tion of L-T_4-I^{131} from subcutaneous injection is relatively rapid in the rat. On the basis that the thyroidectomized rats which received TSR levels of replacement thyroxine were comparable to those controls with intact thyroids, it appears that route of administration of the L-T_4-I^{131} had no effect on the $t\frac{1}{2}$. However, it is, of course, necessary that absorption of the L-T_4-I^{131} be essentially complete prior to utilization of a blood sample for determination of the $t\frac{1}{2}$. By plotting the radioactive counts of the blood samples as a per cent of injected dose on semilog paper, it was found that the rate of disappearance of the L-T_4-I^{131} remained linear after allowing 12 hours for absorption of the L-T_4-I^{131}.

Summary. Data on the biological half-life ($t\frac{1}{2}$) of L-thyroxine-I^{131} (L-T_4-I^{131}) in thyroidectomized, thyroidectomized thyroxine replaced, normal and methimazole treated female rats are presented. Rats thyroidectomized for 7 days had a significantly longer mean $t\frac{1}{2}$ of L-T_4-I^{131} ($P<.005$) of 23.7 $\pm$ 0.7 hours than thyroidectomized rats which received a mean TSR level of replacement thyroxine of 1.0 μg L-T_4/100 g bw and had a mean $t\frac{1}{2}$ of 17.8 $\pm$ 1.1 hours. These thyroxine replaced rats did not differ from intact controls which had a mean $t\frac{1}{2}$ of 19.5 $\pm$ 2.2 hours. Hypothyroidism induced by subcutaneous injection of 400 μg methimazole/100 g bw/day for 30 days had no effect on the $t\frac{1}{2}$ of L-T_4-I^{131} as shown by a mean $t\frac{1}{2}$ of 18.5 $\pm$ 2.1 hours. It was shown also that administration of the tracer dose of L-T_4-I^{131} could be made by either subcutaneous or intracardial injection without effect on the $t\frac{1}{2}$ if proper intervals were maintained between time of injection and collection of the initial blood sample.

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Determination of Carbonic Anhydrase in the Epiphysis of Endochondral Bone.* (30551)

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There is some evidence that carbonic anhydrase (CA) plays an important role in the calcification process in invertebrates. Among other studies, Wilbur and Jodrey(1) showed that acetazolamide, a CA inhibitor, would decrease the rate of calcium deposition in the mantle shell of the oyster. Goreau(2) likewise showed that similar reactions occurred in coral after CA inhibition with acetazolamide.

Among the vertebrates, Scott *et al*(3) and Benesch *et al*(4) have found that egg shell formation in hens is inhibited by unsubstituted sulfonamides. Little evidence is pre-

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sented on the role CA plays in the calcification of bone among mammals.

Kondo and Kuriaki(5) showed CA activity in the whole tissue homogenate of teeth and femur of rats, but did not consider the effect of blood contamination. Simpson(6) showed CA activity was present in costal cartilage and xiphoid process of the rat, but he did not rule out completely the possibility of blood contamination. By using inhibition studies with acetazolamide, he also claimed to find a reduction of 50% in calcium deposition by X-ray diffraction; however, he could not show any difference when he determined the amount of calcium present by chemical methods.

Dulce *et al*(7) found that the tibial epiphysis of the Leghorn rooster and bones of the teleost contained carbonic anhydrase, but no activity was observed in the unossified cartilage of the intervertebral disc of the bird. He postulates that this enzyme aided in the mineralization and demineralization of bone by the local secretion of H^+ ions. Using sulfonamides as CA inhibitors, Benesch *et al* (8) claimed to find calcification defects in the tibias of fetal rats. However, further work by Miller *et al*(9) could not substantiate these findings. Studies by Maren *et al*(10) using acetazolamide on young growing rats showed no difference in the epiphyseal development between control and treated rats.

This paper will attempt to show that the CA enzyme in the epiphysis is similar to that present in blood, and also suggests a possible explanation for its presence in the epiphyseal region of endochondral bone.

Materials and methods. Two groups of male Holtzman rats were used in the experiment. Rats in Group 1 were mature and weighed approximately 500 g. Group 2 contained young rats weighing 100 and 150 g. Blood for analysis was withdrawn by cardiac puncture and the animal sacrificed with ether. The trachea, xiphoid process, costal cartilage, tibia and femur were removed, stripped and scraped free of all surrounding tissue. The bone was then split open and washed free of marrow and blood with distilled water. The epiphysis including the epiphyseal plate had been removed for separate analysis and washed with distilled water to remove as much blood as possible. These tissues were then weighed, placed in cold distilled water and homogenized for 5 minutes on ice to prevent any loss of activity. After settling, an aliquot of the supernate was then tested for the presence of carbonic anhydrase using the method of Maren *et al*(11).

TABLE I. Enzyme Units/g in Bone of Rats.

Tissue	Group 1	Group 2
Tibia, shaft	$<1.0 \pm .1$ *	$1.2 \pm .1$*
Tibia, epiphysis	$12.3 \pm .29$	38.4 ± 3.1
Femur, shaft	$<1.0 \pm .1$	$<1.0 \pm .1$
Femur, epiphysis	10.0 ± 1.8	43.0 ± 6.9
Humerus, shaft	——	<1.0 —
Humerus, epiphysis	——	45.7 —
Radius, shaft	——	<1.0 —
Radius, epiphysis	——	25.6 —
Costal cartilage	<1.0 —	<1.0 —
Xiphoid process	<1.0 —	<1.0 —
Scapulae	——	36.1 —
Skull	——	3.1 —
Blood	1540 ± 1.70	1220 —

* ± S.E.

Group 1 rats weighed approx. 500 g; Group 2 were growing rats at 100-150 g. Values shown above are corrected for enzyme units due to RBC contamination in the tissue by the following formula:

$$\text{E.U./g tissue} = \frac{(\text{E.U./ml whole blood})(\text{mg hgb/g tissue})}{\text{mg hgb/ml whole blood}}.$$

Hemoglobin was determined in all samples by the method of Bing and Baker(12). Blood concentration in the tissue was used to correct for enzyme activity due to the red cell.

Results. The epiphyses of endochondral bone (tibia, femur, humerus and radius) in young rats (Group 2) exhibited CA activity in the range of 30 to 50 enzyme units/g, whereas the epiphyses of mature rats (Group 1) showed about 10-12 enzyme units/g. Cartilage and ossified material, as well as intramembranous bone, typified by the parietal region of the skull, contained little or no activity in either group (Table I). It is apparent that the CA activity is confined to the epiphysis and not in the shaft of endochondral bone.

Inhibition studies(15) by the method using bicarbonate buffer, revealed a small difference among the I_{50}'s for carbonic anhydrase in the epiphyses of femur and tibia and blood using acetazolamide, but no difference using

TABLE II. Comparison of Concentrations Needed to Inhibit 50% of Carbonic Anhydrase from Blood and Bone. Data in 10^{-7} M.

	Acetazolamide	Sulfanilamide
Rat blood	.29	35
Tibia epiphysis	.39	37
Femur "	.73	36

sulfanilamide (Table II). The differences for acetazolamide could be because of the variable amounts of protein in the tissues used. The protein binding sites of the tissue conceivably absorb a small amount of acetazolamide present in the system. This could result in removal of a small but critical amount of drug from reaction with the enzyme. However, by using sulfanilamide, a weak inhibitor, one places a large excess of drug in solution, so that even though saturation of the binding sites may occur, there is still excess drug in the system to react with the enzyme. From this, it appears that the enzymes are similar and are possibly the same enzyme system.

Discussion. As seen in Table I, hyaline cartilage from trachea, xiphoid process, and costal cartilage of young and adult rats contains little or no CA activity. On the other hand, the epiphyseal region of endochondral bone in young animals shows appreciable enzymatic activity. This region is composed of cartilage, osteogenic cells and bone marrow capable of forming hemopoietic cells(13). These osteogenic cells as well as other bone cells are present in the young animal in a greater quantity than in the adult.

Clark(14) showed in his work on embryonic development that CA is present in primitive reticular cells and erythroblasts in high concentration and is probably confined to the bone marrow cell structure. Since multipotential osteogenic cells could possibly develop in hemopoietic cells,† this could explain the CA activity.

† The term osteogenic is used in the sense given by Ham(13), who has concluded that "at least some of the covering and lining cells of bone (osteogenic cells) must be sufficiently undifferentiated to be able to form marrow cells as well as bone cells and that such cells, in periosteal buds, are responsible for marrow formation."

Since these cells are also present to a small degree in adults even after fusion of the epiphyseal plate, the small amount of activity found in adult epiphyseal area would tend to confirm this. This is also supported by the fact that intramembranous bone, typified by the parietal portion of the skull, contains a small amount of CA activity.

However, it can be argued that cartilage from endochondral bone is modified in that it has a reduced amount of chondroitin sulfate and secretes alkaline phosphatase, thus allowing calcification to occur. One may think that CA is necessary for bone formation since calcification depends on $PO_4^=$ and $CO_3^=$. However, ossification is a slow process and the uncatalyzed hydration of CO_2 should therefore provide $HCO_3^=$ and $CO_3^=$ ions at an adequate rate. In fact, studies conducted by Maren *et al*(10) and Miller *et* al(9) showed no changes in bone growth or calcium deposition after CA inhibition. This coupled with negative results obtained from cartilage and ossified bone suggests that the CA of the epiphyseal area is contained in those osteogenic cells destined for hemopoiesis; and that CA plays no role in the calcification of bone but is incidental to the formation of red cells.

Summary. Carbonic anhydrase activity was found in the epiphysis of endochondral bone and not in cartilage or ossified material. These findings, taken together with earlier negative results on epiphyseal growth following CA inhibition, lead to the hypothesis that the CA activity in the epiphyseal region may be due to undifferentiated osteogenic cells, some of which are precursors for hemopoiesis.

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Renal Concentrating Ability in Pyridoxine Deficiency.* (30552)

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Renal concentrating ability depends upon the integrity of a variety of anatomic, physiologic and biochemical functions. Apparently specific disorders of the renal concentrating mechanism have been described in potassium depletion(1), during calcium infusion(2-4), and in protein starvation(5-10). While the mechanism of loss of renal concentrating power is not clear in any of these disorders, the least is known about the effect of protein deprivation on renal concentrating ability. Some studies(6-8) suggest that a defect in urea production is responsible, while studies by Hendrikx and Epstein(9) and by Manitius, Pigeon and Epstein(10) indicate that this is an insufficient explanation for the entire mechanism of the loss of renal concentrating ability.

A function specifically related to metabolism of amino acids and proteins could conceivably be the basis of this lesion. Pyridoxine is a major coenzyme of amino acid metabolism. It has several functions and is particularly involved in transamination reactions required for protein synthesis. It is conceivable that the defect in renal concentrating ability of protein starvation is related to a defect in one of the reactions for which pyridoxine is a coenzyme. Pyridoxine deficiency has already been shown to be associated with microcytic anemia(11,12), hyperoxaluria with stone formation(13), and a number of biochemical abnormalities resulting from defective transamination processes(14). Because of our interest in other aspects of pyridoxine metabolism,§ we were able to examine renal concentrating ability in the pyridoxine deficient rat.

Materials and methods. These studies were conducted on male rats of the Sprague-Dawley strain. The animals weighed 107-147 g at start of the experiment, which was carried out over 5 months. The animals were pair-fed throughout the study. All received the same basic vitamin-free diet (vitamin B-Complex Test Diet, free of vit A, D and B complex, from Nutritional Biochemicals Corp.). Control animals received this diet supplemented by a standard "complete" vitamin mixture (Vitamin Diet Fortification Mixture, Nutritional Biochemicals Corp.). The experimental group received this standard vitamin-free diet supplemented with this vitamin mixture free of pyridoxine and its derivatives. Water intake was unlimited.

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