would produce chronic defects such as neurological changes.

Summary. When tripropionin is added to the diet of rats on a diet deficient in Vit. B_{12} and possibly other factors, there is a fatal toxicity which is not as marked when the diet is supplemented with Vit. B_{12}.

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Occurrence of C^{14}-Oxalate in Rat Urine after Administration of C^{14}-Tryptophan.* (28744)

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Studies from this laboratory have indicated that there is an increase in endogenous oxalate excretion in Vit. B_6 deficiency(1,2). Following administration of tryptophan load tests to human subjects, Gershoff and Prien (3), Farvar *et al*(4), and Faber *et al*(5), have reported increased excretion of oxalate. In the present study the effect of tryptophan on excretion of oxalate by Vit. B_6-deficient and control rats has been investigated.

Methods. Two groups of 18 male weanling Charles River CD rats were fed a purified diet *ad libitum* with or without Vit. B_6 consisting of: casein, 15%; sucrose, 75.7%; corn oil, 4%; salts IV(6), 4%; cod liver oil, 1%; and choline, 0.3%. Four mg of thiamine hydrochloride, 8 mg of riboflavin, 40 mg of niacin, 20 mg of calcium pantothenate, 1 mg of folic acid, 1 mg of menadione, 0.2 mg of biotin, 0.05 mg of Vit. B_{12} and, when used, 4 mg of pyridoxine hydrochloride, were added to each kilo of diet. After 3 weeks the rats were injected intraperitoneally with 5 μc of either DL tryptophan 2-C^{14} or DL tryptophan 3-C^{14} per 100 g of body weight.

The rats were placed in metabolic cages in groups of 3 and 48-hour urine collections were made. Oxalates were extracted and precipitated from the urines as described by Powers and Levatin(7). The calcium oxalate precipitate was dissolved in 1 ml of 20% sulfuric acid and 0.5 ml of 1% manganous sulfate and transferred to a Warburg flask. One ml of 0.03 N potassium permanganate was added from the side arm and the flasks were shaken for 30 minutes. The CO_2 evolved was trapped in the center well on filter paper wet with KOH and the radioactivity determined in a liquid scintillation counter as described by Buhler(8). The total oxalate in the sample was determined by titrating the excess permanganate in the Warburg flask (7). Total urine radioactivity was determined in a liquid scintillation counter after adding 0.2 ml of urine to a mixture of 5 ml of absolute alcohol and 10 ml of a solution of 0.4% PPO and 0.005% POPOP in toluene.

Results and discussion. The data presented in the Table show that tryptophan is a precursor of oxalate in rats and that approxi-

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TABLE I. Effect of Administering Labelled Tryptophan to Rats Fed Diets With and Without Vitamin B_6.

	C^{14} in urine in 48 hr		Urinary oxalic acid, mg/100 g rat/48 hr
	% total	% as oxalate	
DL Tryptophan 2-C^{14}			
+ B_6	3.9	.118	.72
− B_6	8.6	.119	2.23
DL Tryptophan 3-C^{14}			
+ B_6	4.1	.007	1.01
− B_6	7.5	.008	1.85

mately 16 times more labelled oxalate was excreted when tryptophan labelled in the 2-C than the 3-C position was used. Although more oxalates were excreted by the Vit. B_6-deficient rats than their controls, the deficient animals did not convert more of the injected tryptophan to oxalate than the control rats. When either isotope was used approximately twice as much of the C^{14} administered was excreted in the urine of the deficient rats than those receiving Vit. B_6. This was probably a reflection of the less efficient utilization of the tryptophan by the deficient animals.

All known paths for synthesis of oxalic acid in animals involve the intermediate glyoxylic acid. It has been suggested that glyoxylic acid may be derived from serine from C_2 compounds, ethanolamine, glycolaldehyde, and glycolic acid(9). It is probable that this represents a minor pathway in the conversion of serine to glyoxylic acid. In view of Shemin's studies of the formation of glycine from serine(10), it would appear that a serine, glycine, glyoxylic acid pathway involving the loss of carbon-3 of serine would be more likely. This is supported in part by studies in which administration of acetate-1-C^{14} to rats resulted in synthesis of glycine and serine labelled almost entirely in carbon 1(11). It has been reported in *in vitro* studies that the side chain of tryptophan is removed as alanine(12,13) and in *in vivo* studies(14,15) that serine can be derived from the side chain of tryptophan. Gholson *et al*(15) have reported that when DL tryptophan 2-C^{14} was fed to rats 80-96% of the activity observed in alanine recovered from carcass and liver was found in carbon-2. However, considerable randomization of carbons 2 and 3 was observed in serine. The ratio of activity of the 2 and 3 carbons of serine obtained from the total carcass was about 1.4 and in the serine isolated from liver, 3.2. It might be expected that if the formation of oxalate from the side chain of tryptophan proceeded by way of alanine, pyruvate, serine, glycine and glyoxylic acid, the ratio of the activity in urinary oxalate obtained from tryptophan labelled in either the 2 or 3 positions would be of a magnitude similar to that reported above. However, in this study a ratio of approximately 16 was observed. It is difficult to explain these data. It is possible that the labelled oxalate measured in this study was not formed by the metabolic pathway suggested, or that randomization was minimal in the tissue synthesizing oxalate from tryptophan. Nyc and Zabin(11) have shown that serine formed in rats injected with pyruvate-3-C^{14} is equally labelled at carbon atoms 2 and 3. Pyruvate is converted to one or more symmetrical 4-carbon intermediates from which hydroxypyruvate and finally serine are formed. Arnstein and Keglevic(16) have suggested that the serine precursor is more closely related to an intermediate of glucose catabolism such as a triose or glyceric acid, than to pyruvate. This is supported by Koeppe *et al*(17) who found only slight randomization between carbons 2 and 3 of serine and alanine when glycerol 1, 3-C^{14} was administered to rats. It is possible that alanine may be metabolized to serine *via* 3-carbon compounds not including pyruvic acid or that glyoxylic acid may be derived from alanine by a pathway which does not include serine.

It is apparent from these studies that tryptophan is a precursor of oxalate. This probably explains the fact that when normal human subjects are given 10 g DL tryptophan load tests, their 24-hour urinary excretion of oxalate increases about 10 mg(3,5).

When, in unreported studies from this laboratory, trace amounts of glycine 1-C^{14} were administered to control and Vit. B_6-deficient rats approximately 3 times more of the injected C^{14} appeared in the urinary oxalate of the deficient rats than the controls.

In the present work Vit. B_6 deficiency did not appear to affect the percentage of administered C^{14} which appeared in the urine as oxalate. This may have been a reflection of the often reported defect in the conversion of 3-hydroxykynurenine to 3-hydroxyanthranilic acid and alanine in Vit. B_6-deficient rats. Thus, although the Vit. B_6-deficient rats were synthesizing more oxalate than their controls, less labelled precursor was available to them with the result that the specific activity of the oxalate excreted by the Vit. B_6-deficient rats was less than that of their controls.

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Gestation in Salpingectomized-Ovariectomized Progesterone-Treated Rabbits.* (28745)

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Pincus and Kirsch(1) ligated one uterine tube at the isthmus in rabbit at 17 hours after fertile mating; 6 days later, degenerated embryos at the late morula or early blastocyst stages were recovered from the ligated tubes. The embryos on the non-ligated side had entered the uterus and developed normally. Similar results were obtained when the interval between mating and ligation was some 32 hours(2). These results suggested that the uterine tubes were essential to the expansion of blastocyst. It is well known that the fertilized egg does not expand during its 72-hour journey in the uterine tube. At 3 days *post coitum* the utero-tubal junction relaxes, allowing the flow of the luminal fluid of the uterine fluid containing the late morulae and early blastocysts(3). The complex processes of active transport associated with the expansion of the blastocysts are not known.

Ovariectomy does not interfere with the passage of embryos through the uterine tube of the rabbit(2,4,5), rat(6), cat(7) and guinea-pig(8). However, ovariectomy causes termination of pregnancy in the early stages

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