due to coramine was not prevented by the addition of choline or methionine. The fact that a combination of lipotropic factors and high protein diet was not capable of preventing the abnormal liver growth would suggest that this action of coramine is not due to any decrease in factors necessary for transmethylation but to a specific action of the coramine molecule.

Summary. 1. The effect of the ingestion of a series of pyridine derivatives by immature rats on a high protein diet is described. 2. None of the N-methyl derivatives of this series had any appreciable influence on either the fat content or the absolute liver weight. 3. Gamma picoline, nicotinic acid, and nicotinamide produced slight increase in liver fat; beta picoline and coramine produced significant increases in liver fat. In the case of beta picoline and coramine this increase in fat could be prevented by the inclusion of 1.2% methionine or 0.5% choline. The effect of methionine or choline on the metabolism of the other compounds reported was not determined. 4. Coramine produced a great increase in the fat-free liver weight which could not be prevented by the addition of choline or methionine to the diet. 5. The growth inhibition following the administration of beta picoline or coramine was not appreciably affected by the addition of choline or methionine to the high protein diet.

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Growth and Reproduction in Rats on Synthetic Rations.*

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Introduction. It is now generally recognized that rats grow at a rapid rate when given synthetic diets containing only 6 B vitamins. When sulfonamides are added, the growth is greatly retarded but the effect is counteracted by the addition of folic acid and biotin. Ransone and Elvehjem[1] reported that certain folic acid concentrates from liver produced a growth response beyond that which could be attributed to the known vitamins present. Recently evidence has been presented for the presence in fresh liver and other materials, of a factor necessary for continued growth and a normal blood picture in the monkey.[2] Further work has shown the existence of an unknown factor(s) in liver which stimulates the growth of *S. faecalis* when this organism is cultured on a "complete" synthetic medium.[3,4] The present work was undertaken to determine the effect upon growth and lactation of rats receiving a purified ration containing 10 crystalline B vitamins.

Experimental. Male weanling rats (Sprague-Dawley) weighing between 38 and 45 g were used in all growth experiments. They were housed in individual cages and were fed the

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1 Ransone, B., and Elvehjem, C. A., *J. Biol. Chem.*, 1943, **151**, 109.

2 Cooperman, J. M., Ruegamer, W. R., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 101.

3 Cooperman, J. M., Ruegamer, W. R., Snell, E. E., and Elvehjem, C. A., *J. Biol. Chem.*, 1946, **163**, 769.

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TABLE I.
Effect of Liver Supplements on the Rate of Growth in Rats.

Group	Period wk	Supplement	Avg gain per wk g
		Basal ration	
1	4	None	29
2	4	5 cc raw milk per day	33
3	4	0.5 g " liver " "	36
4	4	3% lyophilized liver (Squibb)	38
5	4	1% 1:20 liver powder (Wilson)	35
		Basal ration + double level of B vitamins + cystine	
6	2	None	31
7	2	0.5 g raw liver per day	34
8	2	3% lyophilized liver (Squibb)	37
9	2	1% 1:20 liver powder (Wilson)	35
10	2	1% Lederle liver prep. No. 1432	36
		Corn-soybean meal ration + double level of B vitamins	
11	3	None	36
12	3	0.5 g raw liver per day	43
13	3	3% lyophilized liver (Squibb)	43
14	3	1% 1:20 liver powder (Wilson)	45
15	3	1% Lederle liver prep. No. 1432	44

basal ration and fresh water *ad libitum*. Six rats were used in each group.

The synthetic basal ration was composed of sucrose 73%, casein (Smaco) 18%, corn oil (Mazola) 5%, salts IV 4%, and thiamine 0.3 mg, riboflavin 0.3 mg, niacin 2 mg, pyridoxine 0.2 mg, pantothenic acid 2 mg, folic acid 0.025 mg, biotin 0.01 mg, inositol 10 mg, choline 100 mg, and *p*-aminobenzoic acid 25 mg per 100 g of ration. Vitamins A and D were administered as oleum percomorphum diluted 1:4 with corn oil and fed at the rate of 2 drops per week.

Since milk and liver had been shown to be good sources of the monkey antianemia factor,[2] these substances were used as supplements to the basal ration. Many groups of rats have been studied, and in each case the animals receiving milk or liver supplements grew at a faster rate than those on the unsupplemented ration, but the differences in rate of gain were never large. Typical results are given in Table I, Groups 2 and 3. Lyophilized whole liver at a level of 3% in the diet gave the best response (Group 4). Certain samples of Wilson's 1:20 liver powder have produced some response (Group 5).

When the response was calculated at different weekly intervals, it was found that in practically all cases the greatest difference was observed at the end of 2 weeks. The results for a few groups calculated at the end of this period are given in Table I (Groups 6 through 10). In this instance, the basal ration was supplemented with additional vitamins and cystine in order to eliminate any possible deficiency of known factors. Under these conditions, the differences were still small but the response to each of the supplements was approximately the same as on the original basal ration.

In an earlier report, Jaffé[5] found that an alcoholic extract of fresh liver produced a growth response in rats fed a natural diet. Therefore, the following ration was fed in order to compare the growth response with that obtained with the synthetic ration: whole ground yellow corn 46.35%, commercial soybean meal 46.35%, corn oil (Mazola) 5%, cystine 0.3%, $CaHPO_4$ 0.92%, $CaCO_3$ 0.6%, NaCl iodized 0.44%, $MnSO_4 \cdot 4H_2O$ 0.04%. Vitamins were added at the same levels as in the synthetic ration. The growth on this unsupplemented diet was greater than on the synthetic basal, but the differences between the basal and the supplemented diets were much more consistent. (Table I, Groups 11 through 15).

[5] Jaffé, W. G., *J. Biol. Chem.*, 1946, **165**, 387.

TABLE II.
Results Obtained with *S. faecalis* Assay.

			Readings on Evelyn	
			Series I units	Series II units
Basal medium			72	70
"	"	+ livers of rats fed basal ration alone	53	52
"	"	+ " " " " " " + milk	30	43
"	"	+ " " " " " " + liver	30	46
"	"	+ urine of " " " " alone		45
"	"	+ " " " " " " + milk		36
"	"	+ " " " " " " + liver		37

For details of assay see reference 3.
The values noted represent liver samples added at a level of 90 mg (dry weight) per 10 cc of medium, while the urine represents undiluted samples of 1 cc per 10 cc of medium.

The hemoglobin concentration of the blood was determined after the rats had been on the various rations for 4 and 10 weeks. At both periods, the average figures for animals receiving the supplemented and unsupplemented rations were about the same. Differential leucocyte counts obtained after these rats were on experiment for 10 weeks showed no significant variation from the normal.

At the end of 10 weeks, rats receiving diets similar to those used for Groups 1, 2 and 3 were sacrificed and the livers removed for assay with *S. faecalis*.[3] The readings obtained in the Evelyn colorimeter after a 12-hour incubation period are shown in Table II. The results indicate that there is a substance in the liver of rats which stimulates the growth of *S. faecalis*. However, the livers of those animals that had received milk or liver were much more active in stimulating the growth of this organism than those from rats not receiving milk or liver.

In another series, 24-hour urine samples were collected during the ninth week in addition to removal of the livers at the end of this period. The urine from the rats on the basal group produced some stimulation in growth of *S. faecalis*, but the urine of rats fed milk or liver supplements gave greater stimulation (Table II). When this work was repeated with rats receiving the corn-soybean ration, similar results were obtained.

Lactation Studies. Female weanling rats (Sprague-Dawley) were placed on the synthetic ration and supplemented as in Groups 1, 2 and 3. A single dose of vitamin E (10 mg) was administered by dropper to each rat immediately before each mating. The rats were kept in separate cages until the end of the 10th week of experiment, then each group was placed in a large cage. Females were mated with males from our stock colony at the end of the 13th week. After pregnancy was ascertained by a significant weight increase, the female rats were placed in smaller individual wire cages without false bottoms. One day after parturition all litters were reduced to six. The percentage survival was calculated on the basis of the number alive at the end of the 21-day suckling period as compared to the original number at one day of age.

The original experiment consisted of 6 females in a group, and these were remated after a 3-week rest period. The results obtained are presented as the total of both matings.

Ration	% survival
Basal	73
" + 0.5 g fresh liver/day	95
" + 5 cc raw milk/day	66

Similar series have been carried through with approximately the same results. In every case good reproduction has been obtained but the animals receiving the liver have reared a larger percentage of the young left with the mothers.

Discussion. Although a positive response in growth was obtained whenever a liver preparation was added to the synthetic ration, it is evident that the differences in most cases are so small that it is difficult to use this response as an assay for a new factor in liver. Apparently the supply of at least

one additional factor is limiting when rats are fed the basal ration containing 10 B vitamins, but the degree of deficiency seems to decrease as the animals are continued on this regimen. This type of response suggests that the limiting factor is produced within the body, and that the amount produced is not sufficient to meet the needs of the animal during the first few weeks on the ration.

The most consistent differences were obtained with the mixed diet, and it appears that such a diet is most useful for assay purposes. This may be explained by the fact that the intestinal flora supported by a natural ration differs from that which exists on a synthetic ration. The corn and soymeal is not specific in this respect since other natural rations have given similar responses. Geyer *et al.*[6] have noted greater differences between rats fed a synthetic basal and those supplemented with liver, when the animals were kept in tube cages. Better differences may be obtained when coprophagy is prevented in rats fed the natural ration. Cary *et al.*[7] have reported that extra extraction of casein removes a factor "X" necessary for optimum growth of the rat. This may explain the inability to produce significant differences on synthetic rations containing casein which has not been exhaustively extracted with alcohol.

Previous reports showed that Wilson's 1:20 liver powder was inactive for the stimulation of growth of *S. faecalis;* however the samples used in this work were active for both the rat and *S. faecalis.* Thus different samples vary and the activity is similar for the rat and the microorganism. When the activity of liver or urine from rats fed the basal is compared to that of rats fed the active material, there is always an increased activity in the case of supplemented rats. This change suggests an increased storage of the factor in the liver, as well as increased excretion when a dietary source is fed. Previous work[8] has demonstrated clearly that the B_C potency of the liver was increased when the dietary intake of folic acid was increased.

The fact that the urine and liver of rats fed the unsupplemented ration stimulates growth of *S. faecalis* may be due to the production of the factor by the intestinal flora, to the presence of an undetectable amount of the factor in the basal ration, or to the storage of the factor in the animal body during the pre-experimental period. It is also possible that *S. faecalis* may be stimulated by more than one factor. This last possibility is being investigated.

The results obtained in the lactation studies are in general agreement with reports from other laboratories. Richardson and Hogan[9] reported improved lactation in rats upon addition of a fullers earth eluate of liver, which supplies B_C and probably unrecognized vitamins. A liver filtrate which contained a low level of Vitamin B_C was just as active in this respect. Cerecedo and Vinson[10] reported a beneficial effect on lactation when a folic acid concentrate was added to the diet. It is quite possible that these concentrates are sources of unknown nutrients essential for maximum lactation in the rat. Spitzer *et al.*[11] obtained better lactation when Wilson's 1:20 liver powder was added to a mixed diet which was unsatisfactory for lactation.

In the series reported in this paper, folic acid and biotin were used in crystalline form. The fact that a significant increase in percentage survival of young is attained when fresh liver is added to this diet, indicates that the liver contains a factor(s) necessary for maximum survival of young. It is quite possible that the same factor is concerned in the growth stimulation and in the improved lactation.

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[9] Richardson, L. R., and Hogan, A. G., *Fed. Proc.*, 1945, **4**, 161.

[10] Cerecedo, L. R., and Vinson, L. J., *Arch. Biochem.*, 1944, **5**, 469.

[11] Spitzer, R. R., and Phillips, P. H., *J. Nutr.*, 1946, **32**, 631.

Summary. Although rats grow at a rapid rate on a synthetic diet, it is possible to obtain small increases in the rate of growth by the addition of liver and liver preparations. The differences can also be obtained when the basal ration contains adequate amounts of folic acid. A larger and more consistent response to liver can be demonstrated when a corn-soybean meal ration is used. The addition of liver to the synthetic ration fed female rats increased the percentage of young surviving during the lactation period.

A factor in liver has been shown to stimulate the growth of *S. faecalis*. Assay with this organism indicates that an increased amount of the factor is excreted in the urine and stored in the liver of rats fed liver preparations. It is suggested that more rigorous methods are needed, such as prevention of coprophagy, use of natural rations or more extensive treatment of the components of the synthetic ration in order to devise suitable assay procedures.

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Some Pharmacological Characteristics of Bacitracin II. Absorption and Excretion of Bacitracin in the Dog.*

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Continuing an investigation of the pharmacological characteristics of bacitracin,[1] we have studied the absorption and excretion of the antibiotic in the dog. Because such studies are of fundamental importance in the rational use of the drug, the following data are presented.

Experimental. One female and 5 male mongrel dogs, weighing 7.5 to 11 kg, maintained on a stock diet and water *ad libitum*, were fasted over-night before use. In general, fasting dogs were given 200 cc of water by stomach tube at 9:00 a. m. and again at noon to insure an adequate diuresis. The antibiotic, a single lot designated B-100,† was administered by various routes at approximately 9:30 each morning, and sterile blood samples were taken at specified time intervals thereafter. The blood was permitted to clot and sterile samples of serum, diluted one to 3, were used directly in the assay procedure. Urine samples were collected 7 and 24 hours after administration of the drug. Occasionally 3- and 5-hour urine samples were collected in connection with distribution studies. These, and the routine 7-hour samples were taken by catheter. The 7- to 24-hour specimen was collected in a metabolism cage as a total sample. The urine samples were diluted one to 10 and passed through a Selas filter before testing. For stool analysis, a 1 g aliquot of the well mixed specimen was triturated with 50 cc of water and the clear supernatant fluid was passed through a Selas filter before testing.

The test procedure, involving the inhibition of the Chanin strain of β-hemolytic strep-

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1 Scudi, J. V., and Antopol, W., PROC. SOC. EXP. BIO. AND MED., 1946, **64**, 503.

† We are indebted to Dr. John T. Goorley of the Ben Venue Laboratories for the bacitracin.