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J. F. Feaster
Iowa State College

Victor E. Nelson
Iowa State College

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FURTHER STUDIES ON LACTATION AND REARING OF YOUNG

J. F. Feaster and Victor E. Nelson

The work performed in this laboratory and elsewhere during the past fifteen years has shown conclusively that a diet adequate for growth may not necessarily fulfill the requirements for lactation. The excellent work of Evans and Bishop (1) has conclusively demonstrated that a specific vitamin is required for reproduction. The fact that lactation is not normal on many synthetic and even natural diets suggests the possibility that all of the factors necessary for normal development are not known. The work in this paper was planned in order to increase our knowledge concerning the nutritional factors involved in milk secretion and rearing of young.

Experimental

All of the experiments were performed on rats. The first basal diet had the following composition: casein 18 per cent, salt mixture 185 (2) 3.7 per cent, filtered butter fat 4 per cent, cod liver oil 1 per cent, and dextrin to 100 per cent. This basal diet was fed to all females transferred from the stock ration to the experimental rations at the time of parturition. The second basal diet had the same composition as basal ration number one, except that one per cent wheat germ oil replaced an equivalent amount of dextrin; this basal diet was given to the animals which were carried through successive generations. The casein was prepared from the commercial product by leaching with water acidified with acetic acid; tests made on the animals showed this product to be free of vitamins B and G. Several experiments were performed in order to evaluate various methods for the removal of vitamins B and G from casein. The first method involved leaching with water acidified with glacial acetic acid, so as to make the concentration approximately 0.20 per cent of this acid. The liquid was drained through a cheese cloth and new portions of dilute acid added daily for a period of three weeks. The casein was dried at 85°C. The second method involved further treatment of the casein — purified by the first method — by means of extraction in a continuous extractor for 5 days with hot 60 per cent alcohol (by

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volume) acidified with acetic acid, so as to give a concentration of 0.33 per cent acid. In the third method the casein, prepared by the first method, was dissolved in water by the addition of NH_4OH; acetic acid was added to precipitate the casein, the liquid decanted, and the process repeated for a total of three times. This casein was then dried at 85°C. and extracted for 5 days with hot 60 per cent alcohol (by volume) containing acetic acid to the extent of 0.33 per cent. The final products from the three methods were dried at 85°C. The casein prepared by these three methods when subjected to biological assay was shown to be free of vitamins B and G. To the basal rations were added preparations of vitamins B and G — both separately and together — and the effects noted on lactation and growth.

Some of the results on these preparations are given in table I; basal ration number one was used for the experiments in this table. Fraction 8B is an activated fuller's earth preparation of vitamin B, which was shown by assay not to contain vitamin G. This preparation was made from rice polishings by the following method: four kilograms of rice polishings were stirred intermittently through the day in a battery jar with 95 per cent ethyl alcohol, containing acetic acid (30 ml. glacial acid to 10 liters of alcohol). After 24 hours the supernatant liquid was siphoned into another battery jar, containing four kilos of rice polishings. A battery of four jars was used, and a new jar of rice polishings added each day; and the extract from the new jar of the day before was removed and concentrated in vacuo, so that the temperature remained below 40°C. Fat was removed from the concentrate in a separatory funnel and the concentrate transferred to a flask and neutral 25 per cent lead acetate added to give the maximum precipitate. The lead was removed from the filtrate by means of 20 per cent H_2SO_4. The $PbSO_4$ was filtered through a No. 40 hardened filter paper. The acidity of the lead-free filtrate was adjusted to pH 4.0 to 4.5, seventy-five grams of fuller's earth for each 4 kilos of rice polishings were added, the acidity readjusted to pH 4 or 4.5, and the mixture stirred for 3 hours. The filtrate was again adjusted to a pH of between 4 and 4.5 and 20 grams of fuller's earth added for 4 kilos of rice polishings; following which the pH was again adjusted to between 4 and 4.5, and the mixture stirred for 3 hours. The combined preparations were first air dried and then dried further in a vacuum desiccator over $CaCl_2$; and they were finally dried in a vacuum oven at 40°C. for 12 hours. This preparation is known as the activated fuller's earth preparation and in the table is designated as A. F. E.

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H. L. P. (in Table I) refers to hog liver preparation. This preparation was prepared as follows: fresh hog liver was minced in a food chopper and then stirred into 2 liters of boiling water per kilo of liver. This mixture was boiled for 3 minutes and filtered through a Buchner; the insoluble material was washed with 1 liter of hot water per kilo of liver. The extracts and washings were concentrated under reduced pressure (temp. 50°C.) to a volume of 150 ml., cooled, and 850 ml. absolute alcohol added; the precipitate which formed was dissolved in water and dried on dextrin. One gram of preparation 11F corresponds to 3 grams of hog liver, and one gram of $11F_1$ corresponds to 6 grams of hog liver. D. H. L. refers to dried hog liver prepared by drying minced hog liver in an

Table I — Effect of Preparations of Vitamins B and G on Lactation

Lot No.	Sources of Vitamins B and G	No. of Litters	No. of Young	No. of Young Weaned	Average Weight at Weaning	Percentage Mortality
189	[1] A.F.E. 8B 0.053 g.	4	24	0	0	100
274	A.F.E. 8B 0.318 g.	12	72	50	20.5	30.5
294	A.F.E. 8B 0.318 g.	12	72	44	19.3	38.8
296	[2] H.L.P. $11F_1$ 1.66 g.	12	72	35	18.6	51.3
193	[3] D.H.L. 11A 15%	12	72	17	26.8	76.4
203	D.H.L. 11A 3.3%	6	36	1	18.0	97.2
202	Yeast Auto. 10%	6	36	12	17.5	66.6
192	A.F.E. 8B 0.053 g. D.H.L. 11A 0.20 g.	6	36	1	28.0	97.9
244	A.F.E. 8B 0.106 g. D.H.L. 11A 0.500 g.	10	60	34	30.7	43.3
245	A.F.E. 8B 0.212 g. D.H.L. 11A 0.500 g.	10	60	33	28.9	44.4
246	A.F.E. 8B 0.106 g. D.H.L. 11A 1.0 g.	10	60	4	29.0	93.3
269	A.F.E. 8B 0.212 g. D.H.L. 11A 1.0 g.	12	72	40	31.9	44.4
270	A.F.E. 8B 0.318 g. D.H.L. 11A 1.0 g.	12	72	58	45.2	19.4
272	A.F.E. 8B 0.318 g. Yeast Auto. 10%	12	72	60	35.2	16.6
273	A.F.E. 8B 0.318 g. H.L.P. 11F 1.33 g.	12	72	67	31.2	6.96
275	A.F.E. 8B 0.318 g. H.L.P. $11F_1$ 1.33 g.	12	72	51	34.1	29.2
295	A.F.E. 8B 0.318 g. H.L.P. $11F_1$ 1.33 g.	12	72	60	35.3	16.6
297	A.F.E. 8B 0.318 g. H.L.P. $11F_1$ 1.66 g.	12	72	66	39.3	3.3

oven at 80°C. Autoclaved yeast was prepared by moistening the yeast with distilled water to make a thick paste, autoclaving 5 hours at 15 lbs. pressure, and drying in an oven at 80°C.

The data in Table I are for the most part self-explanatory. The

1 A. F. E. refers to a preparation of vitamin B prepared by adsorption on activated fuller's earth.
2 H. L. P. refers to a preparation of vitamin B from hog liver.
3 D. H. L. is a dried hog liver preparation.

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Table II — Effect of Preparations of Vitamins B and G on Growth, Reproduction and Lactation

Lot No.	Sources of Vitamins B and G	No. of Litters	No. of Young left with mothers	No. of Young Weaned	Average Weight at Weaning	Per Cent Mortality	No. of Young Found Dead	No. of Females on Experiment
307	A.F.E. 8B 0.15 g. H.L.P. 11F$_1$ 1.0 g.	16	85	14	30.6	83.5	0	4
307 2nd gen.	A.F.E. 8B 0.15 g. H.L.P. 11F$_1$ 1.0 g.	7	38	24	32.5	37.0	4	3
307 3rd gen.	A.F.E. 8B 0.15 g. H.L.P. 11F$_1$ 1.0 g.	2	4	0	0	100	0	7
308	A.F.E. 8B 0.30 g. H.L.P. 11F$_1$ 2.0 g.	12	56	19	44.0	64.3	0	4
308 2nd gen.	A.F.E. 8B 0.30 g. H.L.P. 11F$_1$ 2.0 g.	18	87	19	43.7	78.1	7	7
308 3rd gen.	A.F.E. 8B 0.30 g. H.L.P. 11F$_1$ 2.0 g.	3	12	0	0	100	0	6
321	A.F.E. 8B 0.3 g. H.L.P. 11F$_1$ 2.0 g.	5	19	4	45.2	78.6	11	4
321$_a$	A.F.E. 8B 0.6 g. H.L.P. 11F$_1$ 4.0 g.	6	32	15	42.4	53.1	3	4

data in this table were obtained from pregnant females transferred from the growing ration at the time of parturition. The results show that a supply of either vitamins B or G failed to support lactation, indicating that neither one of these vitamins is stored in sufficient amounts by the females while on the growing ration to promote successful rearing of young, when the mothers are transferred to the experimental diet. The data, furthermore, show that dried hog liver, although rich in G, did not give good results for lactation on the levels used (3.3 and 15 per cent). The best results on mortality were obtained by combining the activated fuller's earth and hog liver preparations (lots 273 and 297). The best weaning weight is shown in lot 270, were the animals received activated fuller's earth preparation plus dried hog liver. In these experiments six young were given to each female.

Table II gives the data on the effect of combination of vitamin B preparation (A. F. E. 8B) and vitamin G preparation ($11F_1$) on growth, reproduction, and lactation. Basal diet number two was used in these experiments, and each female was given a maximum of six young to wean. The mortality on most of these rations was very high. On the lower levels of B and G preparations the weaning weights were low, whereas, on the higher levels of these preparations, the weights of the young at weaning were much greater. However, the weights of the young were less and the mortality greater than that obtained by supplementing natural foods with vitamin G preparation. This would seem to indicate that another factor aside from vitamins B and G is associated with lactation. Fraction A. F. E. 8B, at a level of 0.025 gm., plus 0.166 gm. of H. L. P. $11F_1$ gave normal growth; so that the lowest levels of these preparations recorded in Table II are six times the amount required for normal growth. The highest levels of preparations B and G recorded in Table II are 24 times the amount required for normal growth, and still lot 321_a showed a mortality of 53.1 per cent.

Summary

Preparations of vitamins B and G have been administered to lactating females and the effects noted.

Lactation was unsuccessful when a preparation of B or G was fed alone.

Combinations of vitamins B and G preparations gave better results than either one alone, and the lowest mortality of the young was obtained with a combination of activated fuller's earth preparation of B plus hog liver preparation.

The best results on weaning weight of the young was obtained

by a combination of activated fuller's earth preparation of B plus dried hog liver.

Data are also presented on the effect of B and G preparations on growth, reproduction, and lactation; and from these studies it seems very probable that a new factor may be necessary for lactation.

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DEPARTMENT OF CHEMISTRY,
IOWA STATE COLLEGE,
AMES, IOWA.