

powder as a curative procedure to infants on the third or fourth day of life. We found that 5-10 g, given in water, gave a prothrombin response equivalent to 0.5-1.0 μ g of the synthetic vitamin. If the dried milk be extracted with ether, a dose several times as large is required to give the same response. The ether-soluble extract from 10-15 g of dried milk is more than adequate to give this response.

Colostrum is consumed in small quantities during the first 3 days of life, and evidence indicates that it does contain small amounts of vitamin K. In 2 cases (19 and 22 of Table I), nursing problems were such that the infants received water, but neither colostrum nor glucose. Several others, shown in Table I, received glucose, but no colostrum. There is some variation in results, but the general trend is toward lower prothrombin levels. We believe that the colostrum contains enough vitamin K to account for this small difference.

Summary. The vitamin K requirement of the newborn infant is shown to be extremely low (approximately 1 μ g of synthetic vitamin a day). Milk contains enough preformed vitamin K to meet this minimal requirement.

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Absence of Supplementary Relationships in Requirements for Pyridoxin and Essential Fatty Acids.*

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A number of investigators¹⁻⁸ have been concerned with the question of a possible supplementary relation existing between pyridoxin and the essential fatty acids.

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The following materials were generously contributed: thiamin, pyridoxin and α -tocopherol, Merck and Co.; riboflavin, Hoffmann-LaRoche Co.; 92% liver extract, Eli Lilly and Co.; calciferol, Mead Johnson and Co.

¹ Richardson, L. R., and Hogan, A. G., *Univ. of Missouri Research Bull.*, 1936, No. 241.

² Quackenbush, F. W., and Steenbock, H., *J. Biol. Chem.*, 1938, **123**, 97.

³ Salmon, W. D., *J. Biol. Chem.*, 1938, **123**, 104.

⁴ Birch, T. W., *J. Biol. Chem.*, 1938, **124**, 775.

Recent experiments^{9, 10} have indicated that pyridoxin, pantothenic acid and linoleic acid (or arachidonic acid) are necessary for protection against dermatitis.

Experimental. Experiments were designed to test the possible sparing action of linoleic acid upon the pyridoxin requirement of rats and vice versa.

The diet employed differed from the usual fatty acid deficient ration in that the components of the vitamin B complex were supplied in crystalline form (except the filtrate fraction which was given as a liver filtrate). In the usual fatty acid free diet, the vitamin B complex is supplied in the form of an ether-extracted brewers' yeast. Three levels (low, intermediate and high) of an essential fatty acid (ethyl linoleate) were fed in conjunction with two levels of pyridoxin (see table I).

Female rats from different litters were segregated into the several groups. Each litter had a representation in the 3 control groups: namely, the fatty acid deficient, pyridoxin deficient, and high fatty acid-high pyridoxin (groups 1, 4 and 5). The rats were placed on diet 822 and supplements at weaning (21 days) when their average weight was 49 g.

Diet 822.	
Casein (alcohol: ether extracted ¹¹)	24
Sucrose	72
Salts (McCollum 185)	4

The following supplements were fed six times weekly:

Thiamin	15 μ g
Riboflavin	20 "
Liver filtrate ¹²	equivalent to 5 g fresh liver†
Carotene	80 μ g (fed in 2 drops of ethyl laurate)
Calciferol	12 IU (fed in 2 drops of ethyl laurate)
α -tocopherol	0.5 mg (fed in 2 drops of ethyl laurate)

The two levels of pyridoxin fed were 5 and 15 μ g (the daily re-

⁵ Quackenbush, F. W., Platz, B. R., and Steenbock, H., *J. Nutr.*, 1939, **17**, 115.

⁶ Oleson, J. J., Bird, H. R., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1939, **127**, 23.

⁷ Tange, U., *Sci. Papers Inst. Phys. Chem. Res., Tokyo*, 1939, **36**, 482.

⁸ Schneider, H., Steenbock, H., and Platz, B. R., *J. Biol. Chem.*, 1940, **132**, 539.

⁹ Richardson, L. R., and Hogan, A. G., *Proc. Am. Soc. Biol. Chem.*, 1941, 106.

¹⁰ Salmon, W. D., *Proc. Am. Soc. Biol. Chem.*, 1941, 109.

¹¹ Blumberg, H., *J. Biol. Chem.*, 1935, **108**, 227.

¹² Lepkovsky, S., Jukes, T. H., and Krause, M. E., *J. Biol. Chem.*, 1936, **115**, 557.

† In "filtrate factor" tests the equivalent of 10 g of fresh liver stimulated growth to the same extent as did the equivalent of 5 g.

quirement of the rat appears to be approximately 10 μg .^{13, 14}) Three levels of ethyl linoleate, 20, 40 and 80 mg, were given in conjunction with the 2 levels of pyridoxin.

Rats receiving the fatty acid deficient diet (Group 1) exhibited the clinical symptoms of fatty acid deficiency involving a plateauing in growth, scaliness, dandruff, etc. (the classical syndrome of Burr and Burr). 20 mg of ethyl linoleate 6 times weekly (Group 2) protected against the dermatitis, but growth was stimulated to a greater extent with 40 and 80 mg than at the lower level (Groups 3 and 4).

Rats receiving the same diet with 80 mg of ethyl linoleate 6 times weekly but deficient in pyridoxin (Group 5) showed the typical picture of pyridoxin deficiency, namely, subnormal growth and acrodynia (edematous paws, thickened ears, encrusted nose, etc.). Five μg of pyridoxin[‡] did not completely prevent the appearance of the dermatitis. Little difference was apparent between the animals receiving the 3 levels of ethyl linoleate (Groups 6, 7 and 8). Growth was limited by the low pyridoxin intake as was evidenced by the weights of the animals receiving the higher level of pyridoxin.

These results indicate that the two deficiencies are independent of each other. The types of dermatitis produced are easily distinguishable. The scaliness of fatty acid deficiency was observed on a diet

TABLE I.
Growth and Dermatitis in Relation to the Intake of Ethyl Lineolate and Pyridoxin.

Group	No. of Pyridoxin animals	Pyridoxin μg	Ethyl lineolate mg	Gain in wt, 150 days	Type of dermatitis
1. Fatty acid deficient, pyridoxin high	12	15	—	81	Scale
2. Fatty acid low, pyridoxin high	6	15	20	124	None
3. Fatty acid intermediary, pyridoxin high	6	15	40	136	"
4. Fatty acid high, pyridoxin high	12	15	80	145	"
5. Fatty acid high, pyridoxin deficient	12	—	80	72	Severe acrodynia
6. Fatty acid low, pyridoxin low	6	5	20	124	Mild "
7. Fatty acid intermediary, pyridoxin low	6	5	40	120	" "
8. Fatty acid high, pyridoxin low	6	5	80	115	" "

¹³ Dimick, M. K., and Schreffler, C. B., *J. Nutr.*, 1939, **17**, 23.

¹⁴ Reedman, E. J., Sampson, W. L., and Unna, K., *Proc. Soc. Exp. Biol. and Med.*, 1940, **43**, 112.

[‡] Reedman and coworkers¹⁴ found that 2.5 μg of pyridoxin prevented the dermatitis over a 12-week test period.

supplemented with 15 μ g of pyridoxin while the florid dermatitis (acrodynia) characteristic of pyridoxin deficiency was readily produced on a diet supplemented with 80 mg of ethyl linoleate.

Summary. In an attempt to disclose the possibility of a "sparing action" of fatty acids on pyridoxin deficiency, varying levels of ethyl linoleate were fed in conjunction with a subnormal and an optimal level of pyridoxin; the "sparing action" was not observed.

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Failure to Cure or Prevent Graying of Rats with p-Amino Benzoic Acid.*

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Ansbacher¹ has reported that p-amino benzoic acid would cure the characteristic graying of areas of the pelage of rats maintained on a vitamin B deficient diet supplemented with thiamin hydrochloride, riboflavin, pyridoxin hydrochloride, calcium pantothenate, nicotinic acid, inositol and choline chloride. On the appearance of this paper a considerable number of characteristically grayed rats were available to us for experimentation. They had been raised on a somewhat different ration than that employed by Ansbacher, for they received a vitamin B deficient diet supplemented with only three substances, to wit: thiamin hydrochloride, riboflavin and pyridoxin hydrochloride. They were divided into four groups as follows:

(1) Controls: Animals continued on the same diet and supplements which had produced the graying.

(2) Animals receiving daily, in addition to the 3 supplements mentioned, 100 μ g pantothenic acid.

(3) Animals receiving daily, in addition to the 3 supplements mentioned, 3 mg of p-amino benzoic acid.

(4) Animals receiving daily, in addition to the 3 supplements mentioned, both 100 μ g of pantothenic acid and 3 mg of p-amino benzoic acid.

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¹ Ansbacher, S., *Science*, 1941, **93**, 164.