Activity of Certain Vit. B_{12} Analogues in the Chick.* (23265)

M. R. Spivey Fox, George M. Briggs, and Ligia O. Ortiz
Laboratory of Nutrition and Endocrinology, Natl. Inst. of Arthritis and Metabolic Diseases, P.H.S., U. S. Department of Health, Bethesda, Md.

A number of compounds structurally related to vit. B_{12} have been obtained by isolation from substances supporting growth of microorganisms or by chemical modification of cyanocobalamin (as reviewed in 1,2). These compounds differ in their ability to replace vit. B_{12} in various living organisms. The simplest analogues available at present are those cobalamins in which a molecule of water or an anionic group is substituted for the cyanide radical. Rosenblum and coworkers(3,4) have compared absorption and urinary and fecal excretion of chloro-, sulfato-, nitro-, and thiocyanatocobalamin with cyanocobalamin in healthy young men. They found that absorption of the analogues was lower than that of cyanocobalamin, although all compounds appeared to be excreted similarly upon injection. In rats, poor absorption of chloro- and nitrocobalamins was also found. Vit. B_{12} analogues synthesized by microorganisms usually differ from vit. B_{12}, by changes in the nucleotide portion of the molecule. In the case of factor B, no nucleotide is present(5). Vit. $B_{12_{III}}$, or factor III, has a 5-hydroxybenzimidazole moiety replacing 5,6-dimethylbenzimidazole of vit. B_{12} (6-8). Factor B has been found to be inactive in treatment of pernicious anemia(9) and in supporting growth of chicks(9,10), whereas the 5-hydroxy analogue produced a hematological response in patients with pernicious anemia(11,12) and had about 4% of the activity of vit. B_{12} as determined by growth of chicks(10).

In the present investigation the vit. B_{12} activities of acetato-, chloro-, nitro-, and sulfatocobalamin, factor B, and the 5-hydroxy analogue were determined in the young chick.

Methods. Female New Hampshire chicks from a commercial hatchery were distributed into groups of 6 chicks each and maintained on the experiment from 1 to 28 days of age. The chicks were weighed at weekly intervals; diet and water were offered *ad lib.* The chicks were fed a corn-soybean oil meal diet high in fat, which was identical to that used to evaluate the activity of desdimethyl B_{12}(13) except that 1 mg 2-methyl-1, 4-naphthoquinone was added per kg of diet. The analogues were dissolved in ethyl alcohol and appropriate aliquots were added to the diet or diluted with water for parenteral administration.

Results. The 4-week weights of chicks receiving graded levels of vit. B_{12}, acetato-, chloro-, nitro-, and sulfatocobalamin are presented in Table I. At each level of analogue, growth was similar to that with the same level of vit. B_{12}. In only one instance, that of 10 γ nitrocobalamin/kg, was a significant difference seen, and this was borderline. In none of the individual experiments was it possible to demonstrate significant differences between analogue and vitamin. In these experiments each compound elicited similar growth responses when it was injected as when it was fed at the same level in the diet.

In Table II, the activities of factor B and the 5-hydroxy analogue are compared with that of vit. B_{12}. The 5-hydroxy analogue had about one-tenth to one-third the activity of vit. B_{12}. Factor B did not significantly affect growth, although the 4-week weight of chicks receiving 10 γ factor B/kg of diet was somewhat lower than that of the basal group.

Discussion. The good growth of chicks obtained when the cobalamins were fed in the diet indicates that presence of cyanide in the vit. B_{12} molecule is not necessary for maximal utilization in this species. These results differ from the findings of Rosenblum *et al.* (3,4), who demonstrated that more of an oral test dose of cyanocobalamin was absorbed

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than that of the other cobalamins they studied. It is possible that the cobalamins may be converted in the chick's gut to some form which is more easily absorbed or these differences may reflect a basic difference in the absorption mechanism of the chick as compared with man or the rat. Evidence for species specificity of intrinsic factor-binding and subsequent absorption of vit. B_{12} has been demonstrated(14). It does not seem likely that the different experimental approaches, chick growth *vs.* the Schilling test, could account for the differences observed.

From the studies reported to date, vit. B_{12} analogues that support growth of the chick have also been active in patients with pernicious anemia. The young chick is therefore a very useful assay organism for anti-pernicious anemia activity, since microorganisms lack this specificity of response.

Summary. Activity of various vit. B_{12} analogues was determined in young chicks fed a corn-soybean oil meal diet high in fat. The presence of cyanide in the molecule is not necessary for full vit. B_{12} activity in the chick since acetato-, chloro-, nitro-, and sulfatocobalamin had activities equal to that of vit. B_{12} when fed or injected. Factor B had no vit. B_{12}-like activity nor any antagonistic action. The 5-hydroxy analogue of vit. B_{12} had about one-tenth to one-third of the activity of vit. B_{12}.

TABLE I. Growth of Chicks Fed Graded Levels of Vit. B_{12} and Some of Its Analogues (6 Chicks Started per Experimental Group).

	γ of supplement/kg of basal diet	No. of chicks*	Avg wt in g at 4 wk and S.E.
Vit. B_{12}	0	37 (5)	167 ± 9
	5	28 (2)	233 ± 12
	5 inj.†	9 (3)	231 ± 12
	10	23 (1)	248 ± 9
	10 inj.	18 (0)	257 ± 10
	100	42 (0)	290 ± 6
Acetatocobalamin	10	11 (1)	255 ± 12
	10 inj.	10 (2)	241 ± 10
	100	18 (0)	297 ± 10
Chlorocobalamin	5	24 (0)	236 ± 11
	5 inj.	22 (2)	242 ± 13
	10	23 (1)	260 ± 9
	10 inj.	23 (1)	265 ± 10
	100	17 (1)	286 ± 13
Nitrocobalamin	5	22 (2)	230 ± 12
	5 inj.	24 (0)	257 ± 14
	10	23 (1)	284 ± 11‡
	10 inj.	24 (0)	259 ± 10
	100	11 (1)	284 ± 12
Sulfatocobalamin	10	18 (0)	258 ± 10
	10 inj.	18 (0)	282 ± 13
	100	18 (0)	285 ± 13

* No. in parentheses is No. of chicks that died.
† Inj. made 3 times/wk. Amount of compound inj. was equal to amt of same compound consumed during preceding 2-3 day period by group receiving indicated level in the diet.
‡ Wt significantly different ($p = <0.05$) from group receiving same amt of vit. B_{12}.

TABLE II. Growth of Chicks Fed Graded Levels of Vit. B_{12}, the 5-Hydroxy Analogue of Vit. B_{12}, and Factor B (6 Chicks Started per Experimental Group).

	γ of supplement/kg of basal diet	No. of chicks*	Avg wt in g at 4 wk and S.E.	Avg % change in wt†
Vit. B_{12}	0	26 (4)	167 ± 9	
	10	24 (0)	244 ± 8	46
	10 inj.‡	12 (0)	253 ± 8	52
	100	30 (0)	293 ± 9	76
5-hydroxy analogue	10	21 (3)	194 ± 11	16
	10 inj.	23 (1)	174 ± 12	4
	100	22 (2)	229 ± 10	37
	100 inj.	5 (1)	213 ± 18	28
Factor B	10	9 (3)	136 ± 13	−19
	10 inj.	9 (3)	149 ± 12	−11
	100	11 (1)	161 ± 13	− 4
	500	5 (1)	202 ± 7	21

* No. in parentheses is No. of chicks that died.
† Change in wt from basal group (no B_{12}).
‡ Inj. made 3 times/wk. Amount of compound inj. was equal to amt of same compound consumed during preceding 2-3 day period by group receiving indicated level in the diet.

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Effects of Unsaturated Fat on Serum Lipids in Idiopathic Hyperlipemia. (23266)

MARK ALLEN EVERETT, WALTER D. BLOCK, F. A. J. KINGERY, AND ARTHUR C. CURTIS
Department of Dermatology and Syphilology, Medical School, and Institute of Industrial Health, University of Michigan, Ann Arbor.

It has been known for some time that dietary fat influences serum lipid levels. Beveridge *et al.*(1) found that diets high in animal fat produced elevations in serum lipid levels, while diets high in fats of vegetable origin did not. Ahrens and his associates(2) also produced lower serum lipid levels with vegetable oils, especially corn oil. The serum lipid lowering effect of vegetable fat has been attributed by Kinsell(3) and more recently by Bronte-Stewart(4) to its higher content of unsaturated fatty acids. Bronte-Stewart(4, 5), in particular, has shown that the lipid lowering effect of administered fats in normal individuals may be directly proportional to the unsaturated fatty acid content of the diet. It was reported by Lever(6), and confirmed in this laboratory(7), that the intravenous administration of a cottonseed oil preparation (Lipomul I-V)* on some occasions led to a drop in serum lipids in patients with idiopathic hyperlipemia. Administration of the oral product (Lipomul Oral)* failed to lower serum lipids. Since the oral preparation is primarily cocoanut oil, containing almost no unsaturated fatty acids, this may explain the lack of success with the oral product. According to Deuel(8), the iodine number of cocoanut oil is 8-40, while that of cottonseed oil is 105-115.

Methods. Two patients with idiopathic hyperlipemia were placed on diets carefully controlled with respect to fat content. One patient, E.B., was a male 29 years old, the other, D.L., a male 31 years old. Neither patient was hospitalized during the course of the experiments. The following diets were used in this study: Diet I, uncontrolled with respect to fat content; and Diet II, which contained 120 g fat consisting of 50 g of highly purified soybean oil,† 30 g of soy phospholipid concentrate,‡ and 40 g animal fat. Diet III, administered to only one patient (D.L.) had no added soy phospholipid concentrate. This diet, which was isocaloric with Diet II, contained 50 g of soybean oil,† 40 g of animal fat, and 10 g of vegetable fat. The caloric content of all diets was 2400-2600. Serum lipid partitions were done on these patients on fasting blood samples taken

* Supplied by the Upjohn Co., Kalamazoo, Mich.

† Supplied by the Procter and Gamble Co., Cincinnati, O. This soybean oil had the following composition:

Iodine No.	131.9
Total fatty acids	95.9 %
Free " "	.0
OH number	1.6
Unsaponifiable	.5
Tocopherol	.12
Trans fatty acids	.2

Spectrographic analysis yielded the following values:

Saturated fatty acids	16.6 %
Oleic acid	21.2
Linoleic acid	50.4
Linolenic acid	7.7

‡ Purchased from Associated Concentrates, Woodside, L. I., N. Y.