

13741

Choline Deficiency in Rats of Various Ages.*

R. W. ENGEL. (Introduced by A. G. Hogan.)

From the Laboratory of Animal Nutrition, Alabama Polytechnic Institute, Auburn.

Griffith and Wade¹ demonstrated that weanling rats receiving diets deficient in choline developed a hemorrhagic kidney disease within 2 weeks. Later it was shown that this abnormality could be prevented by methionine,² or by betaine,³ as well as by choline. Engel and Salmon⁴ developed a diet which was sufficiently depleted of choline, and of other compounds behaving like choline, to produce 100% mortality in weanling rats. The same diet when supplemented with adequate choline produced good growth. Data are presented herein to show: (1) the approximate minimum requirement of choline for the prevention of the fatal hemorrhagic disease during the first 2 weeks after weaning, and (2) the need for dietary choline for the prevention of the hemorrhagic disease throughout the rapid growth period.

Method. Weanling rats were placed on the choline-deficient diet 31 PMC⁴ at 23 days of age. The rats were individually fed the diet *ad libitum*, and 20 mcg each of thiamin, riboflavin, and pyridoxine, and 100 mcg of calcium pantothenate per day; they were fed adequate amounts of carotene and calciferol once a week. Choline was fed as indicated later. Two groups of rats were retained on the stock diet for 3 and 5 weeks respectively before being transferred to the deficient diet.

The Approximate Minimum Requirement of Choline. Preliminary trials on a few animals had indicated that the approximate daily choline chloride requirement for the prevention of the fatal hemorrhagic conditions was 5 mg. The results of further studies on the minimum requirements of choline are presented in summary form in Table I. Male and female rats from 10 different litters received choline chloride at the 4 mg level. It is of interest that 75% of the fatal cases occurred among the rats from 3 litters, and that there were

* Published with the approval of the Director of the Alabama Agricultural Experiment Station.

¹ Griffith, W. H., and Wade, N. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **41**, 188.

² Griffith, W. H., and Wade, N. J., *J. Biol. Chem.*, 1940, **132**, 627.

³ Griffith, W. H., and Mulford, D. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **45**, 657.

⁴ Engel, R. W., and Salmon, W. D., *J. Nutrition*, 1941, **22**, 109.

TABLE I.
Weight Change and Survival in 23-day-old Rats Receiving Suboptimum Levels of Choline.

No. of rats	Sex	Choline chloride per rat daily, mg	Avg initial wt, g	Avg wt change 1st wk, g	No. surviving 2 wks	Avg wt change* 2nd wk, g	Avg wt change fatal cases 2nd wk, g	Survival %
19	M	4	44	16	11	15	—10	58
15	M	5	47	16	12	12	— 5	80
21	F	4	45	14	17	15	— 7	81
12	F	5	44	15	11	17	— 5	92

*Survivors only.

4 litters represented in which no mortality occurred. The litter variation was not correlated with variation in rate of growth up to the crucial 7- to 10-day period when the hemorrhagic condition developed. Rats from 8 different litters each received 5 mg of choline chloride daily. In this experiment 3 of the 4 fatal cases occurred in rats from one litter. At both levels of choline feeding the male rats were slightly more susceptible to the hemorrhagic disease than the females, a confirmation of the results reported by Griffith,⁵ and by Engel and Salmon,⁴ when the latter workers used diets less severely deficient in choline and in substances behaving like choline.

In view of individual variation, as well as litter variation, it is difficult to establish any exact minimum requirement of choline for the prevention of the fatal hemorrhagic disease. The data suggest that approximately 5 mg of choline chloride per rat daily are necessary for the prevention of the fatal hemorrhagic condition in most cases. Griffith⁶ has reported that for diets containing 18 to 24% of casein and 6% of yeast, 1 to 2 mg of choline chloride per rat daily are needed to prevent kidney hemorrhage. If the methionine supplied by this amount of casein and the choline supplied by this amount of yeast are taken into account, the results reported by Griffith are confirmed in the present experiments.

The Need for Choline Throughout the Rapid Growth Period. Since relatively large quantities of choline were required by the weanling rat for the prevention of the hemorrhagic condition during the first 2 weeks it was important to determine whether this condition could be produced if the rats were supplied with choline through the crucial 2-week period. To this end, weanling rats were fed diet 31 PMC and the usual supplements plus 20 mg of choline chloride per rat daily for 2, 3, 4, and 5 weeks respectively. After these

⁵ Griffith, W. H., *J. Nutrition*, 1940, **19**, 437.

⁶ Griffith, W. H., *J. Nutrition*, 1941, **22**, 239.

periods had elapsed the rats were continued on the same diet without choline and observations were made daily. The results definitely indicate that dietary choline is essential throughout the rapid growing period.

When 20 mg of choline chloride was fed for 2 weeks after weaning and the choline then discontinued, 3 out of 4 rats died with hemorrhagic kidneys between the ninth and the twelfth day. When the choline was fed for 3 weeks, 2 out of 4 rats died, one on the seventh and the other on the fourteenth day after choline was discontinued. Of the 12 rats fed choline for 4 weeks, 6 died of choline deficiency within the succeeding 14-day period, the mean number of days to exitus being 9. Three out of 7 rats which had received choline for 5 weeks died of choline deficiency within 14 days after choline feeding was discontinued.

The results indicate that the susceptibility of growing rats of either sex to choline-deficiency hemorrhagic disease remains practically unchanged between the fourth and the seventh week after weaning. In general, the rats which received choline over longer periods (4 or 5 weeks) usually suffered the greatest weight losses before death. In all but one of these fatal cases the body weight at death was less than it was when choline feeding was discontinued. This is particularly significant since such severe weight losses did not occur in choline-deficient rats during the crucial 7- to 10-day post-weaning period.

The surviving rats were continued on the choline-deficient diet for from 4 to 12 weeks. Their weight gains were very irregular. Eight of the 13 survivors had suffered weight losses during the 2-week period following the discontinuation of choline feeding and showed connective tissue scarring of the kidney cortex at necropsy, an indication that hemorrhage had occurred.

From the previous experiments it was apparent that if rats receiving diets low in choline were deprived of choline at any time during the rapid growing period, they were susceptible to the hemorrhagic kidney disease. It seemed desirable to determine what the result would be if rats were retained on the stock diet until varying weight levels had been reached before they were placed on the choline-deficient diet.

One group of 8 rats was kept on the stock diet for 3 weeks after weaning, and another group of 9 rats was kept on the same diet for 5 weeks. All of these rats were placed on the choline-deficient diet and received the usual vitamin supplements but no choline. Four of the 8 rats which had received the stock diet for 3 weeks after weaning had lost weight after 2 weeks on the choline-deficient diet. All of

these rats survived through the first 2 weeks and resumed growth during the third week. At autopsy 3 weeks later, one of the rats which had lost weight showed severe connective tissue scarring of the kidney surface.

Two of the rats which had received the stock diet for a 5-week period before being placed on the deficient diet lost weight during the second week of the experiment and both rats had connective tissue kidney scars at necropsy.

The data suggest that rats which were fed the stock diet during the preliminary feeding trial were somewhat less susceptible to choline-deficiency disease than those which were fed the choline-deficient diet supplemented with relatively high levels of choline.

Summary. Weanling rats receiving a diet severely deficient in choline required approximately 5 mg of choline chloride per rat daily to prevent the fatal hemorrhagic kidney disease. Choline deficiency was produced within 7 to 14 days at any time during the rapid growing period when choline was omitted from the diet. Weanling rats fed a stock diet for periods of 3 or 5 weeks were somewhat less susceptible to choline deficiency than rats fed the diet low in choline and supplemented with 20 mg of choline chloride daily for similar periods.

13742 P

Alterations in X-Ray Diffraction Pattern of Rat Tibia in Rickets.*

C. I. REED AND B. P. REED.

From the Department of Physiology, University of Illinois, Chicago Colleges.

The use of diffractograms in the study of molecular structure has been discussed extensively by Clark.¹ Numerous studies on bone by this technic have confirmed that there is a characteristic pattern most nearly agreeing with the type pattern of dahlite. Not many reports have given consideration to the possibility that this characteristic pattern may undergo physiological alteration. Roseberry, Hastings and Morse² described briefly some age changes in cultures of osseous

* A part of the expenses of this investigation were borne by a grant from the Nutrition Research Laboratories, part by a grant from the Graduate School Research Fund.

¹ Clark, G. L., *Applied X-Rays*, 3rd, New York, 1940, McGraw-Hill Book Co.

² Roseberry, H. H., Hastings, A. Baird, and Morse, J. K., *J. Biol. Chem.*, 1931, **90**, 395.