brought about by the withdrawal of all protein from the diet on the vitamin A status of normal and of vitamin A-deficient but still growing animals.

EXPERIMENTAL

Diet

The major ingredients of the basal diet for the experimental rats were (% by weight): vitamin-free casein 20, maize starch 58, sucrose 10, maize oil 8, and salt mixture no. 2 (USP XIII, 1947) 4. Vitamins added to the diet were (mg/kg): thiamine hydrochloride 2, riboflavine 4, pyridoxine 4, choline 1000, inositol 1000, *p*-aminobenzoic acid 300, nicotinamide 100, folic acid 2·5, vitamin B_{12} 0·05, biotin 0·1, ergocalciferol 0·042, α-tocopheryl acetate 50, vitamin K 10, calcium pantothenate 10.

Experimental plan

Expt 1. Twenty-four weanling male albino rats of the Wistar strain, weighing 54 ± 1 g, were individually caged and offered the diet described above. Twelve rats received 0·3 ml maize oil containing 17·2 μg vitamin A acetate (USP reference standard, 46 Park Avenue, New York 16, NY) daily and the other twelve were given the same amount of maize oil without vitamin A. The animals were fed *ad lib.* and body-weight and food consumption were recorded. After 4 weeks, the casein was replaced by an isocaloric amount of maize starch in the diet of half of the animals in

each group (groups 3 and 4) whereas the other half (groups 1 and 2) continued on the original diet. After a further 2 weeks, the animals were killed with chloroform.

Expt 2. Two groups of twelve rats each (groups 5 and 6), chosen as described for Expt 1, received the same basal diet as the animals in groups 3 and 4 in Expt 1, except that vitamin A acetate was mixed into the diet of the control group instead of being given separately. Thus, the diet containing vitamin A provided 17·2 μg vitamin A acetate/10 g diet, 10 g being approximately the daily food intake of the control group in Expt 1. The animals were killed with chloroform after 6 weeks on these diets.

Expt 3. Nineteen male weanling rats were put on the vitamin A-deficient diet and each of them was pair-fed with a male litter-mate of identical weight receiving the same diet with 1·72 mg vitamin A acetate added per kg of diet. Each pair of rats was killed when the deficient rat had lost weight for at least 2 consecutive days, usually about 5 weeks after their first receiving the diet.

Tissue sampling

Blood was drawn by heart puncture and spun at 200 ***g*** for 15 min after standing for 30 min in the dark. The serum was stored at $-20°$ under nitrogen. The livers were excised quickly and chilled in ice, and liver homogenates (20%) were made with cold water and stored at $-20°$.

Analytical procedures

Paper electrophoresis of the serum in a barbital buffer of 0·075 ionic strength and pH 8·6 gave four serum protein components, which will be referred to as albumin, and α-, β-, and γ-globulins. Their proportions were determined by densitometry (Beckman Spinco Analytrol) after the paper strips had been stained with bromophenol blue (Brown, Michaels & Kinsell, 1956). Total nitrogen was determined in liver homogenates and in serum by micro-Kjeldahl digestion followed by direct Nesslerization (Umbreit, Burris & Stauffer, 1957). In Expt 3, total serum protein was determined by the biuret method described by Fister (1950). Total lipids were measured by Schneider's (1945) method and lipid phosphorus by the procedure of Taussky & Shorr (1953). Vitamin A determinations were carried out by a modification of the Carr–Price technique described by Roels & Trout (1959). Total and free cholesterol were determined by the procedure of Sperry & Webb (1950).

RESULTS

Body-weight. The growth curves of the animals in Expt 1 are given in Fig. 1.

In Expt 2, the same growth pattern was observed for groups 5 and 6 as for groups 1 and 2 respectively in Expt 1.

When the vitamin A was mixed in the diet of the protein-fed rats (group 5, Expt 2) the increase in body-weight at the end of 4 weeks was 254% of their weight at the beginning of the experiment compared to a 229% increase in the animals in Expt 1 receiving the vitamin A separate from their diet (group 1).

In Expt 3, the pair-fed animals grew at the same rate until both animals in each pair

began losing weight, owing to the reduced food intake of the rats fed *ad lib.* on the vitamin A-deficient diet. During the period of weight loss, each deficient animal lost weight more rapidly than its control despite the same food intake.

Food intake. In Expt 1, the mean daily food intake was 10·9 g/day for group 1. Group 2 ate only slightly less food during the first 4 weeks on the diet; then their food intake fell gradually to about 7 g/day in the 5th week and was reduced further (for several rats to less than 1 g/day) towards the end of the 6th week. There were great variations in food intake between individual vitamin A-deficient animals. When all dietary protein was withdrawn, the food intake of the rats in groups 3 and 4 fell sharply to less than half what it was before the protein withdrawal, but there were again very great variations in food intake between individual animals on the protein-free diet.

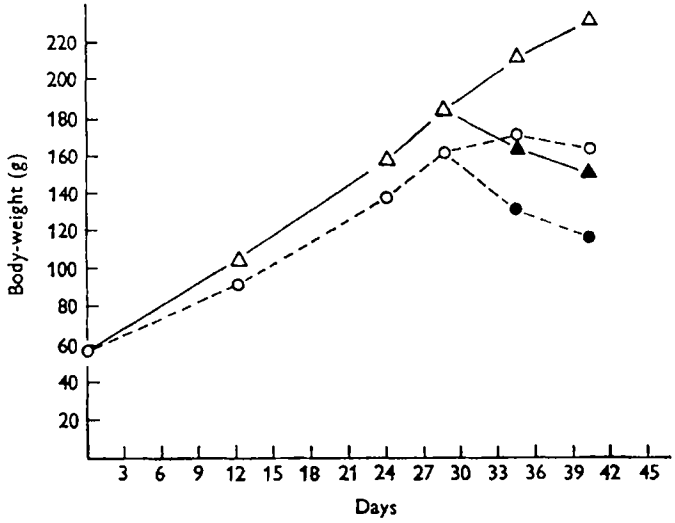

Fig. 1. Expt 1. Mean growth curves for six rats/group. The groups, initially of twelve given or deprived of vitamin A, were after 4 weeks subdivided as shown. △—△, (group 1) + protein + vitamin A; ○ - - - ○, (group 2) + protein − vitamin A; ▲—▲, (group 3) − protein + vitamin A; ● - - - ●, (group 4) − protein − vitamin A.

In Expt 2, the mean food intake for the 6-week experimental period of the animals on the vitamin A-deficient diet was 6·35 g daily compared to 11·4 g for the normal rats. The ratio of weight gain to food intake was 0·52 for normal rats and 0·22 for the vitamin A-deficient rats during the 3rd week of the experiment when the rats on the vitamin A-deficient diet were still growing. Under our experimental conditions, therefore, the efficiency of the food in producing or maintaining body tissues was decreased considerably in vitamin A deficiency. The same phenomenon was observed in Expt 3 during the period of weight loss, when the vitamin A-deficient animals lost weight more rapidly than the controls despite an identical food intake.

In Expt 3, the food intake of the animals on the vitamin A-deficient diet was very similar to that of group 2 (Expt 1) and group 6 (Expt 2). Their pair-fed litter-mate controls received exactly the same amount of food.

Table 1. *Expts 1 and 2. Effect of vitamin A deficiency on liver constituents of rats*

Group no.	No. of animals	Diet	Final body-weight (g)	Expt 1 Total nitrogen (mg/g fresh liver)	Total fat (mg/g fresh liver)	Phospholipids (mg/g fresh liver)	Fresh liver weight (g)	Vitamin A (μg/g fresh liver)
1*	6	+Protein+vitamin A	228	32·1 } $P < 0.01$	70·0 } NS	44·5 } $P < 0.02$	10·0	86 } $P < 0.005$
2†	6	+Protein−vitamin A	160	29·4 }	68·2 }	40·3 }	7·0	2 }
3*	4	−Protein+vitamin A	140	22·5 } NS	74·1 } NS	30·3 } NS	5·8	69 } $P < 0.005$
4†	6	−Protein−vitamin A	110	21·1 }	77·5 }	27·8 }	4·5	1 }

Group no.	No. of animals	Diet	Expt 2 Dry weight of liver (mg/g fresh liver)	Total nitrogen (mg/g fresh liver)	Total fat (mg/g fresh liver)	Phospholipids (mg/g fresh liver)	Total cholesterol (mg/g fresh liver)	Vitamin A (μg/g fresh liver)
5‡	12	+Protein+vitamin A	277·1 } $P < 0.001$	33·5 } $P < 0.001$	60·8 } NS	57·7 } $P < 0.001$	3·41 } NS	228 } $P < 0.005$
6	12	+Protein−vitamin A	256·4 }	27·9 }	60·6 }	49·8 }	2·98 }	1 }

NS, not significant.
* 17·2 μg vitamin A acetate in 0·3 ml maize oil given daily, separate from the diet.
† 0·3 ml maize oil alone given daily.
‡ 1·72 mg vitamin A acetate/kg diet.

Table 2. *Expt 1. Effect of protein deficiency on liver constituents of rats*

Group no.	No. of animals	Diet	Total fat (mg/100 mg liver nitrogen)	Phospholipids (mg/100 mg liver nitrogen)	Vitamin A (μg/100 mg liver nitrogen)
1	6	+Protein+vitamin A	218 } $P < 0.001$	138 } NS	268 } $P < 0.05$
3	4	−Protein+vitamin A	331 }	136 }	307 }
2	6	+Protein−vitamin A	223 } $P < 0.005$	133 } NS	6
4	6	−Protein−vitamin A	358 }	127 }	6

NS, not significant.

Table 3. *Expt* 1. *Effect of vitamin A deficiency on serum proteins of rats*

Group no.	No. of animals	Diet	Total nitrogen (mg/100 ml serum)	Albumin: globulin ratio	Albumin (%)	Globulins		
						% α	% β	% γ
1	6	+Protein+vitamin A	1030	1·04	50·9	20·6	6·8	21·1
2	6	+Protein−vitamin A	900 $\}$ $P < 0·001$	0·75 $\}$ $P < 0·01$	43·0 $\}$ $P < 0·01$	12·1 $\}$ $P < 0·01$	7·6 $\}$ NS	37·5 $\}$ $P < 0·001$
3	4	−Protein+vitamin A	875	0·49	33·2	10·5	13·9	42·1
4	6	−Protein−vitamin A	772 $\}$ $P < 0·001$	0·30 $\}$ $P < 0·05$	22·9 $\}$ $P < 0·001$	12·3 $\}$ NS	13·2 $\}$ NS	51·6 $\}$ $P < 0·001$

NS, not significant.

Table 4. *Expt* 3. *Serum proteins of pair-fed vitamin A-deficient rats*

No. of animals	Diet	Total protein (g/100 ml)	Albumin (%)	Globulins		
				% α	% β	% γ
19 pairs	+Protein+vitamin A	6·1	69·1	11·5	11·3	8·0
	+Protein−vitamin A	5·9 $\}$ NS	61·0 $\}$ $P < 0·001$	11·1 $\}$ NS	13·3 $\}$ $P < 0·001$	14·3 $\}$ $P < 0·001$

NS, not significant.

At the end of the 4th week, most of the animals on the vitamin A-deficient diet showed marked eye lesions, and loss of hair from the ventral part of the abdomen.

Liver constituents. The effect of vitamin A deficiency on some liver constituents of rats in Expt 1 is shown in Tables 1 and 2. It was found that protein deficiency increased the liver fat content both in animals given vitamin A supplements and in vitamin A-deficient animals, whereas the differences due to vitamin A deficiency calculated on a liver nitrogen basis were much less marked.

Protein deficiency did not influence the content of liver phospholipids calculated on a nitrogen basis (Table 2).

The total nitrogen content of the fresh liver of the rats in Expt 2 (groups 5 and 6) was very similar to that of the comparable groups in Expt 1 (groups 1 and 2), and dietary vitamin A caused a significant increase in liver total nitrogen. In Expt 2, the dry weight of the liver was found to be significantly higher in the controls (group 5) than in the vitamin A-deficient rats (group 6). The water content of the liver was increased in the vitamin A-deficient animals. As in Expt 1, vitamin A deficiency had no significant effect on liver fat content, whereas the content of liver phospholipids of the vitamin A-deficient animals (group 6) was lower than those of the controls (group 5). Vitamin A deficiency did not have a significant effect on liver or serum cholesterol. The mean vitamin A content of the livers was 228 μg/g fresh tissue for the control rats with vitamin A mixed in their diet (group 5), but only 86 μg/g for the rats receiving the vitamin A in maize oil, separate from the diet (group 1).

Serum proteins. Table 3 gives the total nitrogen content of the rat serum in Expt 1, and the pattern of the distribution of the major serum protein components separated by paper electrophoresis. The most striking change was observed in the γ-globulins: in the vitamin A-deficient groups the content was higher ($P < 0{\cdot}001$) than in those given vitamin A whether or not the deficient animals received protein.

In Expt 2, results for serum proteins in groups 5 and 6 were essentially the same as those in groups 1 and 2 respectively in Expt 1.

In Expt 3, the rats were pair-fed to eliminate the influence on the serum proteins of the difference in food intake between the normal and the vitamin A-deficient animals. The results are summarized in Table 4. The content of serum albumin was significantly decreased, and that of β- and γ-globulin significantly increased, in the vitamin A-deficient rats. The marked difference in the albumin and globulin percentages between group 1 of Expt 1 and the vitamin A-deficient group in Expt 3 may have been due to the different stages of deficiency the animals had reached when they were killed: whereas all animals in Expt 1 were killed after 42 days on the diet, each pair of animals in Expt 3 was killed when the deficient rat had lost weight for at least 2 consecutive days: i.e. the rats in Expt 3 were generally killed somewhat earlier in the stage of acute deficiency than those in Expt 1.

DISCUSSION

It has been known for a long time that vitamin A deficiency arrests growth, and that growth increases vitamin A requirement (Johnson & Baumann, 1948). In a review article, Moore (1960) concluded very cautiously that knowledge up to the time of his

writing did not point to any close relationship between vitamin A and the general metabolism of protein. Similarly, a summary of our knowledge of the absorption, transport and storage of vitamin A was published by Ganguly (1960) but the author did not discuss the influence of dietary proteins on these mechanisms.

If we compare the liver stores of vitamin A in the control animals (group 1) of Expt 1 (86 μg/g fresh liver) with those of the corresponding group (group 5) of Expt 2 (228 μg/g fresh liver) it is apparent that liver storage and intestinal absorption of vitamin A are higher and growth is better when the vitamin is given with the food than when it is given separately in an oily solution. The animals in group 1 gained 229% of their initial body-weight in 28 days, whereas those in group 5 gained 254%. The presence of protein in the diet of the control group appeared to influence their vitamin A absorption: indeed, the animals deprived of protein but given vitamin A (group 3) had significantly lower ($P < 0{\cdot}01$) liver stores of vitamin A (69 μg/g fresh liver, representing a total liver reserve of 405 μg or 59% of the total quantity of vitamin A they were given by mouth in the experimental period) than their controls (group 1, with 86 μg vitamin A/g fresh liver, representing a total liver reserve of 856 μg or 85% of the total quantity they were given by mouth in the experimental period). The 85% liver storage of vitamin A corresponds well with the figure of over 80% found by Friend *et al.* (1961) in the livers of pigs given a test dose of vitamin A. Our observation of a lowered liver vitamin A reserve in rats due to the withdrawal of protein from their diet for 2 weeks after they had been on a normal diet is in apparent contrast with the observation of Rechcigl *et al.* (1962) who found a higher vitamin A content in the livers of rats fed on a protein-free diet. Murray (1961) could not confirm Rechcigl's findings. Rechcigl *et al.* measured the rate of disappearance of an initial store of vitamin A due to different levels of protein intake on a vitamin A-free diet. We observed the combined effect of vitamin A absorption and utilization in protein deficiency. It seems likely that the decreased absorption of vitamin A in protein deficiency outweighs the reduced utilization due to low protein intake, the net result being a lowered liver storage of vitamin A on a protein-deficient diet.

Esh & Bhattacharya (1960) have also shown that absorption and liver storage of vitamin A in rats are impaired after long periods on low-protein diets or diets containing an inferior quality of protein.

It is not clear at this stage whether the lowered liver storage of vitamin A when protein is completely withdrawn from the diet (group 3) is due to impaired absorption from the gut or whether the transport mechanism is affected. It seems unlikely that the limiting factor for liver storage of the vitamin was directly related to liver proteins in the protein-deficient animals because they stored more vitamin A per 100 mg liver nitrogen (307 μg in group 3) than the normal controls (268 μg in group 1).

Vitamin A deficiency caused a striking change in the serum proteins, similar to that caused by protein deficiency (see Table 3): both vitamin A deficiency alone (when groups 1 and 2 are compared) and protein deficiency alone (when groups 1 and 3 are compared) lowered the content of the serum albumin and α-globulin fractions in rat serum and increased that of the γ-globulins. In the pair-fed animals in Expt 3 (Table 4), both groups had identical serum total proteins, indicating that the lower content of

serum total nitrogen in the *ad lib.* fed animals of Expts 1 and 2 was probably due to the decreased food intake of the deficient rats. The mechanism whereby vitamin A deficiency reduces the content of serum albumin but produces a rise in that of β- and γ-globulin is not clear. A possible explanation is that vitamin A plays a direct or an indirect part in protein synthesis: the observation that vitamin A utilization is reduced when the protein intake is lowered (Rechcigl *et al.* 1962) and increased when dietary protein is raised (McLaren, 1959; Stoewsand & Scott, 1961), and the results of Friend *et al.* (1961), together with our findings presented here that in vitamin A deficiency the content of liver protein is lowered and that of serum proteins, especially the albumins, greatly reduced are arguments in favour of this hypothesis.

Our finding that vitamin A deficiency did not change serum and liver cholesterol levels is in agreement with that of Green, Lowe & Morton (1955).

Liver phospholipids decreased ($P < 0{\cdot}02$) in vitamin A deficiency. The significantly lower levels of phospholipids can perhaps be attributed to an adverse effect of vitamin A on protein metabolism.

SUMMARY

1. In a first experiment, weanling male albino rats were given a vitamin A-deficient diet. Controls received the same diet and 1·72 μg vitamin A daily. After the animals had been 4 weeks on this diet, the protein was withdrawn from the diet of half of them and replaced by starch. These animals were all fed *ad lib.* In another experiment, the rats on the vitamin A-deficient diet were pair-fed with controls receiving vitamin A.

2. The withdrawal of dietary protein reduced the liver store of vitamin A, although reduced protein intake is known to lower the vitamin A requirement in rats. It also lowered the content of serum albumin and increased that of γ-globulin.

3. The results with the pair-fed animals showed that in vitamin A deficiency the content of serum albumin was significantly lowered and that of β- and γ-globulin increased.

4. In vitamin A deficiency the contents of total nitrogen and phospholipids in rat liver were lowered but that of water was increased.

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REFERENCES

Brown, F. F., Michaels, G. D. & Kinsell, L. W. (1956). *Proc. Soc. exp. Biol., N.Y.*, **92**, 587.

Esh, G. C. & Bhattacharya, R. K. (1960). *Symposium on Proteins*, p. 107. Mysore, India: Central Food Technological Research Institute.

Fister, H. J. (1950). *A Manual of Standardized Procedures for Spectrophotometric Chemistry*. New York: Standard Scientific Supply Corporation.

Friend, C. J., Heard, C. R. C., Platt, B. S., Stewart, R. J. C. & Turner, M. R. (1961). *Brit. J. Nutr.* **15**, 231.

Ganguly, J. (1960). *Vitam. & Horm.* **18**, 387.
Green, B., Lowe, J. S. & Morton, R. A. (1955). *Biochem. J.* **61**, 447.
Johnson, R. M. & Baumann, C. A. (1948). *J. Nutr.* **35**, 703.
McLaren, D. S. (1959). *Brit. J. Ophthal.* **43**, 234.
Moore, T. (1960). *Vitam. & Horm.* **18**, 431.
Murray, T. K. (1961). *Canad. J. Biochem.* **39**, 1099.
Rechcigl, M. Jr., Berger, S., Loosli, J. K. & Williams, H. H. (1962). *J. Nutr.* **76**, 435.
Roels, O. A. & Trout, M. (1959). *Amer. J. clin. Nutr.* **7**, 197.
Schneider, W. C. (1945). *J. biol. Chem.* **161**, 293.
Sperry, W. M. & Webb, M. (1950). *J. biol. Chem.* **187**, 97.
Stoewsand, G. S. & Scott, M. L. (1961). *Proc. Soc. exp. Biol., N.Y.*, **106**, 635.
Taussky, H. H. & Shorr, E. (1953). *J. biol. Chem.* **202**, 675.
Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1957). *Manometric Techniques*, 3rd ed., p. 238. Minneapolis, Minn.: Burgess Publishing Corp.

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