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Effect of Dietary Fat on Requirement of Vitamin B_{12} by the Chick.* (22797)

M. R. SPIVEY FOX, LIGIA O. ORTIZ, AND GEORGE M. BRIGGS

National Institute of Arthritis and Metabolic Diseases, P.H.S., U. S. Department of Health, Education, and Welfare, Bethesda, Md.

The young chick's dietary requirement for vit. B_{12} may be influenced by several factors. That the hen's diet affects the vit. B_{12} requirement of her progeny was recognized even before isolation of the vitamin. The requirement of chicks from hens depleted of their vit. B_{12} stores has been shown by most workers to fall between 15 and 30 γ/kg diet(1-4). The vit. B_{12} requirement of chicks from hens fed typical breeder diets was determined by Davis and Briggs(5) to be 1.5 to 2 γ/kg of diet. Similarly, Miller, Norris, and Heuser (6) found that chicks with moderate stores of vit. B_{12} upon hatching required 1.25 γ/kg diet. These values are somewhat below 8 γ, the level that has been tentatively recommended by the Committee on Animal Nutrition of the National Research Council(7). The relationship between vit. B_{12} intake of the hen and requirement of the chick has been quantitated by Milligan, Arscott, and Combs (8). They found that chicks fed a basal corn-soybean oil meal diet (25% protein) require 27, 12, 3, and 0 γ vit. B_{12}/kg diet when the dam's diet contained 0, 4, 8, and 16 γ per kg, respectively.

The influence that certain nutrients of the chick's diet may have upon the requirement of vit. B_{12} has been reviewed by Smith(9). Spivey, Briggs, and Ortiz(10) reported that a severe vit. B_{12} deficiency could be produced in undepleted chicks by the addition of 20% fat to a corn-soybean oil meal diet. The present paper deals with the increase in the vit. B_{12} requirement of chicks receiving this high fat diet.

Methods. The chicks were from a commercial hatchery. They were distributed into groups of 6 chicks each when 1 day old and were kept on the experimental diets for a 4-week period. The chicks were maintained in standard electrically heated batteries with screen wire floors. Feed and water were supplied *ad libitum* and total amount of diet consumed by each group during the experiment was recorded. The chicks were weighed at weekly intervals, and the final weight was the chief criterion of response used in evaluating the results of the experiments. The diets were similar to those used previously(10,11). Diet C29 had the following composition/kg: soybean oil meal 350 g, ground yellow corn 575 g, glucose 10 g, corn oil 5 g, chick salts A 60 g(12), riboflavin 8 mg, vit. D_3 0.02 mg, and 2-methyl-1, 4-naphthoquinone 1 mg. The small amounts of glucose and corn oil served as vehicles for the vitamins. Diet C30 was identical with diet C29 except that 200 g of lard were substituted for an equivalent amount of corn in a kg of diet. The total fat content of diet C29 was approximately 3%, by calculation, and that of C30, 22%. The vit. B_{12} content of each diet was varied in these experiments at levels between 5 and 2,000 γ/kg of diet. For parenteral administration, an aqueous solution of the vitamin was injected subcutaneously in the abdomen

* Part of these data was presented at 39th Meeting of Fed. of Am. Soc. for Exp. Biol., San Francisco, Apr. 11, 1955.

TABLE I. Effect of Dietary Fat on Growth of Chicks Fed Varying Levels of Vit. B_{12}.

Vit. B_{12} in diet (γ/kg)	Diet C29 (3% fat)		Diet C30 (22% fat)	
	No. sig. experiments/ total No. experiments*	4-wk wt (g)	No. sig. experiments/ total No. experiments*	4-wk wt (g)
0	/6	274 ± 7‡	/12	162 ± 5‡
5	1/2	334 ± 13	0/ 6	191 ± 9
5, inj.†	1/1	318 ± 12	2/ 3	226 ± 10
10	1/1	363 ± 19	7/ 8	259 ± 7
10, inj.†	—	—	3/ 3	257 ± 10
20	1/2	321 ± 11	3/ 3	269 ± 12
50	—	—	4/ 5	282 ± 10
100	3/6	343 ± 7	12/12	292 ± 5
200	—	—	3/ 3	291 ± 14
2000	0/1	326 ± 13	3/ 3	302 ± 8

* No. of experiments in which growth was significantly greater (at 1 or 5% level of probability) than that of the control group receiving no vit. B_{12}. There were 6 chicks per experimental group.

† Vit. B_{12} inj. 3 times weekly in the same quantity as that eaten by respective control groups.

‡ Mean ± S.E.

3 times weekly. The vitamin was injected in the same quantity as the mean amount consumed in the diet by the control chicks since the time of the previous injection. At the end of the 4-week experiments, the chicks were killed by decapitation, the livers were removed, and pooled by groups for vit. B_{12} assay. The analyses were carried out according to the USP XV procedure(13) using *Lactobacillus leichmannii.*

Results. The effect of graded levels of vit. B_{12} upon growth of chicks fed either of the 2 diets may be seen from the data presented in Table I. With diet C29, 5γ vit. B_{12}/kg of diet promoted optimal growth in all tests. In some experiments, the chicks grew maximally even in the absence of dietary vit. B_{12}, so the requirement with this diet always fell between 0 and 5 γ vit. B_{12}.

With diet C30, the growth responses to various levels of vit. B_{12} were markedly different from those with diet C29. As reported previously, growth with this diet was always poor in the absence of vit. B_{12}(10). The 5 γ level failed to improve growth significantly in 6 experiments. With increasing additions of vit. B_{12} up to 50 and 100 γ/kg of diet, growth progressively improved to a maximal level. Considerable variation was observed between experiments in the growth response to vit. B_{12}. Although the details are not presented in Table I, 100 γ vit. B_{12}/kg of diet supported growth that was significantly better than that with 50 γ in 3 of 5 experiments where these two levels were fed (average 4-week weight 304 and 261 g, respectively; $p = < 0.05$). In the 2 other experiments maximal growth was attained at 4 weeks with 50 γ vit. B_{12}. Addition of vit. B_{12} in quantities greater than 100 γ/kg of diet resulted in no further improvement in growth. The requirement under these conditions, therefore, falls between 50 and 100 γ/kg of diet.

From the growth data in Table I, it appears that with the low level of 5 γ vit. B_{12} in diet C30, the injected chicks grew better than the controls receiving the vitamin in the diet. When only the weights of chicks are considered from the three experiments in which the vitamin was both fed and injected, the chicks receiving 5 γ vit. B_{12}/kg of diet averaged 171 g ± 10 and the injected groups averaged 226 g ± 10 ($p = < 0.01$). At the lowest part of the vit. B_{12} growth curve, small amounts of the vitamin resulted in appreciable increments in weight gain; therefore, the slight increase in activity due to injecting the vitamin could be demonstrated at this suboptimal level. With 10 γ vit. B_{12}/kg of diet C30 and 5 γ in diet C29, no differences in growth were noted between groups of chicks receiving the vitamin by mouth or by injection.

The livers from most of the groups represented in Table I were analyzed for vit. B_{12}. For ease of presentation, the values obtained

TABLE II. Storage of Vit. B_{12} in Livers of Chicks Fed Varying Levels of Vit. B_{12} in Low and High Fat Diets (6 Chicks/Group).

Vit. B_{12} in diet (γ/kg)	Liver wt (g)	Vit. B_{12} in liver		Vit. B_{12} intake, avg/ chick/4 wk (γ)	Intake stored in liver (%)
		mγ/g	γ/liver		
Diet C29 (3% fat)					
0	7.6	15	.11	.0	
5	8.2	40	.33	2.95	7
5, inj.*	8.0	50	.40	2.95	10
10	9.0	105	.94	6.35	13
100	8.8	605	5.32	62.40	8
2000	7.3	820	5.98	1108.00	0.5
Diet C30 (22% fat)					
0	3.6	35	.13	.0	
5	5.7	35	.20	1.84	4
5, inj.*	6.4	50	.32	1.84	10
10	7.0	70	.49	5.00	7
50	6.5	160	1.04	19.80	5
75	6.8	265	1.80	31.72	5
100	5.7	440	2.51	42.20	6
200	6.8	540	3.67	888.00	0.4
2000	7.6	780	5.93	912.00	0.6

* Vit. B_{12} inj. 3 times weekly in the same quantity as that eaten by respective control groups.

in one experiment only are presented in Table II. The changes demonstrated by these data are representative of all experiments. In the absence of dietary vit. B_{12}, the amount of vitamin in the liver was essentially the same for the 2 diets. Also, with each diet, increasing levels of vit. B_{12} in the diet resulted in increased concentrations of the vitamin in the liver. This progressive increase in liver stores was more pronounced with diet C29 than with diet C30, because the average 4-week consumption of vit. B_{12} was slightly higher with diet C29 at each level of dietary vit. B_{12}. The proportion of the intake retained in the liver was essentially the same up to the 100 γ level with each diet. In general this percentage was slightly lower with diet C30 than with diet C29. With both diets, the extremely high level of 2 mg vit. B_{12}/kg of diet resulted in very high concentrations in the liver, but this represented a very small proportion of the intake. Similarly, 200 γ/kg of diet C30 resulted in a low rate of storage. The injected vitamin appeared to be stored slightly better than that fed in the diet.

Discussion. Incorporation of 20% fat into a corn-soybean oil meal diet not only increases the severity of a vit. B_{12} deficiency in non-depleted chicks but also increases the requirement for vit. B_{12}. This increase is 10 to 20 times the requirement of control chicks fed the low fat diet (3%) and about 2 to 4 times the requirement reported by others for depleted chicks. The high level of fat did not result in depletion of the animal's chief store of the vitamin, since the content of vit. B_{12} in the liver was similar for chicks fed the high and the low fat diets with no dietary source of vit. B_{12} for 4 weeks. Also, the fat did not prevent the normal absorption of vit. B_{12} from the intestinal tract and its subsequent storage in the liver.

Since vit. B_{12} becomes a critically limiting nutrient when the fat content of this diet is increased, these studies suggest a possible role for vit. B_{12} in the utilization of dietary fat. However, the chicks fed diet C30 did not have any apparent difficulty in metabolizing the large amount of dietary fat, even in the absence of vit. B_{12}. All livers appeared normal upon gross observation; the concentrations of water, total lipid, and phospholipid of the livers fell within normal ranges (unpublished data). Extraction of the feces from chicks fed this diet indicated almost complete absorption of the fat in the diet.

It is possible that the effect of fat upon the chick's requirement for vit. B_{12} is indirect and may be mediated through some other nutrient.

The substitution of 20% lard for an equivalent amount of corn increased the caloric density of the diet by approximately 40% and the ratio of calories to dietary protein by about 50%. The carbohydrate content of the diet was similarly reduced. These altered relationships may have been involved in the increased requirement for vit. B_{12} observed with the high fat diet.

Further discussion of the mechanism by which the high fat diet increased the chick's vit. B_{12} requirement will be considered more appropriately in a subsequent report on the "sparing" effect of certain nutrients upon vit. B_{12} under these conditions.

The graded growth response to varying levels of vit. B_{12} which has been obtained, makes diet C30 suitable for assessing the vit. B_{12} activity of concentrates or analogues for the chick. This diet has proven very useful in our laboratory for this purpose(11). The addition of other nutrients, such as methionine or choline, may alter the chick's response to vit. B_{12}, therefore, this diet cannot be used to evaluate the vit. B_{12} activity of low potency materials.

Summary. Incorporation of 20% fat into a corn-soybean oil meal diet increased ten to twenty-fold the vit. B_{12} requirement of non-depleted chicks for optimal growth at 4 weeks of age. The requirement was between 0 and 5 γ per kg of diet (3% fat) and between 50 and 100 γ with the high fat diet. The high dietary fat did not deplete the chick of its vit. B_{12} liver store or alter the absorption of dietary vit. B_{12} and its subsequent storage in the liver.

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Effects of Hypoxia on Iron Absorption in Rats. (22798)

PHILIP S. VASSAR* AND DAVID M. TAYLOR (Introduced by D. H. Copp)
Radiobiological Research Unit, Institute of Cancer Research, Royal Cancer Hospital, London, England.

Many studies have shown that gastro-intestinal absorption of iron is increased, in experimental animals(1,2,3) and human subjects(4), during many diverse types of anemia. Granick(5) has suggested that accompanying hypoxia is probably the only common factor capable of increasing iron absorption.

The present experiments were designed to test the hypothesis that hypoxia is capable of increasing iron absorption without the complicating presence of anemia or dietary changes.

* Present address: Department of Pathology, Faculty of Medicine, University of British Columbia, at Vancouver General Hospital, B.C.