skeleton. Part of the decline in skeletal specific activities which takes place in rats before radiocalcium excretion ceases is also due to skeletal growth.

Data as to the changes of calcium specific activity of the urine and the teeth following the injection of labelled calcium are presented and discussed.

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Toxicity of Glycine for Vitamin B_{12}-Deficient Chicks.*† (18445)

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An unidentified factor which was reported to stimulate growth of chicks fed a vegetable protein diet also lowered the blood non-protein nitrogen level(1). It was later found(2) that a vit. B_{12} concentrate lowered the blood non-protein nitrogen of chicks previously fed a vit. B_{12}-deficient diet. Non-protein nitrogen and urea were found to be higher in zoopherin (vit. B_{12})-deficient rats than in normal rats (3). These and other reports(4,5) indicated that vit. B_{12} is concerned with nitrogen utilization and metabolism. It was the purpose of this experiment to investigate further the vit. B_{12}-nitrogen relationship by feeding chicks a known amount of non-protein nitrogen and observing the effect of vit. B_{12} deficiency.

Procedure. Twenty-eight day-old White Leghorn chicks which had been fed the vit. B_{12}-deficient diet shown in Table I from the day of hatching were used in this experiment. A deficient control group received no treatment, whereas 3 μg of vit. B_{12} per week were injected subcutaneously into the chicks of the supplemented group. The night of the 28th day food was withdrawn from chicks in both groups. The following morning blood was taken from 4 birds of each group by heart puncture, and the birds sacrificed. Protein-free filtrates were prepared from pooled samples of the plasma by tungstic acid precipitation. The filtrates were analyzed for nitrogen by a semi-micro Kjeldahl method and for amino nitrogen using the naphthoquinone-sulfonic acid method(6). Immedi-

TABLE I. Experimental Diet.

	%
Ground yellow corn	43.5
Ground wheat	15
Soybean oil meal (solvent)	37
Dehydrated alfalfa	1
Ground limestone	1.5
Dicalcium phosphate	1.5
Sodium chloride	.5
A and D supplement*	.1
Choline chloride	.1
Methionine	.1
$MnSO_4$	8 g/100 lbs.
	mg per lb.
Riboflavin	4.5
Ca pantothenate	7.5
Niacin	15.0
Thiamin	2.5
Pyridoxine	2.5
Biotin	0.045
Folic acid	0.45
Inositol	4.53
Menadione	4.5
Alpha tocopherol	4.5
Para amino benzoic acid	2.5

* 5,000 I.U. vit. A and 1,000 AOAC chick units vit. D per g.

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TABLE II. Results of Pooled Blood Plasma Analyses.

Time after glycine dose, hr	B_{12} deficient		B_{12} injected	
	N.P.N., mg %	Amino N, mg %	N.P.N., mg %	Amino N, mg %
0	24	9	—	8
2	20	19	35	20
4	43	20	32	16

ately after this bleeding, capsules containing 1 g of glycine were force-fed to all remaining chicks of both groups. Two and 4 hours after the glycine feeding blood samples were obtained by heart puncture and treated as above. Segments of liver and kidney were taken for histological examination. These were fixed in Carnoy's solution and stained with hematoxylin and eosin.

Results. Two hours after the glycine administration, the birds of both the deficient and supplemented groups seemed normal with respect to appearance and behavior. Four hours after the glycine dosage, a dramatic change appeared in the vit. B_{12}-deficient chicks. Two had died and those which remained alive were extremely weak and semicomatose. Several were unable to stand and appeared near death. It was extremely difficult to obtain blood from these birds, probably because of circulatory failure. In marked contrast, the vit. B_{12}-treated chicks appeared normal and lively.

Microscopic examination of histological sections of liver and kidney tissue revealed no difference between the two groups. The results of the blood plasma analyses are shown in Table II.

In a second trial a similar procedure was followed except that food was left before the chicks at all times. Four hours after glycine feeding, the chicks of the vit. B_{12}-deficient group were noticeably listless although they were only slightly affected when compared with the starved B_{12}-deficient chicks of the first trial. The B_{12}-injected birds again seemed normal. No blood or histological examinations were performed on these birds.

Discussion. Both the plasma non-protein nitrogen and amino nitrogen levels showed that the vit. B_{12}-injected birds were able to metabolize the administered glycine better than the vit. B_{12}-deficient birds. In the former group, NPN and amino N were highest 2 hours after glycine administration and had diminished by 4 hours afterwards. In the B_{12}-deficient group, however, NPN and amino N levels did not fall after 2 hours but increased to some extent at 4 hours. These high levels were associated with the toxic effect of the glycine. It was interesting to note that vit. B_{12}-deficient chicks which were on feed during the whole period were not as affected by the glycine as those which were starved. It is possible that energy as well as vit. B_{12} is required in nitrogen metabolism.

Summary. The plasma levels for non-protein nitrogen and amino nitrogen after administration of glycine were higher in vit. B_{12}-deficient chicks than in B_{12}-injected chicks. One gram of glycine, when force fed in gelatin capsules, was toxic to starved, B_{12}-deficient chicks and less toxic to B_{12}-deficient chicks which had not been starved. Chicks which had been injected with vit. B_{12} were able to withstand the glycine whether or not they had been without food.

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Antagonistic Effect of Serum on Bacteriostatic Action of Lupulone. (18446)

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The loss of the antibiotic properties of lupulone when mixed with serum has been noted by Salle, Jann, and Ordanik(1) and by Chin, Chang, and Anderson(2). An effort has been made by this laboratory to determine the cause of this inactivation in order that the