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Decreased Hepatic Gluconeogenesis in Vitamin A-Deficient Rats (41158)

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Abstract. Studies were carried out to investigate the effects of vitamin A deficiency (hypovitaminosis A) on the chemical constituents and gluconeogenic activity of rat liver. Hypovitaminosis A was found not to alter the glycogen, protein, DNA, or RNA content, but to decrease significantly the level of citrate in liver. Free amino acid levels in blood and liver of vitamin A-deficient rats were markedly higher in comparison to pair fed controls. An inhibition of hepatic gluconeogenesis in vitamin A deficiency was indicated by the decreased incorporation of ^{14}C-labeled alanine and bicarbonate into glucose and glycogen by liver slices. Vitamin A deficiency caused a significant decrease in the activities of alanine aminotransferase, ornithine aminotransferase, glucose 6-phosphatase and fructose 1,6-bisphosphatase. Activities of aspartate aminotransferase and tryptophan pyrrolase were found to be unaltered in vitamin A-deficient livers. It is suggested that the inhibition of hepatic gluconeogenesis observed in hypovitaminosis A may be due in part to an impairment in the substrate flux into the gluconeogenic pathway.

There is evidence to show that both vitamin A excess (hypervitaminosis A) and vitamin A deficiency (hypovitaminosis A) cause marked metabolic derangements. In earlier reports from this laboratory, oral administration of large doses of retinol (vitamin A alcohol) to young rats for 2 days was shown to cause marked stimulation of hepatic gluconeogenesis (1–4), deposition of glycogen in the liver (2), increased mobilization and oxidation of fatty acids, and fatty liver formation (5–7). Investigations into the mechanism of retinol-induced stimulation of gluconeogenesis have revealed an activation of key gluconeogenic and certain amino acid-degrading enzymes, and an increase in the hepatic NADH:NAD ratio (2–4). Hypovitaminosis A, on the other hand, has been reported to inhibit gluconeogenesis as evidenced by decreased incorporation of ^{14}C-labeled acetate, lactate, and glycerol into rat liver glycogen (7). However, the mechanism by which vitamin A deficiency causes decreases in hepatic gluconeogenesis is not yet understood. Thus, a detailed study of hepatic gluconeogenesis in hypovitaminosis A was undertaken. This communication deals with changes in hepatic gluconeogenesis, alterations in the activities of certain gluconeogenic and amino acid-metabolizing enzymes, and the level of citrate caused by vitamin A deficiency.

Materials and Methods. *Animals and diet.* Vitamin A deficiency was produced in young rats by maintaining them on a vitamin A-deficient diet (8) prepared in the laboratory (please see Table I for the composition of diet). Weanling male rats of Wistar strain were divided into two groups: control and vitamin A deficient. Animals in both the groups were pair-fed with the vitamin A-deficient diet for a period of 6 weeks. Rats of the Control group were given 2000 IU of vitamin A (retinol) orally once a week. Weighed amounts of the diet were supplied to each rat and the food intake and body weight were recorded daily. Pair feeding was carried out by restricting the food consumption each day in all animals to the lowest amount of food consumed by an individual animal on the previous day. The quantity of diet supplied each day was increased or decreased by 1–2 g depending upon their daily consumption. However, in

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TABLE I. COMPOSITION OF VITAMIN A-DEFICIENT DIET[a]

Ingredients	Amount of ingredient present/kg diet
Fat free casein[b]	240 g
Sucrose	659 g
Salt mixture[c]	50 g
Cottonseed oil	30 g
Choline dry mix[d]	10 g
Water soluble vitamin mixture[e]	10 g
Vitamin D and E preparation[f]	1 ml

[a] Adopted from Wolf *et al.* (8).

[b] Casein was washed in water, acetone and petroleum ether. The washed casein was kept overnight in an oven at 105°.

[c] The salt mixture was composed of NaCl, 243.0 g; $K_3C_6H_5O_7 \cdot H_2O$, 533.0 g; K_2HPO_4, 174.0 g; $Ca(H_2PO_4)_2 \cdot H_2O$, 800.0 g; $CaCO_3$, 368.0 g; $MgCO_3$, 92.0 g; $FeC_6H_5O_7 \cdot 3H_2O$, 36.0 g; $MnSO_4$, 2.8 g; $CuSO_4 \cdot 5H_2O$, 400 mg; $K_2Al_2(SO_4)_4 \cdot 24H_2O$, 200 mg; KI, 100 mg; $CoCl_2 \cdot 6H_2O$, 200 mg; $ZnCO_3$, 100 mg; NaF, 2 mg.

[d] 20% choline chloride premixed in corn starch.

[e] 100 g of water-soluble vitamin mixture contained: thiamine hydrochloride, 250 mg; riboflavin, 150 mg; calcium pantothenate, 400 mg; nicotinic acid, 1 g; pyridoxine hydrochloride, 60 mg; biotin, 6 mg; folic acid, 40 mg; 2-methyl-1,4-naphthaquinone, 40 mg; cyanocobalamin, 0.5 mg; inositol, 2 g; *p*-aminobenzoic acid, 60 mg and cellulose sufficient to make up to 100 g.

[f] 1 ml of this preparation contained 2800 IU of vitamin D and 60 mg of vitamin E.

the event a rat was found to be eating a considerably lesser amount than the rest of the animals, that particular one was discarded. The rats were considered to be vitamin A deficient when net gain in their body weight on four consecutive days did not exceed 1 g, provided that in 2 of these days the rats did not gain any weight at all (9). At this stage animals were sacrificed after fasting them for 18–20 hr.

Analysis. Levels of citrate in the liver were determined by the method described by Stern (10) using TCA extracts of the tissue prepared immediately after sacrificing the animals.

Protein was estimated by the method of Lowry *et al.* (11). Glycogen, DNA, and RNA were determined by the methods described earlier (12). Vitamin A content was estimated by the method of Carr and Price as described by Embree *et al.* (13). Amino acid levels in blood and liver were measured as reported elsewhere (3).

Incorporation of ^{14}C-labeled precursors into glucose and glycogen by liver slices. About 300 mg of uniformly thin liver slices were suspended in 3 ml Krebs–Henseleit bicarbonate buffer (pH 7.4) containing 50 μmole (1 μCi) of L-[U-^{14}C]alanine or 72 μmole (1 μCi) sodium [^{14}C]bicarbonate, or 30 μmole of [1-^{14}C]glycerol in Erlenmeyer flasks and incubated at 37° with constant shaking in an atmosphere of 95% oxygen and 5% CO_2. To each of the flasks containing [^{14}C]bicarbonate as precursor, 90 μmole of glycerol, 120 μmole of pyruvate, and 60 μmole of sodium acetate were also added. The flasks containing [^{14}C]bicarbonate or [^{14}C]alanine were incubated for 3 hr, whereas those containing [1-^{14}C]glycerol were incubated for 1 hr. At the end of incubation, slices were removed, quickly washed with chilled distilled water, and dropped in 30% KOH. Glycogen was isolated, purified, and assayed for radioactivity as described earlier (3).

After removing the slices, the medium was treated with $Ba(OH)_2$ and $ZnSO_4$, and an aliquot of the supernatant was used for glucose estimation (14). Another aliquot was used for the preparation of glucosazone according to the procedure described earlier (2). The glucosazone was dissolved in ethanol and aliquots of the solution were used for measuring radioactivity in a windowless gas flow counter with an efficiency of about 40%.

Enzyme assays. Suitable homogenates were prepared from fresh liver and used immediately for enzyme assays. Methods of assay were always standardized and experimental conditions giving linear rates of reaction with respect to periods of incubation and enzyme concentrations were followed.

Activities of alanine aminotransferase (L-alanine:2-oxoglutarate aminotransferase, EC 2.6.1.2) and aspartate aminotransferase (L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1) were measured by the colorimetric procedure (2), using 700 *g* supernatant of the liver homogenates prepared in 0.1 *M* potassium phosphate (pH 7.4). One unit of alanine aminotransferase or 1 unit of aspartate aminotransferase catalyzes the formation of 1 μmole of pyruvate or 1 μmole oxalacetate per minute, respectively.

Ornithine aminotransferase (L-ornithine:2-oxoglutarate aminotransferase,

EC 2.6.1.13) was assayed as described earlier (3) in 700 *g* supernatant of 10% liver homogenate prepared in 0.1 *M* potassium phosphate (pH 8.0) containing 10 m*M* 2-mercaptoethanol. One unit of enzyme produces 1 μmole of pyrroline-5-carboxylate per minute.

A 100,000 *g* supernatant of liver homogenate in 20 m*M* potassium phosphate (pH 7.0) containing 0.14 *M* KCl was used for the assay of tryptophan pyrrolase (L-tryptophan:oxygen oxidoreductase, EC 1.13.1.12) (3). One unit of the enzyme produces 1 μmole of kynurenine per hour under the conditions of the assay.

Glucose 6-phosphatase (D-glucose 6-phosphate phosphohydrolase, EC 3.1.3.9) activity was assayed in 700 *g* supernatant of the liver homogenate prepared in 0.1 *M* potassium citrate (pH 6.5) by measuring the orthophosphate released (15). One unit of enzyme releases 1 μmole of P_i per minute.

Activity of fructose 1,6-bisphosphatase (D-fructose-1,6-diphosphate, 1-phosphohydrolase, EC 3.1.3.11) was measured in 700 *g* supernatant of the liver homogenate in 50 m*M* sodium lactate buffer (pH 5.0) by the method of Freedland and Harper (16). One unit of enzyme releases 1 μmole of P_i per minute.

Statistical analysis of the results (mean ± SEM and *t* test for significance of difference between two means) was carried out according to the standard procedures (17).

Vitamin A (Prepalin) was supplied by Glaxo Laboratories India Ltd., Bombay, India. L[-U-^{14}C]Alanine and [^{14}C]bicarbonate were purchased from Bhabha Atomic Research Center, Bombay, India. [1-^{14}C]-Glycerol was obtained from Radiochemical Center, Amersham, England. All other substrates and cofactors used in this study were purchased from Sigma Chemical Company, St. Louis, Missouri.

Results. Figure 1 illustrates the growth and food intake of vitamin A-deficient and pair fed control rats. As can be noted, vitamin A-deficient animals gained weight in a similar fashion as controls up to the end of the fifth week. After this period, body weight of the vitamin A-deficient animals reached and remained at plateau throughout the sixth week. All animals were sacrificed at the end of 6 weeks. It can be noted that food intake had stayed within the range of 7–9 g per day throughout the duration of the experiment. The slight depression in food take seen between 3 and 4 weeks is not of any significant difference when the actual values and variations are taken into account.

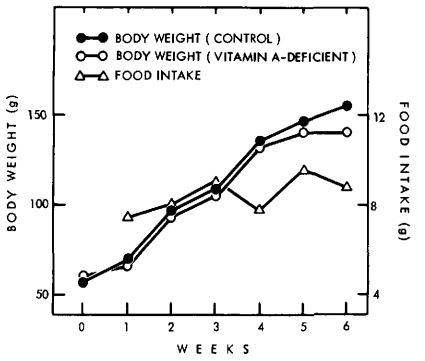

FIG. 1. Body weight and food intake of vitamin A-deficient and pair fed controls. Details of the diet and treatment of animals are given under Materials and Methods. Each value plotted is the average of six animals.

In order to get an overall picture of the metabolic changes caused by vitamin A deficiency, the major chemical constituents of liver were estimated (Table II). Vitamin A deficiency did not bring about any change in the weight, protein, DNA, RNA, or glycogen content. The level of citrate in liver was significantly decreased as a result of hypovitaminosis A. No detectable amount of vitamin A was found in the livers of vitamin A-deficient rats.

As a possible means of assessing the gluconeogenic activity of the liver, incorporation of ^{14}C-labeled precursors into glucose and glycogen was measured using liver slices. Vitamin A deficiency caused a marked decrease in the incorporation of [^{14}C]alanine and bicarbonate into glucose (Table III) and of alanine, bicarbonate, and glycerol into glycogen (Table IV). It is evident from the data presented in Table V that vitamin A deficiency increased the level of free amino acids in blood by 23% and that in the liver by 50%.

The effect of vitamin A deficiency on the activities of glucose 6-phosphatase and fructose 1,6-bisphosphatase was examined.

TABLE II. EFFECT OF VITAMIN A DEFICIENCY ON WEIGHT AND MAJOR CHEMICAL CONSTITUENTS OF RAT LIVER

Constituents	Control	Vitamin A deficient
Liver wt (g)	4.72 ± 0.26[a] (22)[b]	4.32 ± 0.23 (22)
Liver wt/100 g body wt (g)	2.96 ± 0.09 (22)	2.96 ± 0.08 (22)
Protein (mg/g fresh liver)	217 ± 8 (8)	211 ± 7 (8)
DNA (mg/g fresh liver)	2.26 ± 0.07 (8)	2.37 ± 0.06 (8)
RNA (mg/g fresh liver)	9.08 ± 0.36 (8)	8.13 ± 0.34 (8)
Glycogen (mg/g fresh liver)	3.71 ± 0.23 (6)	3.04 ± 0.11 (6)
Citrate (μmole/g fresh liver)	0.27 ± 0.01 (5)	0.18 ± 0.01* (5)
Vitamin A (μg/g fresh liver)	38.80 ± 9.30 (7)	Amount not detectable (7)

[a] Each value given is mean ± SEM.
[b] Figures in parentheses indicate the number of animals used.
* Significantly different from controls at $P < 0.01$.

The results (Table VI) indicate that in comparison to the pair fed controls, vitamin A-deficient animals had significantly lower activities of glucose 6-phosphatase and fructose 1,6-bisphosphatase in the liver.

Activities of certain amino acid-degrading enzymes of the liver were measured in order to find out whether hypovitaminosis A had caused any impairment in amino acid degradation. It is evident from the results presented in Table VI that vitamin A deficiency brought about a marginal but statistically significant decrease in the activity of alanine aminotransferase and an appreciable decrease in ornithine aminotransferase activity. Deficiency of vitamin A, however, did not cause any alteration in the activity of aspartate aminotransferase or tryptophan pyrrolase.

Discussion. A decrease in hepatic gluconeogenesis in hypovitaminosis A is indicated by lowered incorporation of ^{14}C-labeled alanine and bicarbonate into glucose and glycogen, and [^{14}C]glycerol into glycogen by liver slices. In an earlier report from this laboratory a marked increase in hepatic gluconeogenesis from alanine, bicarbonate, and glycerol in rats fed excess doses of vitamin A has been demonstrated (1–3). The fact that feeding of excess vitamin A stimulates gluconeogenesis and vitamin A deficiency lowers this process suggests that vitamin A may be directly or indirectly involved at one or more steps in hepatic gluconeogenesis.

It is generally accepted that regulation of hepatic gluconeogenesis could be brought about by a direct control of gluconeogenic

TABLE III. INCORPORATION OF L-[U-^{14}C]ALANINE AND [^{14}C]BICARBONATE INTO GLUCOSE BY LIVER SLICES FROM NORMAL AND VITAMIN A-DEFICIENT RATS

Precursor	^{14}C incorporated into glucose (cpm/g liver/hr)		Percentage decrease
	Control	Vitamin A-deficient	
L-[U-^{14}C]Alanine	3307 ± 131[a]	1742 ± 18*	47
[^{14}C]Bicarbonate	2452 ± 45	1336 ± 123*	46

[a] Each value is mean ± SEM of the results obtained from five rats.
* Significantly different from controls at $P < 0.01$.

TABLE IV. INCORPORATION OF ^{14}C-LABELED PRECURSORS INTO GLYCOGEN BY LIVER SLICES FROM NORMAL AND VITAMIN A-DEFICIENT RATS

Precursor	^{14}C incorporated into glycogen (cpm/g liver/hr)		Percentage decrease
	Control	Vitamin A deficient	
L-[U-^{14}C]Alanine	2024 ± 85[a]	1333 ± 41*	34
[1-^{14}C]Glycerol	4321 ± 220	2161 ± 179*	50
[^{14}C]Bicarbonate	1002 ± 69	586 ± 19*	42

[a] Each value is mean ± SEM of data obtained from five rats.
* Significantly different from controls at $P < 0.01$.

activity in the liver by regulating the supply of precursors from extrahepatic tissues such as muscle and adipose tissue (18, 19). Among the various substrates that are utilized by liver for gluconeogenesis, amino acids are of major quantitative importance and there are indications that their supply could regulate the rate of gluconeogenesis in the liver (18, 19). In the present study, vitamin A deficiency significantly elevated the levels of free amino acids in blood and liver. The increase in amino acid levels could be due to either their increased mobilization from extrahepatic tissues or their decreased utilization for gluconeogenesis in the liver. The decrease in the incorporation of [^{14}C]alanine into glucose and glycogen by liver slices from vitamin A-deficient animals suggests an impairment in the utilization of amino acids for gluconeogenesis. The decreased utilization of amino acids for gluconeogenesis may be due to a marked inhibition of the activities of certain amino acid-catabolizing enzymes such as alanine aminotransferase noted in the present study (Table VI). Hypervitaminosis A, on the other hand, has been shown to decrease free amino acid levels in blood and liver and increase the activities of alanine and ornithine aminotransferases (3). These changes paralleled an overall increase in hepatic gluconeogenic ability and activation of the key enzymes of gluconeogenesis (2). The fact that gluconeogenesis not only from alanine but also from glycerol was inhibited in vitamin A deficiency indicates a block at some step or steps subsequent to the formation of triose phosphate, the step at which glycerol is expected to enter gluconeogenic pathway. A marked inhibition of the activities of fructose 1,6-bisphosphatase and glucose 6-phosphatase in the livers of vitamin A-deficient rats noted in this study lends support to this postulate.

As citrate has been shown to activate fructose 1,6-bisphosphatase (20), and inhibit phosphofructokinase (21), the marked decrease in the level of this metabolite in the liver during vitamin A deficiency noted in the present study would favor channelling of carbon skeletons in the direction of glycolysis and against gluconeogenesis.

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TABLE V. EFFECT OF VITAMIN A DEFICIENCY ON THE LEVELS OF AMINO ACIDS IN BLOOD AND LIVER OF RATS

Tissue	Amino nitrogen		Percentage change
	Control	Vitamin A deficient	
Blood, mg/100 ml (6)[a]	11.81 ± 0.96[b]	14.54 ± 0.77*	+23
Liver, mg/g (7)	0.48 ± 0.02	0.72 ± 0.03**	+50

[a] Figures in parentheses indicate number of rats used.
[b] Each value presented is mean ± SEM.
* Significantly different from control at $P < 0.05$.
** Significantly different from control at $P < 0.01$.

TABLE VI. EFFECT OF VITAMIN A DEFICIENCY ON THE ACTIVITIES OF CERTAIN KEY GLUCONEOGENIC AND AMINO ACID METABOLIZING ENZYMES OF RAT LIVER

Enzyme	Activity (units/g liver)	
	Control	Vitamin A deficient
Glucose 6-phosphatase	21.30 ± 0.74[a] (5)[b]	18.13 ± 0.27* (5)
Fructose 1,6-bisphosphatase	7.23 ± 0.39 (5)	4.49 ± 0.24* (5)
Alanine aminotransferase	29.58 ± 0.35 (5)	24.37 ± 1.28* (5)
Aspartate aminotransferase	14.64 ± 0.33 (5)	13.02 ± 0.31 (5)
Ornithine aminotransferase	1.90 ± 0.19 (5)	1.09 ± 0.08* (5)
Tryptophan pyrrolase	22.25 ± 1.20 (6)	22.65 ± 1.49 (6)

[a] Mean ± SEM.
[b] Figures in parentheses indicate number of animals used.
* Significantly different from control at $P < 0.01$.

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