noted in monkeys as reported by Cooperman *et al.*[4]

Preliminary observation in Mink 1 at the present time indicates that an additional factor in fresh liver is required. After the response in body weight to folic acid therapy this animal lost 40% of the total body weight in a period of 3 weeks. Inositol, *p*-aminobenzoic acid, vitamin E and K at levels given in Table I were added to the ration with no response in body weight. The feeding of 10% fresh liver for 18 days resulted in a regain of 63% of the body weight loss; hemoglobin rose from 10.7 at the time of deficiency to 17.3 g per 100 cc of blood.

The possible role of biotin in the nutrition of the mink is still obscure. A response to biotin in Mink 2 was obtained. However, further efforts to produce an uncomplicated biotin deficiency has been unsuccessful to date.

The role of *p*-aminobenzoic acid, inositol, α-tocopherol and 2-methyl-1,4-naphthoquinone has not been established in our preliminary work.

The practical aspect of these studies indicates the importance of considering the folic acid content of mink rations. Further studies as to the requirement of the known members of the vitamin B complex and additional factors in relation to growth, maintenance, reproduction, and development of fur are necessary.

Summary. 1. Purified rations have been successfully employed in studying nutritional requirements of adult mink. 2. Feeding highly purified vitamin B complex free rations of varying protein levels supplemented with thiamine, riboflavin, niacin, pyridoxine, pantothenic acid and choline produced a severe deficiency in 10 to 13 weeks. The characteristic symptoms are given. 3. The administration of synthetic folic acid to 8 mink at the time of severe deficiency symptoms resulted in an immediate recovery, whereas 3 mink not receiving folic acid died. 4. Preliminary observation indicates the existence of another factor present in liver distinct from the known vitamins which is seemingly necessary for maintenance of body weight and hemoglobin regeneration.

We wish to acknowledge our indebtedness to Merck and Co., Rahway, N. J., for the crystalline vitamins; to Wilson Laboratories, Chicago, Ill., for the various liver preparations; to Dr. B. L. Hutchings of Lederle Laboratories, Inc., Pearl River, N. Y., for the synthetic folic acid.

[4] Cooperman, J. M., Elvehjem, C. A., McCall, K. B., and Ruegamer, W. R., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 92.

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Toxicity of Choline in the Diet of Growing Chickens.

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Choline has a wide potential use in rations for poultry. Because of the probability of this substance's being fed in amounts greater than needed for maximum benefits, it is desirable that information on the toxicity of the compound for poultry be obtained.

One of the authors (P.B.P.) found that an oral dose of 5 g was fatal to 5 kg rabbits within 30 minutes, whereas with rats, Hodge and Goldstein[1] found that 6.7 g per kg body weight given by stomach tube to rats starved for several hours "represented the LD_{50} for albino rats."

Hodge[2] later reports the results of supply-

[1] Hodge, H. C., and Goldstein, Max R., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 281.

[2] Hodge, H. C., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 212.

TABLE I.
Effect of Dietary Level of Choline on Growth of Chickens.

	Series 1				Series 2	
Lot No.	1	2	3	4	5	6
% choline added	0.05	1	2	4	0.05	1
Avg gain in g*	1201	1043	1035	915	1089	972
Index of gains, controls 100	100.0	86.8	86.2	76.2	100.0	89.2

* Avg of mean gains of males and females.

ing varying percentages of choline chloride in the feed and in the drinking water of rats over a 4 months' period. Amounts up to 1% in the feed did not retard growth, but the inclusion of 1% or more in the drinking water resulted in reduced growth rates. In his study Hodge was unable to attribute any consistently abnormal histopathological findings to the effect of the choline chloride, and indications were that where the larger percentages of choline were used the resultant retarded growth rates were due to limited intakes of feed and water in the respective series of the experiment.

In the past the possible toxicity of choline to chickens has been given but little consideration. However, the authors, in blood choline studies, had previously fed mature hens about 3% of choline chloride over a 3 months' period and had observed a decline in egg production and a partial molt, the new feathers being curled, and presenting an overall frizzled appearance.

In the present studies the effect of different levels of choline chloride in the diet on the growth and fat deposition in chickens was determined. In the report of this work the terms choline and choline chloride are used interchangeably.

Experimental. One-week-old hybrid chicks (New Hampshire Red × White Leghorn) were divided into 8 even-weight lots of 16 birds each. They were reared according to standard procedure, and the experiment covered a period of 11 weeks. The experiment was conducted with 2 series of diets. The basal rations contained 0.05% of choline chloride which is considered adequate to meet the needs for maximum growth. The basal ration used in the first series was a general starter type ration with slight modifications in the second series. Choline chloride was added to the experimental diets at levels of 1%, 2% and 4% in the first series and 1% in the second series.

The results obtained, as summarized in Table I, show that with increasing levels of choline in the diet there is a retardation of the rate of growth. The addition of 1% choline brought about decreases in gains of 13.2% and 10.8% respectively in the 2 series. The addition of 2% and 4% of choline to the diet resulted in decrease in the rate of gain of 13.8% and 23.8% respectively as compared with the gains made on the basal ration.

The data in Table I have been treated statistically by taking the gains for the males and for the females in each lot. The significance of the difference in the mean gains between the males on the basal ration (lot 1) and the males in lot 2 receiving 1% of choline has been tested according to Fisher's t value. Likewise the t value for the significance of the means between the males in lot 1 and the males in lot 3, and the males in lot 4 have been calculated. Similarly the t values were calculated for the females between lot 1 and each of the other lots. The significance of the difference in mean gains for the females between lots 5 and 6, and for the males of the 2 lots, was also tested. The addition of 1% or more of choline to the basal ration produced a significant ($P = 0.05\%$) decrease in the rate of gain for the males, while in the case of the females the decrease in rate of gain was significant at the 2% level of choline and highly significant at the 4% level of choline in the diet. The decrease in rate of gain for the females in the 1% level of choline was not significant, but on the basis of the average t value for males and females on this level the difference becomes significant at this level in both series I and II.

Other than the reduced rate of gains no

gross pathological manifestations were observed as a result of the inclusion of amounts of choline chloride up to 4% of the ration.

Four representative specimens from each lot were slaughtered, and showed appreciable differences in body fat deposits. Carcasses of chickens fed the diet with 4% added choline had considerably less subcutaneous, abdominal and mesenteric fat than those fed the 1% and 2% levels, which in turn had less fat deposits than chickens receiving 0.50% of choline in the diet.

Summary. Various levels of choline chloride were added to rations fed to growing chickens. The addition of 1%, 2% and 4% reduced the rate of gain by about 12%, 13.8% and 23.8% respectively. As much as 4% of choline chloride added to the diet produced no toxic manifestations other than retarded growth.

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Ulnar-Femoral Nerve Anastomosis in Paraplegic Rhesus Monkey.*

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In seeking a method of bridging the gap in neural continuity caused by transection of the spinal cord, the following experiment was carried out.

The left ulnar nerve of a rhesus monkey was dissected from the axilla to the forearm and transposed subcutaneously to the left flank. The left femoral nerve was then exposed through a retro-peritoneal approach, and divided close to its origin where its proximal end was sutured to the distal end of the transposed ulnar nerve. For the sake of convenience, the left forelimb was then amputated at the shoulder.

Five and one-half months later the spinal cord was transected at D 4. Following transection it was evident that struggling movements resulted in active flexion of the left thigh despite the atrophy which had followed femoral nerve section.

Faradic stimulation of the anastomosed ulnar-femoral nerve through the intact skin, both above and below the point of nerve suture elicited contractions of the ipsilateral psoas and vastus muscles (innervated by the sutured femoral nerve).

Ten days after cord transection, the right motor cortex was exposed under ether anesthesia, and stimulated electrically. Stimulation of the exposed leg area, as expected, yielded no response. Stimulation of the forelimb area, however, with the same strength of current, elicited contractions of the contralateral (left) shoulder muscles and also simultaneous contractions of the left psoas and vastus muscles, innervated by the anastomosed femoral nerve. An ensuing generalized convulsive seizure involved not only the musculature above the level of the cord transection but also the above-mentioned muscles of the left thigh, which were the only muscles that participated in the convulsion below the level of cord transection. These movements were phasic and coordinated with the phasic movements of the upper extremities.

Electrical stimulation of the segments of the cervical cord giving rise to the transposed ulnar nerve yielded contractions of the ipsilateral thigh muscles while stimulation of the cervical cord elsewhere at the same current strength failed to do so.

On exposing the anastomosed ulnar-femoral nerve, it was found that direct electrical stimulation of the nerve both above and below the point of suture elicited flexion of the left thigh. There was no evidence that stimulation of the skin (or other structures) or of the anastomosed femoral nerve itself, distal to the suture line, caused a pain reaction. Microscopic studies of the anastomosed

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