The Assay of Vitamin B_{12}

7. Effect of Dietary Lactose and of the State of Maternal Nutrition on the Growth Response of the Rat to Vitamin B_{12}*

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Vitamin B_{12} improves the growth of rats maintained on diets of vegetable origin (Emerson, Wurtz & Zanetti, 1949). The effect is greater in animals from parents fed on such diets based on soya with adequate added minerals and vitamins (Emerson *et al.* 1949; Dryden, Hartman & Cary, 1949; Bosshardt, Paul, O'Doherty, Huff & Barnes, 1949). Addition of lactose to the diet depresses the growth of rats (Ershoff, 1949); the effect may be overcome by the administration of vitamin B_{12} (Hartman, Dryden & Cary, 1949).

The work described here was carried out during development of a procedure for assaying vitamin B_{12} on rats, and is concerned with the effects of dietary lactose and of the nutrition of the mother on the response of young rats to vitamin B_{12}.

EXPERIMENTAL

Animals. Albino rats of the WAG strain (Bacharach, Cuthbertson & Thornton, 1949) were used throughout. In tests to determine the growth-promoting effect of vitamin B_{12} equal numbers of weanling male and female animals, weighing about 30 g, were used. The mothers of the experimental animals were from the breeding colony and had been maintained on stock diet RBSS 10*a* (Table 1) with weekly supplements of cabbage and 3 g liver, or had been placed on the soya-glucose deficient diet RB 12 S/2 (Table 1) before mating. The effect of vitamin B_{12} supplements on growth rate was studied in normal stock weanlings and in first, second and third litters from does maintained on the soya-glucose diet.

Maintenance of animals. The mothers were housed in wooden breeding cages with wood-wool litter; food and water were unrestricted.

The young animals to be used in the growth experiments were weaned at 21 days, and were subsequently maintained in wire cages on screen bottoms, two animals in each cage, for 28 days. During this period their supply of water and experimental diet was unrestricted. The diets used were one or other of the soya-diets RB 12 S/2 and RB 12 S/4 (Table 1).

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Part 1. Cuthbertson, W. F. J. & Lloyd, J. T. (1951). *J. gen. Microbiol.* **5**, 416.
Part 2. Lees, K. A. & Tootill, J. P. R. (1951). *Biochem. J.* **50**, 455.
Part 3. Cuthbertson, W. F. J., Pegler, H. F. & Lloyd, J. T. (1951). *Analyst*, **76**, 133.
Part 4. Emery, W. B., Lees, K. A. & Tootill, J. P. R. (1951). *Analyst*, **76**, 141.
Part 5. Cuthbertson, W. F. J., Pegler, H. F., Quadling, C. & Herbert V. (1951). *Analyst*, **76**, 540.
Part 6. Harrison, E., Lees, K. A. & Wood, F. (1951). *Analyst*, **76**, 696.

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Procedure with experimental animals fed on the soya-glucose diet RB12S/2. Weanling rats from mothers on normal stock diet were used and also animals from the first, second and third litters of mothers maintained on the soya-glucose diet RB12S/2. The animals were divided into groups, each containing equal numbers of males and females, and were dosed with vitamin B_{12} by stomach tube twice a week. Observations were made for 28 days.

Table 1. *Percentage composition of the diets*

Stock diet RBSS 10*a*

Wholemeal wheat flour	75
Full-cream, roller-dried milk	20
Dried yeast	5

Supplement of liver, 3 g, and green leaves, 3 g/week

Soya diets ...	RB12S/2	RB12S/4
Full-fat soya flour	70	72
Glucose	24	—
Lactose	—	22
Salts (Hubbell, Mendel & Wakeman, 1937)	4	4
SKADE 3*	2	2
Vitamin supplement†		

* SKADE 3 (fat-soluble vitamin solutions):

Vitamin A concentrate	2×10^5 i.u.
Calciferol	0·25 mg (10^5 i.u.)
α-Tocopheryl acetate	14·2 g
Menaphthone	100 mg
Arachis oil to	1000 g

† Vitamin supplement/kg diet (mg):

Inositol	220	Aneurin HCl	30
Nicotinic acid	100	Riboflavin	30
Ca-D-pantothenate	100	Pyridoxin HCl	8
p-Aminobenzoic acid	75	Folic acid	1

Biotin 0·2

Procedure with experimental animals fed on the soya-lactose diet RB12S/4. In these experiments the weanlings were obtained from the stock colony or from mothers maintained on the soya-glucose diet. The weanlings were themselves maintained on the soya-lactose diet, but were otherwise treated in the same way as the animals that in the preceding experiment had been fed on the glucose diet.

RESULTS AND DISCUSSION

Soya-glucose diet. The growth increments found are summarized in Table 2, which shows the growth also of normal animals on stock diet under comparable conditions. The figures show that normal weanling rats on the soya-glucose diet grew at a fair rate even in the absence of vitamin B_{12}, but that weanlings from mothers maintained on the soya-glucose diet grew poorly unless vitamin B_{12} was given. It can be seen also

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Table 2. *Mean weight increases (g) of groups of weanling rats maintained for 28 days on a soya-glucose diet (RB12S/2) deficient in vitamin B_{12}, and given various supplements of vitamin B_{12}*

(Vitamin B_{12} was administered twice weekly by stomach tube)

Exp. no.	Origin of animals	No. of animals	Maternal diet	Supplement of vitamin B_{12} (μg/day) 0	0·04	0·08	0·16	0·32	0·64	1·0	2·5
1	Stock colony	8	Stock RBSS 10*a*	58·5	—	—	—	—	—	69·1	72·0
2	First litter	12	Soya-glucose RB 12 S/2	46·8	54·1	58·5	68·9	72·5	68·0	—	—
3*a*	Second litter	16	RB 12 S/2	48·5	50·3	53·4	50·3	59·0	—	—	—
3*b*	Second litter	8	RB 12 S/2	—	47·7	51·1	61·2	63·1	69·9	71·3	77·5
4	Third litter	6	RB 12 S/2	49·9	—	—	50·7	—	60·4	—	64·1
			Experimental animals fed on stock diet RBSS 10*a*								
5	Stock colony	8	RBSS 10*a*	—	—	—	—	—	—	72·0	—

After elimination of differences due to sex and treatment the standard deviation of an observation is $\pm$10·5 g, i.e. the standard error of the mean weight increase of a group of eight animals is $\pm$3·7 g.

Table 3. *Mean weight increases (g) of groups of eight weanling rats maintained for 28 days on a soya-lactose diet (RB12S/4) deficient in vitamin B_{12} and given various supplements of vitamin B_{12}*

(Vitamin B_{12} was administered twice weekly by stomach tube. Numbers in parentheses indicate the number of animals that died in each group)

Exp. no.	Origin of animals	Maternal diet	Supplement of vitamin B_{12} (μg/day) 0	0·04	0·16	0·64	2·56	5·0	7·5
6	Stock colony	Stock RBSS 10*a*	45·8 (1)	50·1	55·2	64·5	65·9 (1)	67·7	70·6
7	Stock colony	Stock RBSS 10*a*	36·9	—	—	—	—	—	—
8	First litter	Soya-glucose RB 12 S/2	25·1	32·2	37·0	46·0	48·2	54·2	—

After elimination of differences due to sex and treatment the standard deviation of an observation is $\pm$9·2 g, i.e. the standard error of the mean weight increase of a group of eight animals is $\pm$3·3 g.

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that in the absence of vitamin B_{12} the growth rates of animals from the first, second and third litters of mothers on the soya-glucose diet were about the same. The responses of the animals given different doses of vitamin B_{12} in Exps. 1 and 2 show that a normal growth rate could be achieved by administering daily from 0·16 to 2·5 μg vitamin B_{12}. Consequently, it may be concluded that the rats on the soya-glucose diet were deficient only in vitamin B_{12}.

Comparison of the responses of first, second and third litters to vitamin B_{12} in Exps. 2, 3*a*, 3*b* and 4 shows that the amount of vitamin B_{12} required for growth was greater the longer the mothers had been maintained on the deficient diet. Thus, for a growth increment of about 60 g in 28 days, supplements of 0·08, 0·32 and 0·64 μg vitamin B_{12} a day were needed by animals from the first, second and third litters, respectively. Exps. 2, 3*a*, 3*b* and 4 show that the slope of the curves relating the amounts of vitamin B_{12} given to the growth increments became less with successive litters from mothers on the deficient diet. The fact that successive litters responded progressively less well to vitamin B_{12} might have been due to a decrease in the animals' ability to utilize vitamin B_{12}. This decrease may itself have been due to a continuous decrease in the reserves of some other factor needed for the utilization of vitamin B_{12}.

For assay purposes weanlings from the first litters of mothers on the soya-glucose diet would appear to be the most suitable, for supplements of vitamin B_{12} restored the growth rates to normal, whereas the growth obtained in absence of vitamin B_{12} was small. The response of such animals was satisfactory, for a daily supplement of 0·16 μg vitamin B_{12} induced during the observation period a growth response exceeding by about 20 g that of rats receiving no supplement.

Soya-lactose diet. The growth responses to graded supplements of vitamin B_{12} are shown in Table 3. Comparison of Tables 2 and 3 shows that the growth of normal weanlings on the soya-lactose diet was somewhat less than that of animals on the soya-glucose diet and born of mothers maintained on that diet (Table 2, Exp. 2). Normal growth could be restored by supplying adequate vitamin B_{12}, but the amount needed by animals on the soya-lactose diet was much greater than by those on the soya-glucose diet; thus 7·5 μg vitamin B_{12} a day were needed for maximum growth of stock animals on the soya-lactose diet, but only from 0·16 to 0·3 μg for that of animals on the soya-glucose diet even when their mothers had been maintained on the deficient diet.

With the soya-lactose diet the offspring showed even more markedly the effect of their mother's having received the soya-glucose diet, as can be seen from Exp. 8 (Table 3). The first litters from mothers fed on the soya-glucose diet grew only 25 g in 4 weeks on the soya-lactose diet, and supplements of up to 5 μg vitamin B_{12} a day did not allow them to grow normally.

From these results it would appear that normal stock animals given the soya-lactose diet could be used for vitamin B_{12} assay. The low growth caused by this diet could be completely remedied by adequate supplements of vitamin B_{12}, but the animals were less sensitive to vitamin B_{12} than those fed on the soya-glucose diet after their mothers had been maintained on the same diet.

The growth responses of the male rats were significantly higher, by about 20 %,

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Table 4. *The effect of sex on the mean weight increases in g of groups* of rats fed on either diet RB12S/2 or RB12S/4 and dosed with varying amounts of vitamin B_{12} over a period of 28 days*

(Vitamin B_{12} was administered twice weekly by stomach tube)

Origin of animals	Parental diet	Experimental diet	Sex	Vitamin B_{12} (μg/day)								Standard deviation	Regression in bracketed range	Precision
				0	0·04	0·08	0·16	0·32	0·64	2·56	5·0	7·5	*s*	*b*	*b/s*
First litter	RB12S/2	RB12S/2	♂	51·7	[63·3	60·2	77·1]	75·6	73·5	—	—	—	±9·83 ($n'=29$)	+22·9	2·33
First litter	RB12S/2	RB12S/2	♀	43·3	[46·5	55·2	52·8	67·2	63·3]	—	—	—	±4·86 ($n'=23$)	+15·2	3·13
Stock colony	RBSS10*a*	RB12S/4	♂	43·5	[56·0	—	61·2	—	74·5]	73·3	74·2	73·6	±7·30 ($n'=20$)	+15·4	2·11
Stock colony	RBSS10*a*	RB12S/4	♀	51·3	[44·2	—	49·2	—	54·5]	60·2	62·2	65·8	±8·58 ($n'=21$)	+8·58	1·00

* Each group contained not more than seven and not less than three animals.

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than those of the female animals in the same experimental conditions. The different responses of male and female rats fed either the soya-glucose or soya-lactose diet, and given various supplements of vitamin B_{12} are demonstrated in Table 4. From these results it is clear that on either diet the vitamin B_{12} requirement of the female rat is greater than that of the male. The statistical data presented in Table 4 show that male and female rats should be satisfactory for assay purposes if an appropriate range of vitamin B_{12} concentrations is employed.

SUMMARY

1. Weanling rats were maintained on a soya-glucose diet deficient in vitamin B_{12}. They required more vitamin B_{12} for growth when their mothers had been maintained on the same deficient diet than when the mothers had been maintained on a stock diet.

2. The offspring of does on the deficient diet gave graded growth responses to graded doses of vitamin B_{12} when maintained on the deficient diet.

3. The growth of such animals could be restored to normal by supplements of vitamin B_{12}.

4. The response to vitamin B_{12} decreased in successive litters from mothers on the deficient diet.

5. When the deficient diet contained lactose instead of glucose the growth rate was depressed, but the effect could be overcome by means of vitamin B_{12}.

6. Normal stock rats given the soya-lactose diet showed graded growth responses to graded doses of vitamin B_{12}.

7. When the offspring of mothers maintained on the soya-glucose diet were given the soya-lactose diet, their growth was adversely affected to a greater extent than that of the offspring of mothers maintained on a stock diet.

8. The vitamin B_{12} requirement of male rats was greater than that of females.

9. It is concluded that rats reared to weaning on stock diet could be used for vitamin B_{12} assay if the soya-lactose diet were given, but for this purpose it would be preferable to use animals from the first litters of mothers maintained on the soya-glucose diet and themselves maintained on this diet.

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