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Received November 2, 1955. P.S.E.B.M., 1956, v91.

Transmethylation Reactions *in vivo* and *in vitro* in the Young Calf.* (22305)

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Johnson *et al.*(1) have shown that the young calf on a "normal" methionine intake requires a dietary source of choline and the present investigation was undertaken to determine whether this was replaceable by methionine. Earlier work has shown that rat liver slices and homogenates methylate homocysteine to form methionine with either choline or betaine as methyl donor. In studies by Borsook and Dubnoff(2), guanidoacetic acid was shown to be methylated by methionine in guinea pig liver homogenates. Bernheim and Bernheim(3) demonstrated that rat liver homogenates could oxidize acetylcholine, but found guinea pig liver to be inactive in this oxidation. Kensler and Langemann(4) reported that human and rabbit liver resembled guinea pig liver in this respect. We have now studied these enzyme systems in the calf.

Methods. Methionine-choline interrelationship. Male calves of various dairy breeds, 24 hours old, were fed a synthetic milk diet previously described by Hopper and Johnson (5) except that alpha protein was used as the protein source in the diet. Various levels of DL-methionine were added to the diet at time of feeding to give the total dietary methionine values indicated in Table I. The vitamin premix contained all the vitamins, except choline, which was a variable in the experiment. When choline was administered, it was a constituent of the vitamin premix. All animals were fed *ad lib.* twice daily from nipple pails. At the end of the trials, all animals were sacrificed and liver samples obtained for ether extract determinations. Post mortem examinations of all animals were conducted for the detection of histological lesions.

In vitro studies using calf liver homogenates. Liver samples were obtained from calves which had been fed the complete synthetic milk diet and compared with liver samples from 175-200 g, male rats fed commercial laboratory pellets. These samples were handled and the study of the methylation of homocysteine by betaine was conducted by

* The authors are grateful to Dr. J. O. Alberts and Dr. D. A. Willegan, College of Veterinary Medicine, for autopsy of animals, and to Dr. K. E. Gardner, Department of Dairy Science, for securing the calves.

This investigation was supported in part by funds donated by Merck and Co., Rahway, N. J., and Armour and Co., Chicago, Ill. Thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, calcium pantothenate, nicotinic acid, choline and vit. B_{12} were generously supplied by Merck and Co. through courtesy of Dr. L. Michaud. Folic acid was generously supplied by Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y., through courtesy of Dr. T. H. Jukes. Lard emulsion in feeding the animals was generously supplied by Armour and Co., Chicago, Ill. through the courtesy of Mr. B. M. Shinn.

† This paper is taken from a thesis submitted by the senior author to the graduate college, University of Illinois in partial fulfillment of requirements for the Degree of Doctor of Philosophy in Animal Nutrition. Present address: Department of Animal Husbandry, University of Nebraska, Lincoln.

TABLE I. Effect on Various Levels of Dietary Methionine on Growth and Liver Ether Extract of Animals on Choline-Deficient Diets, in 15 Calves.

Days on trial	Total dietary methionine, %*	Dietary choline, %	Daily gain, lb	% ether extract in liver (dry matter basis)
35	.4	0	.26	9.75
35	"	0	.19	3.65
43	"	0	.35	6.42
41	"	0	.34	4.17
38	.8	0	.66	38.96
35	"	0	.86	23.32
44	1.2	0	.91	13.65
43	"	0	.70	13.41
33	"	0	1.00	9.52
33	"	0	1.40	18.00
41	1.6	0	.34	37.68
41	"	0	.27	26.57
41	.8	.2	.90	14.04
41	"	"	.54	4.90
33	"	"	1.20	9.56

* The basal ration contained 0.3% methionine, and DL-methionine was added to give these total values.

procedures outlined by Williams *et al.*(6) and by Mistry *et al.*(7). The methylation of guanidoacetic acid by methionine was studied according to the method of Borsook and Dubnoff as used by Mistry *et al.*(7). Choline oxidase activity was determined by the method of Richert and Westerfeld(8).

Results. In vivo studies. As noted in Table I, good growth was observed in the calves receiving the choline-deficient diets containing 0.8 or 1.2% DL-methionine. However, the livers of the calves receiving only 0.8% DL-methionine contained a considerably greater amount of ether extract. This indicates a deficiency of labile methyl groups at this level of methionine in the absence of choline. Treadwell(9) has reported that the young rat has a definite need for choline when the diet contains 0.8% methionine. Neumann *et al.*(10) found the young pig required 0.1% choline when fed a "synthetic milk" ration containing 0.8% methionine. The calves receiving a total of 0.4% methionine in the diet‡ and no choline exhibited very small daily gains. This result was due to a methionine deficiency as well as a deficiency of labile methyl groups and the extremely poor growth presumably accounts for the failure to find choline-deficiency fatty livers. Diets supplying a total of 1.6% methionine supported very poor growth and liver samples from these animals during the feeding trial indicated that the 1.6% level of methionine was apparently "toxic," as the animals scoured severely throughout the trial. Liver ether extract data for the animals receiving 1.2% methionine, are similar to those from the normal animals receiving 0.8% methionine and 0.2% choline, as seen in Table I. Histological study of liver revealed fatty infiltration and some kidney damage in the methyl-deficient animals, confirming the liver fat data. The results indicate that the young calf can, by transmethylation, synthesize its required choline from methionine as has been shown for the rat, for the pig, and for man.

‡ This diet also contained approximately 0.15% cystine, supplied in the alpha protein.

In vitro studies. The results of methionine formation by calf liver homogenates are shown in Table II. Although there was some transmethylase activity in calf liver homogenates, the activity was much less than that of rat liver homogenates even though both species were on diets which were satisfactory for growth.

The results of creatine formation by calf liver homogenates are presented in Table II. Under the same experimental conditions, over 4 times as much creatine was formed by rat liver homogenates as by calf liver homoge-

TABLE II. Results of Enzyme Studies in Calf and Rat Liver Homogenates.

	No. of animals	Results
Methionine synthesis from homocysteine + betaine		Avg methionine formed/100 mg dry matter of liver tissue/hr, μg
Calf	2	59
Rat	3	88
Creatine synthesis from guanidoacetic acid + methionine		Avg creatine formed/100 mg dry matter of liver tissue/hr, μg
Calf	2	6.9
Rat	2	29.6
Choline oxidase activity		$\mu l/O_2/10$ min./flask
Calf	2	4.78
Rat	2	56.6

nates. However, both species were able to methylate guanidoacetic acid to form creatine. The low values found in this study for calf liver are of the same magnitude as those reported by Cohen(11) for beef liver. Cohen (12) found that addition of folic acid and glutamic acid did not bring the levels into line with those for the rat.

The choline oxidase activity of calf liver homogenates as compared to rat liver homogenates is shown in Table II. These results indicate that calf liver possesses a much lower choline oxidase activity than rat liver. The low activity of the enzyme would indicate a slow choline turnover similar to that found by Handler(13) for the guinea pig.

Summary. 1. Using a synthetic milk diet, calves receiving 1.2% DL-methionine exhibited no demonstrable choline requirement, as measured by growth and liver ether extract. On a diet supplying 0.8% methionine, fatty livers developed in the absence of choline. 2. A study was made of the ability of calf liver homogenates to methylate homocysteine to form methionine, when betaine served as methyl donor; to methylate guanidoacetic acid, when methionine served as the methyl donor, and to oxidize choline. It was found that while calf liver homogenates would carry out all of these reactions, the activity of the calf liver enzymes was very much less than that of rat liver homogenates.

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Received November 28, 1955. P.S.E.B.M., 1956, v91.

Pernicious Anemia. I. Remission by Small Oral Doses of Purified Vitamin B_{12}.* (22306)

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During studies on pernicious anemia, it was found, contrary to previous experience, that partial remission occurred in a patient with pernicious anemia in relapse who received minimal doses of purified vit. B_{12} by mouth. Pernicious anemia is generally considered to be the result of a heredofamilial atrophy of gastric and duodenal mucosa, with absence of "intrinsic factor," a material necessary for absorption of vit. B_{12} from the gastrointestinal tract into the blood stream. Megaloblastic erythropoiesis of pernicious anemia in relapse can be restored to normal by overcoming the deficiency in intrinsic factor by (1) parenteral administration of vit. B_{12}, thus circumventing the abnormal gastrointestinal tract; (2) oral administration of massive doses of vit. B_{12} (1000 to 9000 μg/wk), through a sort of "mass action" effect; or (3) oral administration of vit. B_{12} together with some outside source of intrinsic factor. How-

* Aided by grants from the Squibb Institute for Medical Research and the A. A. List, Anna Ruth Lowenberg, and Frederick Machlin Research Funds.