seconds in dog 5. Dog 5 then developed ventricular fibrillation and died. In order to observe what might occur if epinephrine is inadvertently injected into the intraspinous ligaments instead of intrathecally, 1 mg was injected at this site in dog 6. A-V nodal rhythm, ventricular premature beats appeared 30 seconds later.

Summary and Conclusions. Epinephrine was administered intrathecally to 5 dogs anesthetized with cyclopropane. The characteristic changes which occur after intravenous epinephrine is given did not occur. At the end of one hour epinephrine was still present in sufficient quantities in the spinal fluid to cause disturbances in cardiac rhythm when the spinal fluid was withdrawn and given intravenously. Presumably the passage of epinephrine from the intrathecal space into the blood stream does not occur or occurs so slowly that it is of little consequence in dogs anesthetized with cyclopropane. The same is not true if epinephrine is given intravenously or into the interspinous tissues.

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Further Studies on Nutritional Requirements of the Cotton Rat. (*Sigmodon hispidus hispidus*).*

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Previous studies[1] showed that the cotton rat (*Sigmodon hispidus hispidus*) requires thiamin, riboflavin, pantothenic acid, vitamin B_6, nicotinic acid and inositol in the diet for optimum growth. Since these animals are very susceptible to dental caries[2,3] and are also susceptible to virus infections[4-6] it was of interest to extend the results obtained on their nutritional requirements. Studies have been carried out on the rate of growth obtained when diets low in choline, biotin, folic acid or nicotinic acid were fed. Preliminary studies on the reproduction performance have been conducted with the use of purified diets with and without the addition of liver extract.

Experimental—Care of Animals. Weanling cotton rats, 15-25 g in weight, obtained from our stock colony, were used in the growth studies. Litter mates and males and females were distributed as uniformly as possible among the groups within each experimental series. The animals were housed singly in screened bottom cages and food and water were provided *ad libitum*. The rate of growth for the different groups was observed over a period of 4-6 weeks.

In the reproduction studies one male and 1 or 2 females were housed in a colony cage with the screened bottom removed. Wood shavings and a metal can were included in each cage. The animals were placed together at an early age, usually before they were 6

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weeks of age, since when adult animals are placed together, a high mortality often results. The animals usually remain in the can during the day, a factor which reduces the excitability of the animals considerably. Examinations were made for litters and the number of young counted, but the litter was disturbed as little as possible. The male was left with the female and several pairs have consistently produced a litter every month. The young were weaned at 14-17 days of age, sexed and weighed. Data were obtained, therefore, on the number of young born, number weaned, weight of young at weaning and the number of litters per female. Greater difficulties are inherent in obtaining data on reproduction as complete as can be obtained with the white rat. Discussions of methods of care and handling of cotton rat colonies have been reported.[1,7,8]

Composition of Diets. The basal ration used in the growth studies was composed of the following: (in %) sucrose 67, purified casein 24, mineral mixture 4, corn oil 4.7, vitamin A & D conc. 0.3 and the following B vitamins (per 100 g) thiamin 250 μg, riboflavin 300 μg, pyridoxine 250 μg, Ca pantothenate 2 mg, nicotinic acid 2.5 mg, choline 100 mg, inositol 100 mg, *p*-aminobenzoic acid 30 mg, biotin 10 μg and pteroylglutamic acid 200 μg. The vitamin deficient diets were prepared by omitting the appropriate vitamin from the B vitamin mixture.

This ration was modified for the reproduction studies by increasing the amount of casein to 30% and the vitamin A & D concentrate to 0.6% at the expense of the sucrose. The amounts of the B vitamins added were doubled with the exception of nicotinic acid, choline, inositol and *p*-aminobenzoic acid. For some groups 4% of 1:20 liver extract was added at the expense of the entire ration.

Results and Discussion. Growth studies. A severe choline deficiency could not be consistently produced either when the basal diet without added choline previously described was fed or when a peanut meal ration[9,10] low in choline and methionine was used. When animals with an initial weight of 15 g were used, more failed to survive than when larger animals were used. A total of 5 animals out of 24 fed the deficient diets died within the first 2 weeks on experiment. The average growth rates of the surviving animals were somewhat lower than for the groups fed 100 mg of choline chloride per 100 g of ration in addition to the basal ration. The variation was rather great, and although the results did not warrant extensive studies on the quantitative requirements, they appear to be qualitatively similar to those obtained with the white rat.

In previous studies[1] crystalline biotin and pteroylglutamic acid were not available. The present studies showed that the omission of either or both these vitamins did not appreciably affect the rate of growth. Eight to 19 animals were used in each group and the average rate of growth per week obtained when both vitamins were included was 10.0 g, biotin omitted 9.7 g, pteroylglutamic acid omitted 9.2 g and both omitted 9.4 g. Therefore it is concluded that dietary supplements of these factors are not needed when tested with the purified diet described. In accord with previous work[1,11] the addition of liver extract to the ration increased the rate of growth.

Other findings revealed that dietary tryptophan was an effective precursor of nicotinic acid for the cotton rat as well as other animals.[12,13] Consequently, studies were conducted to determine the effect of the amount of casein (tryptophan) in the diet on the dietary requirement of nicotinic acid. From the results shown in Table I, it can be seen

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TABLE I.
Effect of the Level of Casein in the Diet on the Growth Response With and Without Nicotinic Acid Supplementation.

Level of casein fed %	Rate of gain* g/wk —N. A.	Rate of gain* g/wk +N. A.	Comments on group receiving the —N. A. diets
12	1.7 (10)	5.6 (7)	5 dead at 4 wks
18	5.7 (16)	7.1 (12)	4 " " 6 "
24	8.7 (7)	8.0 (6)	0 " " 6 "

* The supplemented groups received 1.0 mg nicotinic acid/100 g ration. The number of animals in each group is given in parentheses.

TABLE II.
Reproduction Studies.

Dietary regimen	No. of pairs	Total No. of litters	Avg No. young per litter	Avg wt of young and age at weaning*
Stock†	5/6‡	25	4.5	21.5 (14.6)
Basal	2/5	2	2.0	20.0 (14)
Basal — biotin or pteroylglutamic acid	2/11	2	1.0	None weaned
Basal + 4% 1:20 liver extract	5/7	17	2.8	22.1 (14.9)

* The average age at weaning is given in parentheses.
† Rockland rat pellets, complete.
‡ No. of pairs producing young/total No. of pairs in the group.

that the response due to nicotinic acid supplementation decreased as the casein level was increased. In previous work an 18% casein diet was used, and although nicotinic acid was again shown to increase the rate of growth, this effect disappeared when 24% casein was fed.

Reproduction Studies. Some of the animals that were used in studying the effect of ingesting diets with and without the addition of biotin and pteroylglutamic acid on the rate of growth were used for reproduction studies. One male and one female were placed in colony cages after 6 weeks on experiment and fed the basal diet described for reproduction studies with the appropriate vitamin omitted. The reproduction performance of these animals was compared with that for pairs that received stock ration or the basal ration + 4% 1:20 liver extract. These tests have been conducted over a 9 months period and are summarized in Table II. Data obtained on animals from pairs that died within the first 6 months of this period were not considered in these tabulations. It can be seen that the purified diets which contained pteroylglutamic acid and biotin or only one of these supplements failed to support successful reproduction. Only 4 pairs of a total of 16 produced young, and each of these pairs only produced one litter which was also small in size. Since inadequate reproduction was obtained when both pteroylglutamic acid and biotin were included, the data obtained do not permit an accurate evaluation of the essentiality of either biotin or pteroylglutamic acid.

The addition of 4% of 1:20 liver extract to the basal diet did improve the reproduction performance, however. Five of a total of 7 pair produced young and 2 of the pairs have produced 5 and 6 successive litters when this diet was fed. The litters were smaller in size, however, than those that were obtained from pairs fed stock ration. These data suggest, therefore, that liver extract contains factors which are necessary for reproduction in the cotton rat, but that the size of the litters is still not optimal. It is possible that the addition of cellulose to the purified rations may be beneficial since Howell and associates[14] have recently demonstrated that the addition of 10% cellulose to a purified diet prevented trichobezoar in the cotton rat and

[14] Howell, S. R., Schlack, C. A., McCay, C. M., and Taylor, B. L., *Science*, 1948, **107**, 424.

increased the rate of gain. A variable accumulation of hair in the stomach was also observed in the present study for stock pairs fed purified diets. Further work is needed to determine the value of adding cellulose to the ration on the reproduction performance.

The weight of the young at weaning for both sexes was similar, *i.e.* 20.5 for 32 males and 21.0 for 44 females from a pair fed stock ration. It can be seen from Table II that the weaning weights of young were similar for the groups receiving different diets. This observation cannot be accurately evaluated, however, since smaller litters were weaned by the groups fed purified diets, and thus would result in a variable stress of lactation.

It is recognized that the purified diets used favor the development of dental caries and this may have affected the reproduction performance. The animals ate the rations readily, however, and reached a mature weight approximating that obtained when stock ration was fed. A few pairs were fed starch as the carbohydrate, but their performance did not appear to be improved over that for the groups fed sucrose diets.

Summary. The cotton rat (*Sigmodon hispidus hispidus*) was shown not to require a dietary source of biotin or pteroylglutamic acid for optimum growth when a purified ration was fed. The growth response obtained with nicotinic acid supplementation was dependent on the casein (tryptophan) content of the diet in that as the casein level was increased the growth response attributable to the addition of nicotinic acid was decreased. A poor reproduction performance was observed when purified diets were fed. Improvement was noted when liver extract was fed in addition to the purified diet or when stock ration was fed.

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Pyridoxine, Choline and l-Methionine Content of Parenteral Liver Extract.

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In the nonspecific use of parenteral liver extract, the physician is not much interested in a statement of antianemia potency as he is in knowing the amounts of the several biologically active substances (factors of the B complex) present. Numerous requests for such information have made it necessary to analyze the various types of liver extract. The advent of the several microbiological assays made it possible to determine the amounts of several of the B factors. A previous article[1] contains data for total solids, total nitrogen, riboflavin, niacin, pantothenic acid, and folic acid (*L. casei factor*) present in several samples of refined, concentrated extracts and in the dilute, crude preparations. In this brief paper, data is presented for the pyridoxine, choline, and *l*-methionine content of twelve different commercial extracts.

Materials and Methods. The liver extracts, conforming to U.S.P. standards, included in this report represent random samples purchased from a wholesale druggist and unselected samples of our own products. The total amounts of pyridoxine, choline, and *l*-methionine extracted from whole liver by hot, acidified water (pH 5.0—85°C) are shown, numbers 34 and 35 in Table I. The method of Hochberg, Melnick, and Oser[2] has been used for the determination of pyridoxine. The microbiological method described by Stokes, *et al.*[3] has been used for the estimation of *l*-methionine. Choline has

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