sized that early embryogenesis in *Rana pipiens* might occur at the expense of preformed purine-containing nucleic acid fragments stored in the ovum and that nucleic acid synthesis from smaller molecules involving PGA might begin only at neurulation, the earliest stage that PGA antimetabolites affect development in this species.

Summary. Fetal development in the rat was severely affected by a 36-hour period of pteroylglutamic acid deficiency when instituted early in the second week of pregnancy. The incidence of fetal death or abnormality was higher when the vitamin-deficient diet was started on the 8th day of pregnancy than on the 7th day but decreased rapidly thereafter with increasing fetal age.

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Vitamin E Deficiency and Biosynthesis of Glycine, Serine, and Methionine in Rabbits.* (22542)

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While studying the effect of vit. E deficiency on the incorporation of formate-C^{14} and glycine-1-C^{14} into the nucleic acids of various rabbit tissues, it was found that the deficiency also altered the level of isotope incorporation into the proteins of those tissues. The tissue protein exhibiting the greatest difference was that of skeletal muscle(1). This paper reports the results of the isolation of glycine, serine, and methionine from skeletal muscle of normal and vit. E-deficient rabbits following injections of either sodium formate-C^{14} or glycine-1-C^{14}.

Methods. New Zealand rabbits of both sexes were placed on a purified diet deficient in vit. E(2). Controls were given 4 mg of α-tocopherol acetate per kilo of body weight twice weekly. When symptoms appeared in 3-4 weeks, one deficient and one control rabbit were injected with either sodium formate-C^{14} (specific activity, 2.85 μc/mMole) or glycine-1-C^{14} (specific activity, 0.58 μc/mMole) at a level of 10 μc per 100 g of body weight. After 4 hours the animals were killed and the skeletal muscles of the hind legs were removed.

This tissue was homogenized in an equal volume of water and extracted with cold 10% trichloroacetic acid, alcohol, alcohol-ether, and hot 10% sodium chloride. The protein residue was then dried. The specific activities of these protein samples were determined by counting small aliquots in an end-window Geiger-Muller tube with window thickness of 2 mg per sq. cm. All values were corrected to infinite thinness. Approximately 10 g of the protein was hydrolyzed according to the method of Stein and Moore(3). The amino acids were separated with an ion exchange column, using Dowex 50-X4, using the method of Moore and Stein(4) with modifications. The hydrolysate was placed on the column in a citrate buffer at pH 3.1. The effluent was collected in 4 ml fractions at a rate of 50 ml per hour at 32°C. Each fraction was assayed for C^{14} content by counting an aliquot which had been evaporated to dryness.

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TABLE I. Incorporation of Sodium Formate-C^{14} and Glycine-1-C^{14} into Protein, Glycine, Serine, and Methionine in Skeletal Muscle from Normal and Vit. E-Deficient Rabbits.

Compound inj.	Diet	Protein, c.p.m./mg	c.p.m./μMole		
			Glycine	Serine	Methionine
Glycine-1-C^{14}	Normal	2.1	53	14	0
	Vit. E-deficient	5.6	49	13	0
Na formate-C^{14}	Normal	1.9	—	11	0
	Vit. E-deficient	11.8	—	37	41

All values were corrected to infinite thinness. The glycine content was determined by the method of Alexander *et al.*(5), serine by the method of Frisell *et al.*(6), and methionine by the method of Dubnoff(7). The specific activities were calculated for each amino acid. Two animals were used per group and the agreement was excellent.

Results. The protein from the skeletal muscle of vit. E-deficient rabbits injected with glycine-1-C^{14} contained 2.5 times as much radioactivity as the protein from similarly treated normal controls (Table I). However, the radioactivities of the glycine and serine isolated from the skeletal muscle protein of normal and vit. E-deficient rabbits were essentially the same. Since the glycine-serine interrelationship is well known, it would be expected that a major percentage of the radioactivity of any protein following an injection of glycine-1-C^{14} would be found in the glycine and serine. This does not appear to be the case in the skeletal muscle protein of vit. E-deficient rabbits. The location of the radioactivity, not accounted for in glycine and serine, is unknown. Inasmuch as only the methylene portion of glycine has been shown to be a methyl group precursor, the lack of activity found for methionine in both normal and vit. E-deficient rabbits was to be expected. The injection of sodium formate-C^{14} into normal and vit. E-deficient rabbits resulted in 6 times as much radioactivity in the skeletal muscle protein of the deficient animals. The deficiency resulted in higher specific activities for serine and methionine (Table I). These two amino acids, taken together, contain approximately the same percentage of the protein activity whether from deficient or normal animals. Considering the lack of activity in the methionine of the normal rabbits, the high specific activity of the methionine from the deficient animals is striking. This increased utilization of formate in vit. E deficiency is in agreement with previous observations. It has been reported that following formate-C^{14} injections, tissue purines (1) and urinary creatinine(8) of deficient rabbits have much higher activities than the same compounds from normal animals.

The data of formate utilization may be explained partially by the excretion of creatine and allantoin. Both compounds have been shown to be excreted in great quantity in vit. E-deficient rabbits(2,9), and the biosynthesis of each is known to involve 1-carbon fragments. Presumably, an elevated excretion is the result of an increased rate of biosynthesis which would in turn mean a decreased availability of 1-carbon fragments. Therefore, following the injection of sodium formate-C^{14}, all compounds requiring 1-carbon fragments would be found to have higher specific activities in the vitamin E-deficient rabbits than in the normal controls.

Summary. Injections of sodium formate-C^{14} resulted in no activity in the methionine from the protein of skeletal muscle from normal rabbits but such activity did appear in the methionine of vit. E-deficient rabbits. The specific activities of glycine and serine were essentially the same in normal and in deficient skeletal muscle protein following injections of glycine-1-C^{14}. In spite of this the activity of the protein from deficient rabbits was much higher than that of the normal controls.

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Effect of Intravenously Administered Amino Acids on Blood Ammonia.* (22543)

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In a study of the metabolism of parenterally administered glycine Handler, Kamin and Harris(1) reported that glycine infused into dogs at rates exceeding 1 mg amino N/kg/min. was invariably found to be lethal, although after varying periods of time. These findings were interpreted by Handler *et al.* as a manifestation of the toxicity of glycine. Doolan *et al.*(2) in studies on renal clearance of amino acids in humans observed a severe reaction in a subject receiving a 5% solution of glycine injected intravenously at a mean rate of 0.67 mg amino N/kg/min. Because of the symptoms associated with the reaction to glycine these authors suggested that an elevation in blood ammonia might be responsible for the toxicity otherwise attributed to the amino acid itself. To investigate this possibility the effect in dogs of intravenous administration of amino acids on the levels of ammonia in the blood was studied.

Procedure. One liter of an amino acid solution was infused intravenously over a 2-hour period to adult male mongrel dogs under sodium pentobarbital anesthesia. Blood samples were drawn prior to the infusion and at 30-minute intervals thereafter. Heparin was used as the anticoagulant. Each sample was analyzed for ammonia, urea and amino acid nitrogen. Ammonia nitrogen was determined by the method of Conway(3); urea nitrogen by the method of Van Slyke and Kugel(4), and amino acid nitrogen by the method of Hamilton and Van Slyke(5).

In the first series of experiments glycine was infused in doses ranging from one to 7 mg amino N/kg/min. In a similar study DL-alanine was infused at a rate of 3.5 mg amino N/kg/min. To compare the blood ammonia levels that follow the infusion of a mixture of amino acids with those after infusion of a single amino acid, an acid hydrolysate of casein fortified with DL-tryptophan† was infused at rates of 3.5 or 0.58 mg amino N/kg/min.

Results. In Table I the concentrations in blood or plasma of ammonia, urea and amino acid nitrogen after the infusion of the various amino acids are given. The data were derived from individual experiments under the conditions described. It is apparent that significant increases in blood ammonia occurred after the infusions of glycine at levels above one mg amino N/kg/min. At the maximum infusion rate used (7 mg amino N/kg/min) the dog expired in respiratory failure immediately after the 2-hour infusion. At lower rates of administration no symptoms were elicited. The elevations of ammonia in the blood were directly related to the amounts of amino acid administered, indicating an immediate relationship between the dose of amino acid and the occurrence of ammonia intoxication. The amino nitrogen levels reflect the concentration in the blood of glycine that was being infused. Conversion to urea of the nitrogen derived from the infused amino acid was responsible for the rise in blood urea nitrogen since it was proportional

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