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Effect of Various Dietary Supplements on Growth and Lactation in the Albino Mouse.*

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In the studies of the specific requirements of vitamins of the B complex for normal lactation it has been concluded that thiamin,[1] riboflavin,[2] pantothenic acid,[3] factor W,[4] the filtrate fraction,[5] and vitamins L_1 and L_2[6] are necessary for normal lactation in the rat. Some of the recently developed strains of brewer's yeast have been claimed good sources of all the above mentioned vitamins, and it is a well known fact that purified diets containing an adequate amount of yeast will provide for normal reproduction and lactation in the rat.

All of these studies have been conducted on rats and, while it is well known that mice are very difficult animals to raise, there is essentially nothing known about specific dietary factors needed for lactation in this species. The present communication is the first of a group of studies on specific dietary requirements of the mouse.

Four groups of 25 Strain A female mice each were placed at weaning on the purified diets A, B, C, and D shown in Table I. A fifth group was given a commercial food widely used in small animal colonies (Purina Fox Chow), and a sixth was fed this same commercial food together with daily supplements of fresh lettuce leaves and dried brewer's yeast. The 4 purified diets were made in such a manner that the protein, fat, and carbohydrate contents were the same for each diet. Furthermore, in the diets containing liver extract and dehydrated grass† (C and D) the amount of yeast was decreased so that with a margin of safety these diets did not contain

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1 Evans, H. M., and Burr, G. O., *J. Biol. Chem.*, 1928, **76**, 263.

2 Haussemann, D. L., and Hetler, R. A., *J. Nutrition*, 1931, **4**, 127.

3 Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 625.

4 Sure, B., and Beach, A., *J. Nutrition*, 1940, **19**, 57.

5 Morgan, A. F., and Sims, H. D., *Science*, 1939, **89**, 565.

6 Nakahara, W., Inukai, F., and Ugami, S., *Sci. Papers Inst. Phys. Chem. Research*, 1939, **36**, 327.

† We are indebted to the Cerophyl Laboratories for a generous supply of this material.

TABLE I.
Percentage Composition of Diets.

Ingredient	Experiment 1 A	B	C	D	Experiment 2 A	B	C	D
Glucose	34	34	34	30.5	19	19	19	15.5
Casein	31	31	31	28.5	28	28	28	25.5
Lard	20	20	20	19.5	19	19	19	18.5
Yeast*	8	8	6	5.7	8	8	6	4.7
Salt mixture†	7	7	7	5.8	6	6	6	5.8
Ascorbic acid		0.1				0.1		
Liver extract‡			2				2	
Dehydrated grass§				10				10
Wheat bran					20	20	20	20

*Anheuser Busch, Strain G.
†Hubbel, R. B., Mendel, L. B., and Wakeman, A. J., *J. Nutrition*, 1937, **14**, 273.
‡Liver fraction B, Wilson Laboratories, Chicago.
§Cerophyl, Cerophyl Laboratories, Kansas City, Mo.

more thiamin, riboflavin, or pantothenic acid than the basal diet A. The mice in the 6 groups were placed 5 to a cage. Those mice receiving the purified diets were given weekly supplements of 0.02 cc each of cod liver and wheat germ oil. Food consumption was measured daily and the mice were weighed every other day. At approximately 100 days of age the mice were bred and the resulting pregnant females were individually caged. Careful litter records were kept through 2 successful pregnancies. At the end of this period the diets were altered so as to contain 20% wheat bran (Table I, Experiment 2). Mice receiving these 4 diets were bred and litter records again kept through 2 successful pregnancies.

The growth rates of the mice receiving the 4 purified diets (Experiment 1) were definitely greater than mice on either the commercial food alone, or the commercial food supplemented with lettuce and yeast. Ascorbic acid in the pure form or as a component of the dehydrated grass had no effect on growth rate or food intake.

Litter records are presented in Table II. Several observations appear to be significant. The basal purified diet is definitely inadequate for lactation. In the absence of wheat bran addition of ascorbic acid or liver extract does not increase the survival of mice through the suckling period. Both grass and wheat bran materially improve the diet for lactation and their effects may be additive. The addition of both ascorbic acid and wheat bran to the basal diet seems to have a greater effect than wheat bran alone.

Wheat bran is very rich in phytin, and as this compound has recently been shown to be a dietary essential for the mouse,[7] feeding experiments with both inositol and phytin were carried out to see

[7] Wooley, D. W., *Science*, 1940, **92**, 384.

TABLE II.
Litter Record of Mice.

Group diet	No. litters	No. litters weaned	% litters weaned	No. mice born	No. mice weaned	% mice weaned	Avg No. mice per litter
		Experiment 1.					
A Basal	37	3	8.1	188	5	2.7	5.1
B " + Ascorbic acid	39	1	2.6	181	1	0.6	4.6
C " + Liver extr.	42	1	2.4	226	2	0.9	5.4
D " + Grass	41	12	29	240	45	18.8	5.9
Commercial diet	41	27	66	246	135	55	6.0
" " (supplemented)	39	32	82	247	155	63	6.3
		Exper. 2 (Added Wheat Bran).					
A Basal	31	11	35	142	33	23	4.6
B " + Ascorbic acid	29	11	38	116	42	36	4.0
C " + Liver extr.	41	13	32	217	44	20	5.3
D " + Grass	33	17	52	187	75	40	5.7
		Exper. 3 (Adults Started on Diets).					
Basal	17	7	41	88	22	25	5.2
" + Inositol	15	2	13	71	6	8.5	4.7
" + Phytin	15	3	20	86	8	9.3	5.7

if either of these substances was the active constituent of the wheat bran. Three groups of 10 adult female mice each were placed on the basal purified diet. One of these groups received 100 mg of inositol per 100 g of diet and a second group received 100 mg of phytin per 100 g of diet.

The weaning record of approximately 2 litters per mouse is shown in Table II, Experiment 3. Neither of the 2 supplements enhanced lactation. On the contrary, it would appear that both inositol and phytin in the amounts administered had a deleterious effect. In contrast to Experiment 1, the mice used in this study had been raised to maturity on the commercial diet and were then placed on the basal purified diet. They were bred immediately following the change of diet. It will be seen in Table II that the lactation record of the controls in Experiment 3 was superior to the controls in Experiment 1 that had received the same diet from the time of weaning. This is perhaps due to the storage of certain dietary essentials for lactation that were not entirely depleted during the period of gestation when the mice in Experiment 3 were receiving the inadequate basal diet.

It is well recognized that ascorbic acid is not necessary in the diet of the rat. This has been assumed to be true for the mouse, but Kleiner and Tauber[8] have reported a few observations suggesting that the addition of small supplements of ascorbic acid to the diet of mice results in an increased growth rate. Such an effect of ascorbic acid is not substantiated by the results of the present study.

[8] Kleiner, I. S., and Tauber, H., *Food Research*, **1936**, **1**, 399.

Essentially the only observation in the literature regarding specific vitamins necessary for lactation in the mouse is that of Nakahara, *et al.*[9] These investigators came to the conclusion that vitamins L_1 and L_2, which are supposed to be necessary for lactation in rats, are also necessary for mice but that mice are more resistant to the development of L_1 and L_2 deficiency than rats. Furthermore, they state that brewer's yeast is an adequate source of these two lactation vitamins. Contrary to the above mentioned investigation, the results of the present study show that diets containing 8% brewer's yeast are completely inadequate for lactation in the mouse. However, 2 supplements, namely dehydrated grass, and wheat bran will effectively increase lactation of mice raised on a purified diet containing brewer's yeast.

According to calculations of the amounts of thiamin, riboflavin, and pantothenic acid in the yeast (Anheuser Busch Strain G‡), dehydrated grass (cerophyl§), and wheat bran[10, 11] used in these studies, the level of these substances in the diets with dehydrated grass and wheat bran supplements was not higher than in the basal diet. It is concluded that some other component of grass and bran exerts the favorable effect upon lactation. This substance is not inositol or phytin, because when added these compounds did not improve lactation. Furthermore, as the addition of a liver preparation that has been used by other investigators as a potent source of factor W did not result in an increased lactation, it would seem probable that this factor is not of concern in interpreting the results of the studies presented here. It would appear likely that mice either require more of some lactation promoting substance than do rats, or that they require some substance not necessary in the diet in rats.

Summary. On the basal diets studied, ascorbic acid has been shown to have no influence on the growth rate of mice. Purified diets containing 8% yeast are not adequate for lactation in mice. Both dehydrated grass and wheat bran effectively increase lactation, but these two substances when added to a basal purified diet alone or combined do not provide for as good lactation as does a stock commercial diet. It has been concluded that the lactation-promoting effect of the dehydrated grass and wheat bran is not due to an increase

9 Nakahara, W., Inukai, F., Ugami, S., and Takahashi, K., *Sci. Papers Inst. Phys. Chem. Research*, 1939, **36**, 318.

‡ Personal communication from Anheuser Busch, Inc.

§ Personal communication from Cerophyl Laboratories.

10 Fixsen, M. A. B., and Roscoe, M. H., *Nutrition Abs. and Rev.*, 1937, **7**, 823.

11 Jukes, T. H., *J. Nutrition*, 1941, **21**, 193.

of thiamin, riboflavin, pantothenic acid, factor W, or inositol in the diet.

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Effects of Sulfanilamide, Sulfapyridine, Sulfathiazole and Sulfanilylguanidine upon Colon-Typhoid-Dysentery Group.

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Recent studies of Marshall, *et al.*,[1] indicate that sulfanilylguanidine compares favorably with the *in vitro* effects of sulfanilamide and sulfapyridine upon microörganisms, particularly those of the colon-typhoid-dysentery group. Following incorporation of sulfanilylguanidine in the diet of normal mice the *in vitro* effects were largely reproduced under *in vivo* conditions in that such medication caused a distinct reduction in the usual number of coliform organisms in the stools.

Since earlier observations[2, 3, 4] indicated that sulfathiazole was equal to, or slightly superior to, sulfanilamide and to sulfapyridine in its bacteriostatic action upon organisms of the colon-typhoid-dysentery group, it appeared worth while to further study the effects of sulfanilamide, sulfapyridine and sulfanilylguanidine upon additional organisms of the group mentioned and to compare them with sulfathiazole and sodium sulfathiazole under corresponding experimental conditions.

The *in vitro* test procedures used in evaluating the several compounds were, with slight modifications, those described by Marshall.[1] Small inocula (0.2 cc) containing 50 to 750 organisms of highly diluted 18-24-hour cultures in tryptone water, were exposed to 5 cc quantities of varying drug concentrations ranging from 100 to 200

[1] Marshall, E. K., Jr., Bratton, A. C., White, H. J., and Litchfield, J. T., Jr., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 163.

[2] Long, P. H., and Bliss, E. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 324.

[3] Lawrence, C. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 162.

[4] Libby, R. L., and Joyner, A. L., *J. Inf. Dis.*, 1940, **67**, 67.