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Effect of Major Dietary Constituents on Growth Response of Rats to Vit B_{12}.* (24206)

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Hartman *et al.*(1) reported that high protein rations produced a deleterious and sometimes fatal effect in Vit. B_{12} deficient rats. Bosshardt *et al.*(2) found that rations high in fat had a sparing action in mice upon the requirement for Vit. B_{12}. McCollum and Chow(3) reported that casein rations high in carbohydrate increased the Vit. B_{12} deficiency in female rats. The purpose of this study was to determine which of the 3 basic dietary constituents—fats, carbohydrates, and proteins—would produce the greatest growth response to Vit. B_{12} when plant protein rations were used.

Method. Adult female Sprague-Dawley rats (100 days old) were placed on a corn-soybean ration(4) at mating and maintained on this ration during pregnancy and lactation. The young had access to this ration until they were placed on experimental rations at weights of 40 ± 5 g. The care and handling of animals have been described previously(4). The rations (Table I) were adjusted so that the same percentage of calories was derived from protein in the high carbohydrate and high fat rations. Protein was isocaloric in the high protein and high protein-high fat rations. All animals received, in addition, 2 drops of Halver Oil weekly.

Results. The results are tabulated in Tables II and III. The differences in growth between the supplemented and unsupplemented rats on the high carbohydrate and high fat rations were not great. Similar results were also obtained in rats on the low protein diet in Series I. Growth differences obtained between supplemented and deficient animals fed the high protein and high protein-high fat diets were in every instance highly significant ($P<0.01$). Large variations in growth occurred with these diets with the greatest variation in the non-supplemented groups.

TABLE I. Composition of Rations.

Ingredients	High carbohydrate	High fat	High protein, high fat	High protein	Low protein
Drackett protein*	24.	35.	66.5	54.	16.2
Corn starch	62.5	16.5		32.5	70.5
Hydrogenated fat†		35.	20.		
Corn oil	5.	5.	5.	5.	5.
Salts XIV‡	4.	4.	4.	4.	4.
Vit. mix§	2.	2.	2.	2.	2.
Alphacel‡	2.	2.	2.	2.	2.
Methionine	.5	.5	.5	.5	.5
Vit. B_{12} mix‖ when indicated	.1	.1	.1	.1	.1

* Drackett Assay Protein G-1 81% protein (N × 6.25). † Crisco. ‡ Nutritional Biochemicals. § *J. Biol. Chem.*, 1954, v206, 765. ‖ 5% 0.001 Triturate B_{12} (Merck), 95% sucrose.

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TABLE II. Effect of Diet on Growth Response of Rats to Vit. B_{12}.

Ration	Avg growth in g, 5 wk						Diff. in g	
	$-B_{12}$			$+B_{12}$				
Series I ♂								
High Carbohydrate	161	10.4*	(8)†	183	4.0	(7)	22	10.5‡
Fat	177	8.8	(8)	212	4.1	(7)	35	9.7
Protein	117	12.4	(8)	180	5.2	(7)	63	13.4
Protein, High Fat	93	10.4	(8)	174	5.7	(7)	81	11.9
Low Protein	164	3.4	(10)	176	2.2	(10)	12	4.0
Series I ♀								
High Carbohydrate	106	5.3	(8)	119	3.4	(7)	13	6.3
Fat	114	5.6	(8)	131	4.0	(7)	17	6.8
Protein	96	7.8	(8)	122	3.4	(7)	26	8.5
Protein, High Fat	89	13.7	(8)	132	4.8	(7)	43	14.5

* Stand. error of mean. † Figures in parentheses indicate No. of animals. ‡ Stand. error of diff.

TABLE III. Effect of Diet on Growth Response of Male Rats to Vit. B_{12}.

Ration	Avg growth in g, 5 wk						Diff. in g	
	$-B_{12}$			$+B_{12}$				
Series II								
High Carbohydrate	132	2.9*	(6)†	147	2.9	(6)	15	4.1‡
Fat	178	9.9	(6)	187	6.4	(6)	9	11.8
Protein	124	13.8	(6)	172	4.5	(6)	48	14.5
Protein, High Fat	149	9.4	(6)	185	7.4	(6)	36	12.0
Series III§								
High Carbohydrate	100	3.0	(6)	113	4.8	(6)	13	5.6
Fat	106	4.0	(6)	115	4.8	(6)	9	6.2
Protein	56	5.5	(6)	89	5.9	(6)	33	8.1
Protein, High Fat	73	6.0	(6)	95	1.9	(6)	22	6.2

* Stand. error of mean. † Figures in parentheses indicate No. of animals. ‡ Stand. error of diff. § Three wk growth period in Series III.

Discussion. The greater growth differences observed in rats on high protein diets were in accord with the work of Hartman *et al.*(1). Numerous reports have implicated Vit. B_{12} in protein metabolism(5,6). In recent studies a decreased incorporation of labeled amino acids into protein was observed in Vit. B_{12} deficient animals(7). On a growth basis it appears that rats on adequate protein intake (high carbohydrate and high fat diets) utilized protein almost to the same extent with or without Vit. B_{12}. Since the lack of Vit. B_{12} was more detrimental to growth of rats on high protein rations, it apears that this vitamin plays a role in the utilization of excess protein for non-protein functions.

The fat sparing action found in mice by Bosshardt *et al.*(2) and in rats by McCollum and Chow(3) was seen in Series II and III; however, high levels of fat appeared to increase the deficiency in rats fed the high protein rations in Series I. Spivey *et al.*(8) found that high fat diets increased the Vit. B_{12} deficiency in chicks. McCollum and Chow(3) and Cuthbertson *et al.*(9) demonstrated that high carbohydrate diets produced a greater Vit. B_{12} deficiency in rats than high protein or high fat diets. In our work, less growth difference was observed with rats on high carbohydrate rations. These contrasting results might be explained on the basis of different type rations used, and the relative degree of coprophagy practiced. It has been shown that the nature of the ration probably has an influence on the intestinal synthesis of Vit. B_{12} and thus on the benefits of coprophagy(10). Barnes *et al.*(11) reported that the production of Vit. B_{12} deficiency was closely related to the extent of coprophagy practiced by the rat.

Summary. 1) Rats born to dams maintained on Vit. B_{12} deficient diets from mating

to weaning were fed rations high in carbohydrate, fat, protein, and protein and fat with and without Vit. B_{12}. 2) The greatest degree of deficiency was consistently observed in rats on high protein and high protein-high fat rations. Since Vit. B_{12} increased ability of rats to utilize excess protein, one of the important functions of this vitamin appears to be the metabolism of excess amino acids for non-protein functions. 3) Differences in growth on various diets, however, may be due to degree of intestinal synthesis of Vit. B_{12} and extent of coprophagy.

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Cytopathogenic Effects of Hemadsorption Virus Type I.* (24207)

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Several viruses causing human disease induce formation of large multinucleated cells in tissue culture preparations. These include measles(1), mumps(2), croup associated virus(3-4), and 2 recently described myxoviruses. Chanock, *et al.*(5), have termed these latter hemadsorption viruses Types I and II. They were isolated from children with respiratory illnesses and appear distinct from previously described viruses. Attention was called to the fact that hemadsorption virus Type I has been isolated from uninoculated HeLa cell cultures. The unique cytopathogenic changes caused by this virus in HeLa cell cultures have been followed by time-lapse phase-contrast cinemicrography.

Materials and methods. Hemadsorption virus Type I (Mill's strain) was obtained from Dr. Wallace Rowe following its isolation from uninoculated HeLa cultures. The virus pool for these studies was second passage LoHi‡ virus titering approximately 10^5 $T.C.D._{.50}$ per ml. Photographic chambers were constructed by attaching #1 cover slips on each side of perforated stainless steel slides. The top cover slip was placed slightly eccentrically, leaving a small opening at one edge for access to the chamber. This was subsequently sealed with a drop of paraffin. A suspension of small clumps of HeLa cells in Eagle's basal medium, 5% calf serum, 5% cord serum, penicillin 100 units and streptomycin 100 μg/ml, was introduced into the chamber which was then sealed and inverted until cell attachment occurred. These preparations were carried on growth medium until colonies reached optimal size for photography, at which time the growth medium was removed, the cells washed twice with buffered

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‡ Designates a stable cell line established from explants of a lymph node from patient with rheumatoid arthritis. The strain grows on glass in an epitheloid pattern with a mean generation time of 24 hours. The viral spectrum is similar to that of HeLa. Tumors have been produced by inoculation of irradiated cortisone treated mice.