Reprinted from ARCHIVES OF BIOCHEMISTRY
Vol. 27, No. 2, July, 1950
Printed in U. S. A.

Reproduction of Mice Kept on Rations Low in Vitamin B_{12}

Reproduction failures of rats and mice on synthetic or whole-plant rations have been described and attributed to a lack of animal protein factor (APF) or vitamin B_{12} (1–4). We have succeeded in breeding mice, kept on whole-plant rations, for several generations; nearly normal reproduction and mortality rates were observed although the animals showed symptoms of vitamin B_{12} deficiency.

The experimental diets contained 22–4% of crude protein (N × 6.25). Diet III had the following composition: expelled sesame-oil cake-meal, 46.5%; ground yellow corn, 46.5%; cottonseed oil containing 0.2% of percomorphum and 0.2% of wheat germ oil, 5%; U.S.P. salt containing 0.1% of $CoCl_2$, 2%; 10 B-complex vitamins as described (4). Albino mice were kept on a commercial stock ration, containing 24% of crude protein (13.5% of animal origin) prior to the start of the experiments. Pregnant females were put in individual screen-bottomed cages and given diet III. Litters were

TABLE I

Reproduction of Mice Kept on Different Diets

Diet	No. of litters	No. of litters weaned	Weight of young at 4 weeks	Weight change of mothers during lactation	No. of young dead between 2nd and 4th week
III, 1st generation	6	6	15.2	0	2
III, 2nd generation	10	9	9.2	0	5
III, 3rd generation	12	10	9.2	0.7	11
III, 5th generation	13	13	11.5	0	6
I, 2nd generation	9	8	10.3	−0.5	4
Control	10	10	15.4	0.5	4

TABLE II

Growth Response of Mice Raised on Diet III to Single Injections with Crystalline Vitamin B_{12}

No. of mice	Dose of vit. B_{12}	Wt. gain/day/animal, 2 weeks before injection	Wt. gain/day/animal, weeks after injection
	μg.	*g.*	*g.*
18	0.01	0.24	0.30
25	0.5	0.21	0.63

reduced to seven. The mothers were killed 7 weeks after the birth of the litters and these were kept together on diet III until females became pregnant; the mice were then separated and the same operation repeated. In the course of 18 months, five generations of mice were obtained by brother–sister breeding.

In a previous study, a very poor reproduction in rats and mice on a soybean–corn ration had been observed (4). Therefore, pregnant female mice, raised on diet III and born from mothers raised on diet III, were put on this soybean–corn ration (diet I) which differed from diet III in that sesame meal was replaced by commercial solvent-extracted soybean meal. The animals and their litters were treated as described and 3 generations were raised on this diet.

The young of the experimental groups grew more slowly than the controls but the mortality was nearly the same in both groups (Table I). Single intraperitoneal injections of 0.5 μg./animal of crystalline vitamin B_{12} (Merck) resulted in growth improvement for a period of 2 weeks, while 0.01 μg. was without effect (Table I). Kidney hypertrophy has been observed in rats deficient in vitamin B_{12} (5,6); the ratio between body weight and weight of the kidneys in mice raised on diet III in the 3rd and 4th generation was measured and found to be 50.1 ±5.2[1] in 55 adults weighing 20–32 g., while in 35 controls weighing 22–32 g. this ratio was 55.5 ±6.7.[1] (For difference between means, $p. < 0.01$.)

Diets I and III were analyzed with *Lactobacillus leichmannii* and found to possess 0.4 and 0.5 μg./100 g. of vitamin B_{12} activity, while with an assay procedure using *Lactobacillus lactis* Dornier, 0.7 and 0.4 μg./100 g., respectively, were found.[2]

These results indicate that mice were able to reproduce on diets low in vitamin B_{12}, according to microbiological assay, and insufficient to cause normal growth and to prevent kidney hypertrophy.

References

1. Van Landingham, A. H., and Lyon, H. B., *Arch. Biochem.* **13**, 475 (1947).
2. Zucker, T. F., and Zucker, L. M., *Arch Biochem.* **19**, 323 (1948).
3. Bowland, J. P., Ensminger, M. E., and Cunha, T. J., *Arch. Biochem.* **16**, 25 (1948).

[1] Standard error.

[2] The *L. leichmannii* assay was made by the Wisconsin Alumni Research Foundation, the *L. lactis* Dornier assay by Merck and Co., Research and Development Division. We are deeply indebted to Dr. H. B. Woodruff for this assay.

4. JAFFÉ, W. G., *Z. Vitamin —, Hormon —, Fermentforsch.* **2**, 493 (1949).
5. HARTMAN, A. M., DRYDEN, L. P., AND CARY, C. A., *Arch. Biochem.* **23**, 165 (1949).
6. ZUCKER, L. M., AND ZUCKER, T. F., *Arch. Biochem.* **16**, 115 (1948).

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Received March 16, 1950