TABLE I. Average Thickness of Mouse Periosteum (in $\mu \pm$ AD) Taken from the Anterior Aspect of the Mid-Shaft of the Femur.*

Age (wk)	1	5	8	26	52
Osteogenic layer	76.9 ± 17.5	53.9 ± 11.3	32.0 ± 6.3	18.5 ± 7.7	14.1 ± 10.5
Periosteum (fibrous + osteogenic layers)	136.3 ± 20.9	90.3 ± 15.3	87.6 ± 14.3	60.8 ± 10.2	48.1 ± 13.2

* 15 areas were measured in each case.

can only be proved by serial killing after a single injection of label.

Summary. An autoradiographic study of cell proliferation in intact and fractured femoral periosteum of mice using H^3-thymidine showed that osteogenic cells are a relatively quiescent cell population awaiting a signal for proliferation and transformation as in fracture repair. Osteogenic cells constitute a self-sustaining cell population, which becomes diminished in size with increasing age. Osteoblasts are in part self-reproducing and in part are produced by transformation of pre-osteoblasts.

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Epinephrine Metabolism in Phenylketonuria.* (26734)

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A possible disturbance in the metabolism of epinephrine in phenylketonuria has been suggested. Cawte(1,2) has demonstrated an increased rise of blood pressure following an intravenous dose of adrenalin among phenylketonurics and attributed this to an impairment of epinephrine production. Weil-Malherbe(3) has shown a decrease of plasma epinephrine and norepinephrine in various forms of mental defect, with phenylketonurics being among the lowest.

The present paper will describe the decrease of dopamine, norepinephrine, and epinephrine in phenylketonuria and discuss evidence for the *in vivo* inhibition of dopa decarboxylase by phenylalanine derivatives.

Materials and methods. Two groups of phenylketonurics were studied. Eight patients† (ages 9-15) had received no specific treatment and 6 patients (ages 3-7) had been treated with low-phenylalanine diet from 6 months to 4 years. Identical studies were carried out in 8 normal and 8 mentally-retarded controls of comparable age and sex to the untreated phenylketonurics.

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TABLE I. Summary of Values in Controls and Phenylketonuries.

	Controls		Phenylketonuries		
	Normal (8)	Retarded (8)	Untreated (8)	Treated (6)	P*
		Plasma			
Phenylalanine (mg %)	1.92 ± 1.83	2.02 ± .76	37.00 ± 4.40	8.46 ± 4.74	<.001
Tyrosine (")	1.95 ± .34	2.08 ± .48	1.93 ± .42	1.05 ± .19	.8
Norepinephrine (μg/l)	3.00 ± .75	3.13 ± .97	1.30 ± .46	1.93 ± .57	.01–.02
Epinephrine (")	1.81 ± .72	1.91 ± .56	.78 ± .23	1.27 ± .40	.02–.05
		Urine			
Dopamine (μg/24 hr)	52.97 ± 4.22	56.35 ± 5.13	26.65 ± 3.18	38.61 ± 2.77	<.001
Norepinephrine (")	9.62 ± 1.70	10.49 ± 3.30	3.46 ± 1.14	6.63 ± 1.02	.005–.01
Epinephrine (")	5.94 ± .39	6.42 ± 1.88	1.70 ± .67	3.33 ± .90	<.001

Numbers in parentheses indicate number of subjects tested. Values expressed as means and stand. dev.

* t test of difference between controls and untreated phenylketonurics.

Twenty-four hour urine specimens were collected in dark bottles containing 10 ml 6N HCl. Urine norepinephrine and epinephrine were determined by the method of von Euler and Lishajko(4) and dopamine by the method of Carlsson and Waldeck(5). During the collection, a blood sample was obtained using heparinized syringes. Plasma phenylalanine was determined by the method of LaDu and Micheal(6), tyrosine by the method of Udenfriend and Cooper(7), and norepinephrine and epinephrine by the method of Price and Price(8).

Results. The data are summarized in Table I. No significant difference exists between the controls of normal intelligence and those who are mentally retarded and the 2 groups may be treated as one. While plasma phenylalanine levels were markedly elevated in the untreated phenylketonurics, no alterations in plasma tyrosine levels could be detected. The decrease of plasma tyrosine in the treated phenylketonurics could in part be attributed to a relative deficiency of tyrosine in the low-phenylalanine diet(9).

There is a uniform decrease of epinephrine-like compounds in the untreated phenylketonurics. In the plasma, the decrease of norepinephrine ($.02<P<.01$) and epinephrine ($.05<P<.02$) is moderately significant. In the urine, the decrease of dopamine ($P<.001$), norepinephrine ($P<.005$) and epinephrine ($P<.001$) is highly significant.

In all instances, this decrease is reversible when the phenylketonuric is treated with a low-phenylalanine diet. The inverse relationship between individual values for plasma phenylalanine and epinephrine-like compounds is shown in Table II. The negative correlation coefficient in each group is highly significant ($P<.001$).

Discussion. Holtz(10) has shown that the primary pathway of epinephrine formation is as follows:

$$\text{Tyrosine} \longrightarrow \text{Dopa} \xrightarrow[\text{Decarboxylase}]{\text{Dopa}} \text{Dopamine} \longrightarrow \text{Norepinephrine} \longrightarrow \text{Epinephrine}$$

A decrease of dopamine, norepinephrine, and epinephrine in phenylketonuria could be caused either by a deficiency of tyrosine(1) or a decrease of dopa decarboxylase(11). In the present study, levels of tyrosine in the plasma appear to be unaltered by the excessive phenylalanine. Although the decrease of epinephrine-like compounds could be caused by a failure of tyrosine to dopa, or by the use of an alternative pathway, it is most likely due to a decrease of dopa decarboxylase. Previously, Hartman *et al.*(12) and

TABLE II. Correlation between Plasma Phenylalanine and Various Epinephrine-Like Compounds.

Compound	Correlation coefficient*
Plasma — Norepinephrine	−.82
Epinephrine	−.65
Urine — Dopamine	−.73
Norepinephrine	−.73
Epinephrine	−.71

* $P<.001$ in all instances.

Fellman(13) have demonstrated *in vitro* inhibition of dopa decarboxylase by phenylpyruvic, phenyllactic, and phenylacetic acids. Since these derivatives of phenylalanine are known to be present in excessive quantities in phenylketonuria and this change is reversed by a diet low in phenylalanine content (13), the present data would appear to provide evidence for the *in vivo* inhibition of dopa decarboxylase.

Pare *et al.*(14) have indicated that the decrease of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in phenylketonuria is due to an inhibition of 5-hydroxytryptophan decarboxylase by phenylalanine metabolites. These findings have recently been confirmed in normal rats made phenylketonuric by feeding excessive amounts of both phenylalanine and tyrosine(15). Studies carried out *in vitro* by Yulwiler(16) and Hess *et al.* (17) have indicated that the pyridoxal-phosphate dependent decarboxylases may represent closely related or identical enzyme systems. The similarity between the changes caused by the derivatives of phenylalanine upon 5-hydroxytryptophan and dopa decarboxylase would seem to provide further *in vivo* evidence for this hypothesis.

Summary. Data have been presented showing a decrease of norepinephrine and epinephrine in the plasma and of dopamine, norepinephrine, and epinephrine in the urine of phenylketonuric children. These changes were reversed when the patients were treated with a low-phenylalanine diet. Since plasma tyrosine levels remained unchanged, this decrease appears to be caused by inhibition of dopa decarboxylase by the derivatives of phenylalanine. This would provide further *in vivo* evidence that the pyridoxal-phosphate dependent decarboxylases represent related or identical enzyme systems.

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Effects of Vitamin B_{12} and Methionine on Excretion of Formiminoglutamic Acid by the Chick.* (26735)

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Young rats fed a diet deficient in Vit. B_{12} were found by Silverman and Pitney(1) to excrete large amounts of formiminoglutamic acid (FGA), a metabolite of histidine that normally is excreted in negligible amounts in urine. They found that supplements of either Vit. B_{12} or methionine caused a drop in FGA

* Part of these data were presented at Fifth International Congress on Nutrition, Sept. 1-7, 1960, Washington, D. C.