15122

Effect of Vitamin K Deficiency on Liver Lipids in the Chick.*

JOHN B. FIELD AND HENRIK DAM.

From the Department of Biochemistry and Pharmacology, School of Medicine and Dentistry, University of Rochester, Rochester, N.Y.

In a series of recent communications[1-4] Honorato and his associates have indicated that 2-methyl-1,4-naphthoquinone possesses lipotropic properties analogous to choline and suggest that the former substance serves as a "methyl-donor." They report that avitaminosis K in the chick is accompanied by a fatty degeneration of the liver which is prevented by the administration of 2-methyl-1,4-naphthoquinone as well as by choline.[4] These results are at variance with the early observations of Dam and his group,[5,6] as well as other laboratories,[7] in the studies which led to the isolation and identification of vitamin K. These previous studies did not reveal any correlative pathological change in deficient chicks other than alterations resulting exclusively from the characteristic hemorrhagic syndrome. If the absence of vitamin K produces fatty liver degeneration, a route through which the naphthoquinone may function to maintain normal plasma prothrombin levels is suggested. In a previous paper Emmel and Dam[8] reported that no histological indication of fatty degeneration could be seen in the livers of chicks reared on a vitamin K-free diet. The trials herein reported present an elaboration of this study[8] and the amount of total lipid and phospholipids in the liver as determined by chemical methods is given.

Methods. One-day-old White Leghorn chicks were placed on a basal synthetic diet consisting of: alcohol-extracted casein, 150 g; dried brewer's yeast, Fleischmann Type 2019 (ether extracted), 75 g; gelatin, 80 g; McCollum's salt mixture No. 185 with Mn

TABLE I.

	Basal synthetic diet	Basal plus choline	Basal plus vit. K	Control natural diet
Prothrombin levels in 4-week-old chicks.				
Avg clotting time, sec.	106.6	73.0	20.5	20.2
Range	60.0->200	46.2-91.3	16.5-30.0	20.0-20.5
Total lipids in livers of 4-week-old chicks.				
Lipids in % wet wt. of liver, avg	4.93	4.08	5.23	3.64
Range	3.51-7.00	2.79-5.34	3.25-7.75	3.43-3.94
Lipids in % dry wt of liver, avg	17.68	16.57	18.34	14.05
Range	13.48-25.22	12.55-19.70	12.32-25.60	13.75-14.28
Total phospholipid (calculated as P) in livers of 4-week-old chicks.				
Mg P in $CHCl_3$ extr. per g wet liver, avg	.85	.80	.82	.80
Range	.76-93	.66-.95	.65-.93	.76-.86
Mg P in $CHCl_3$ extr. per g dry liver, avg	3.08	3.17	2.89	3.09
Range	2.73-3.67	2.41-3.84	2.06-3.32	2.72-3.44

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2 R. Honorato C., and A. Cassis, *Rev. med. y. alimentacion.*, 1942-3, **5**, 141.

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4 S. Topelberg G. and R. Honorato C., *Rev. Soc. Argentina Biol.*, 1943, **19**, 409.

5 Schonheyder, F., Thesis, 1936, University, Copenhagen.

6 Dam, H., Schonheyder, F., and Tage-Hansen, E., *Biochem. J.*, 1936, **30**, 1075.

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8 Emmel, V. M., and Dam, H., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 11.

and Cu added, 50 g; gum arabic, 50 g; *l*-cystine, 1 g; cod liver oil, 50 g; *d,l*-alpha-tocopherol acetate, 50 mg; and cornstarch, 544 g. This diet was supplemented either with 5 g choline chloride (replacing 5 g cornstarch) or 30 mg of a vitamin K compound, the tetra sodium salt of 2-methyl-1,4-naphthohydroquinone diphosphoric acid (Synkayvite, Hoffmann-La Roche). After 2, 3, and 4 weeks blood samples were withdrawn by cardiac puncture into sodium oxalate solution without anesthetic. The prothrombin time of the plasma was determined by adding 0.2 ml of a mixture of $CaCl_2$ and an emulsion prepared from brains of vitamin K-deficient chicks to 0.1 ml oxalated chick plasma at 38°C. Plasma fibrinogen was determined by a modification of the colorimetric method of Folin and Ciocalteu.[9] The chicks were sacrificed after 4 weeks by decapitation and the livers removed. Livers not taken for immediate analysis were frozen. Total lipids were determined by extraction with chloroform after preliminary grinding of the liver with sodium sulfate. The phospholipid content was measured by oxidizing the lipids obtained from a 10 ml chloroform aliquot with sulfuric acid and hydrogen peroxide (Superoxol, Merck) and determining the P- content by a standard colorimetric procedure.[10]

Results and Comment. In all cases determinations were made on groups consisting of an average of 9 (5 to 12) chicks. The plasma prothrombin time of chicks receiving either the synthetic diet supplemented with vitamin K or a commercial growing chick ration was approximately the same while the prothrombin time from chicks on the synthetic diet, with or without added choline, was markedly prolonged (Table I). The plasma prothrombin time from chicks receiving added choline was perhaps somewhat less than that of chicks given the synthetic diet alone but the qualified significance of this difference does not permit of interpretation.

The total lipid content and the total phospholipid content of the livers of the chicks after 4 weeks is also summarized in Table I. The lipid content of the livers from chicks raised on the various diets was quite similar. In fact, a typical high-lipid "fatty liver" was not obtained inasmuch as the lipid content did not in any case exceed 7.75% (on a wet basis). Apparently sufficient choline was furnished by the yeast contained in our synthetic diet to prevent the complications of a choline deficiency. Therefore it is surprising that Topelberg and Honorato[4] using the Almquist and Klose ration low in vitamin K,[11] which also contains 7.5% yeast, state that they found a high lipid content in the livers of their unsupplemented chicks. Contrary to their report the total lipids of the livers from our chicks given vitamin K were slightly greater than the liver lipids from other chicks receiving the synthetic diet, while that of the choline-supplemented chicks was somewhat less.

The variations in total lipids were quite unrelated to the observed prothrombin times. Marked hypoprothrombinemia was typical of the chicks receiving the vitamin K-deficient diet as well as chicks consuming the same diet supplemented with choline, while the prothrombin times of the vitamin K-supplemented chicks were the same as those from chicks receiving the control natural ration. Plasma fibrinogen levels are often a sensitive indication of liver integrity.[12] The fibrinogen levels of all chicks receiving the basal synthetic diet with and without its supplements were similar.† The plasma fibrinogen of chicks on a vitamin K-free diet in which the prothrombin time was drastically prolonged resembled the fibrinogen levels in chicks receiving vitamin K.

The status of choline as a coagulant, aside from its capacity to reduce hypocoagulability resulting from failure of the liver in cases of fatty infiltration that arises in choline defi-

9 Reiner, M., *Manual of Clinical Chemistry*, 1941, New York.

10 Youngsberg, G. E., and Youngsberg, M. V., *J. Lab. Clin. Med.*, 1930, **16**, 158.

11 Almquist, H. J., and Klose, L. R., *Biochem. J.*, 1939, **33**, 1055.

12 Foster, D. P., and Whipple, G. H., *Am. J. Physiol.*, 1921-1922, **58**, 407.

† A difference in the fibrinogen levels of chicks receiving the synthetic diet in contrast to those given the natural diet indicates that plasma fibrinogen can be influenced by diet. Our observations on this subject will soon be reported elsewhere.

ciency,[13] is still obscure. For example, Bellows and Chinn[14] indicate that intraocular hemorrhages were obtained in young rats raised on a diet low in choline and methionine while Zunz and Vessoelovsky[15] did not observe any change in the clotting time of rabbits injected intravenously with choline or its homologues. In unpublished studies, it was found that choline and methionine had no effect on the hypoprothrombinemia induced in rats by 3,3′-methylenebis (4-hydroxycoumarin) and gave inconclusive results in correcting the hypocoagulability (prothrombin and fibrinogen deficiency) induced by chloroform liver intoxication in dogs maintained on an adequate dietary regime.‡

Summary. 1. The hypoprothrombinemia induced in chicks by feeding a vitamin K-free diet was not significantly influenced by the addition of choline to the diet. 2. Chicks raised on a vitamin K-free diet exhibited the typical hypoprothrombinemia but their fibrinogen levels and total liver lipids resembled those of control chicks. 3. The total lipids of the livers of chicks receiving synthetic diets were somewhat increased over the lipids from chicks given a natural ration but this condition was not corrected by vitamin K.

[13] Henry, E. M., *Biol. Symposia,* 1941, **5**, 177.

[14] Bellows, J. G., and Chinn, H., *Arch. Ophthamol.,* 1943, **30**, 105.

[15] Zunz, E., and Vessoelovsky, O., *Arch. Int. Pharmacodynamic.,* 1938, **60**, 146.

‡ Field, J. B., and Link, K. P. These observations were made during the course of studies on the anticoagulant, 3,3′-methylenebis (4-hydroxycoumarin) and will be published in the near future.

15123

Inhibition of Cytochrome Oxidase (Paraphenylendiamine Oxidase) of the Thyroid Gland by Thiouracil and Other Compounds.

K. E. PASCHKIS,* A. CANTAROW, AND E. K. TILLSON

From the Jefferson Medical College and Hospital, Philadelphia, Pa.

It is believed that an oxidizing enzyme plays an important role in the formation of thyroid hormone. Inasmuch as thiourea, thiouracil, sulfonamides, and other compounds have been found to suppress formation of thyroid hormone[1,2,3] it appeared of interest to study the influence of these agents on oxidizing enzymes of the thyroid gland. The experiments reported here deal with the influence of thiouracil, sulfonamides, para-aminobenzoic acid, glutathione, cysteine, ascorbic acid, and certain xanthines on the cytochrome oxidase (paraphenylendiamine oxidase) in the thyroid gland.

Methods. The cytochrome oxidase activity of thyroid tissue was determined by a modification of the method of Galli-Mainini.[4] Rats were stunned by a blow on the neck and exsanguinated by opening the chest and cutting the heart. The thyroid glands were dissected free from connective tissue, rapidly weighed on a torsion balance, and immediately dropped into a test tube containing 5 cc phosphate buffer solution pH 7 and a small amount of sand. The tissue was triturated with a glass rod. Five cubic centimeters of a freshly prepared 0.2% aqueous paraphenylendiamine solution was added. The test tubes were then shaken in a mechanical shaker for 45 minutes at 37°C. The samples were immediately filtered into a colorimeter tube and read in the Evelyn photoelectric colorimeter against a blank treated in identical manner (blank =

* J. Ewing Mears Fellow in Physiology and Medicine.

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[2] Astwood, E. B., Sullivan, J., Bissel, A., and Tyslowitz, R., *Endocrinol.,* 1943, **32**, 210.

[3] Astwood, E. B., *J. Pharmacol. Exp. Therap.,* 1943, **78**, 79.

[4] Galli, Mainini C., *Rev. Soc. Argent. de biol.,* 1943, **19**, 205.