cytes as a result of prednisone therapy. In the present study, 2 of 13 patients had lymphocytes which were moderately sensitive to cortisol succinate. The parallel *in vivo* and *in vitro* findings suggest further studies to determine whether there is a correlation between *in vitro* sensitivity of leukemic lymphocytes to cortisol and *in vivo* response of the leukemic patient.

Summary. Cortisol succinate, cortisol, and corticosterone in small doses were toxic to lymphocytes from rat blood and rabbit thymus. The toxicity was indicated by a reduction in *in vitro* survival time of the lymphocytes, according to a slide-chamber method. Minimal toxic effects were produced by 0.01 μg/ml of cortisol succinate and cortisol and by 0.1 μg/ml of corticosterone. In contrast, lymphocytes from the blood of normal individuals and from non-leukemic patients were resistant to 10 μg/ml of cortisol succinate. These experiments suggest a species difference in the sensitivity of lymphocytes from man as compared to those from the rat and rabbit.

Similar tests were done on the lymphocytes from the blood of 13 patients with chronic lymphocytic leukemia or with lymphosarcoma in leukemic phase. In general, the leukemic lymphocytes were more sensitive to cortisol succinate (10 μg/ml) than normal lymphocytes. Two of the 13 patients had lymphocytes which were very sensitive to the corticosteroid.

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Experimental Phenylketonuria in Rats.* (26929)

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Auerbach, Waisman and Wyckoff(1) found that rats fed a diet containing 5% DL-phenylalanine or 2.5% each of DL-phenylalanine and L-tyrosine showed a 2-fold increase in plasma phenylalanine levels and an increased excretion of urinary phenylpyruvic acid. Similar observations were reported recently by Huang *et al.*(2). However, Neill and Langford(3) failed to demonstrate any phenylketonuria in rats fed a synthetic diet containing as high as 3.75% each of L-phenylalanine and L-tyrosine. The slightly elevated plasma phenylalanine levels obtained by previous investigators(1,2) were much lower than those found in phenylketonuric patients or in experimental phenylketonuric monkeys(4,5). The increased excretion of phenylpyruvic acid by rats fed DL-phenylalanine(1,2) is in all probability due to presence of the D-isomer, since D-phenylalanine can be deaminated by D-amino acid oxidase in kidney to form phenylpyruvic acid(6). Therefore, it seemed appropriate to obtain additional information in rats fed excessive quantities of the L-isomer of phenylalanine before the observations made in rats, monkeys and man could be compared biochemically.

Methods and materials. Eighteen-day-old

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TABLE I. Individual* Daily Food Intakes of Rats Kept in Normal and in Light-Dark Rooms.

Normal		Light-Dark	
Total 24 hr	8:00 a.m.-3:00 p.m.	Total 24 hr	8:00 a.m.-3:00 p.m.†
g		g	
16.4	.0	14.3	1.4
16.1	.9	15.3	3.9
19.4	1.4	17.5	4.9
20.0	.4	16.9	3.6
16.2	.2	17.2	3.0
18.4	.0	18.4	3.3
19.4	1.4	13.8	1.7
17.0	1.3	18.7	4.1
18.6	.6	20.2	4.9
17.2	.8	17.5	6.5
Avg 17.9	.7	17.0	3.7

* These values are avg of 3 consecutive days' intake of individual rat.

† The time-switch in Light-Dark room was so arranged that the 3 hr dark period occurred from 11:00 a.m. to 2:00 p.m.

male rats† were fed a ground commercial chow diet with or without L-phenylalanine. The animals were kept in individual cages either in an ordinarily window-lighted animal room or in a dark room equipped with an automatic timeswitch set to have alternate 3 hour light and dark periods throughout 24 hours. This is referred to as the "light-dark room".

Plasma phenylalanine was determined by the method of Udenfriend and Cooper(7). Urine samples were tested qualitatively for phenylketones with 10% ferric chloride or with saturated 2,4-dinitrophenylhydrazine or by the Phenistix‡ method. Quantitative analyses for phenylpyruvic acid were made by the method of Berry and Woolf(8). The phenylalanine hydroxylase activities of the rat livers were determined by the method of Udenfriend and Cooper(9). Food intakes were recorded several times daily at the desired intervals.

Results and discussions. It was previously noted in experimental phenylketonuria in monkeys that total daily intake of phenylalanine, as well as frequent feedings of this amino acid were 2 important factors necessary to produce sustained high plasma phenylalanine levels which in turn caused excretion of urinary phenylpyruvic acid. Since it is known that rats are nocturnal feeders the light-dark room was used in order to increase the frequency of feedings in the rats.

Table I presents daily food intakes of 2 groups of 10 rats fed a normal diet and kept either in a normally-lighted room or in the light-dark room. No significant difference in total daily food intake was observed between these 2 groups. However, the rats kept in the normal room consumed only about 4% of their total intake during the day (8:00 a. m. to 3:00 p. m.) whereas the animals kept in the light-dark room consumed 21% of their intake during the same period which included 3 hours of darkness. The results showed that rats consumed their food mainly during the dark, and that the light-dark cycle stimulated a more frequent intake.

Rats fed diets containing high phenylalanine appeared to change their eating pattern as shown in Table II. Total 24 hour intake remained the same in the 2 rooms at any percentage of phenylalanine added to the diet. The rats fed the higher levels of phenylalanine consumed less diet than at lower levels and these rats ate frequently despite darkness or light in contrast to rats fed normal diet or diets containing less phenylalanine. These changes in food intake cannot now be completely explained, but in all probability are a reflection of the high phenylalanine intake.

Table III presents ranges of plasma phenylalanine values of rats together with body weight and daily phenylalanine intake as af-

TABLE II. Food Intakes* of Rats Fed Diet Containing L-Phenylalanine Kept in Normal or in Light-Dark Rooms.

Phenylalanine in diet, %	Normal Total 24 hr	Normal 8:00 a.m.-3:00 p.m.	Light-Dark Total 24 hr	Light-Dark 8:00 a.m.-3:00 p.m.
	g		g	
.0	17.9	.7 (3.9)†	17.0	3.7 (20.9)
3.5	14.0	1.2 (8.5)	12.8	2.6 (20.2)
5.0	6.2	1.1 (17.7)	6.6	1.5 (22.8)
7.0	8.1	1.9 (23.5)	8.7	2.3 (26.5)

* These values are avg intake of 10 animals for 3 consecutive days at end of 3rd wk except for the group fed 5% L-phenylalanine diet which was recorded at end of first wk.

† Values in parentheses expressed as % of total 24 hr intake.

† Holtzman Rat Co., Madison, Wisc.

‡ Ames Co., Elkhart, Ind.

TABLE III. Correlation of Body Weight and Phenylalanine Intake with Plasma Phenylalanine Levels.

Phenylalanine in diet, %	Duration of exp., wk	Body wt, g*	Daily phenylalanine intake/100 g body wt, g†	Range of plasma phenylalanine, mg/100 ml		Activity§ of liver phenylalanine hydroxylase
				Midmorning	Midafternoon	
			Normal room			
	1	62.0	.06			
0	2	101.3	.06	.9– 1.8‡	.9– 1.8	
	3	151.7	.05			
3.5	2	113.0	.44	2.5– 2.7	1.0– 2.2	
5.0	1	52.0	.60	4.1– 9.9	3.5– 9.9	
	2	84.0	.57	2.2– 5.5	2.8–14.0	
7.0	1	36.0	.74	25.8–38.0	23.4–26.0	
	3	58.0	.98	12.0–34.0	16.5–29.0	
			Light-dark room			
	1	68.0	.07			6.7 ± .3‖
0	2	100.6	.06	.9– 1.8	.9– 1.8	7.3 ± .3
	3	152.3	.04			7.6 ± .3
3.5	2	100.6	.42	2.3– 5.3	3.1– 4.4	3.0 ± .5
5.0	1	50.0	.81	16.5–19.2	20.5–21.1	
	2	76.6	.60	8.2–21.2	6.2–10.7	
7.0	1	39.0	.98	18.2–70.6	17.5–30.2	3.3 ± .5
	3	52.9	1.15	16.0–36.0	12.0–28.8	4.5 ± .8

* Avg initial body weights are 38.5–40.5 g. Ten rats in each group.
† Values based on final body wt and avg daily intake of last 3 days of experiment.
‡ Range of all normal animals studied.
§ μM of tyrosine formed/hr/g of wet liver at 25°C.
‖ Stand. error of mean.

fected by diets containing 3.5, 5, or 7% of the amino acid. Blood samples of some rats were taken in the midmorning and others in the midafternoon to insure an accurate observation of changes in plasma phenylalanine levels as affected by diet. These data indicate that 3.5% L-phenylalanine diet neither affected growth of rats nor influenced plasma phenylalanine values when the animals were kept in the normal room, whereas a slight increase in plasma phenylalanine levels was noted in the animals kept in the light-dark room. It will be remembered that this difference may be due to the frequent feedings of the animals kept in light-dark rooms (Table II).

Rats fed the 5% L-phenylalanine diet did not grow as well as those on normal diet. Significantly higher plasma phenylalanine values than normal were observed in these animals regardless of the room. However the animals kept in the light-dark room appeared to have more consistently elevated plasma phenylalanine levels. Presumably when rats receive various quantities of phenylalanine orally, plasma phenylalanine levels of these animals reach a peak within a short time (less than half hour, unpublished data) then gradually return to normal values. Therefore, animals which receive repeated small dosages of phenylalanine (by frequent feedings in a light-dark room) may not have as high a peak value of plasma phenylalanine but will exhibit persistently elevated plasma phenylalanine values as compared with rats which receive one large feeding of the compound.

Prolonged and extraordinarily high plasma phenylalanine values comparable to those of phenylketonuric patients and monkeys were found in rats receiving the diet containing 7% L-phenylalanine despite the room in which the animals were housed. High plasma phenylalanine values were observed not only in samples taken in the morning but also in samples taken in the afternoon. From the eating patterns of the animals (Table II) it was no surprise that high plasma phenylalanine could be maintained throughout the whole day. However, growth of these rats was adversely affected by this highest level

of L-phenylalanine and 10% mortality was noted in some groups during the first week.

The extraordinarily high plasma phenylalanine values of rats receiving 7% L-phenylalanine may be simply the result of overloading the metabolic capacity of the phenylalanine hydroxylase enzyme system. Freedland, Krakowski and Waisman(10) indicated that excess dietary phenylalanine decreased liver phenylalanine hydroxylase activity of rats kept in a normal animal room. The present results show that lowered phenylalanine hydroxylase activity by excess dietary phenylalanine was also obtained in the rats kept in a light-dark room (Table III). The activity of this enzyme was reduced to 40-60% of the value in the controls regardless of concentration of the amino acid and duration of experiment. The rats grew less on high phenylalanine diets; liver weight expressed as percentage of body weight remained the same. The phenylalanine hydroxylase activity therefore would always be lower in experimental animals compared to normal controls whether the activity was expressed per 100 g body weight or per total liver.

The results in Table III also indicate that concentration of dietary amino acid was more important to attain high plasma phenylalanine values than duration of experiments or than the room in which the animals were placed. This agrees with the findings(11) that concentration of phenylalanine or tryptophan is more important to modify the tissue serotonin content than duration of experiments.

The rats fed diet containing 3.5% L-phenylalanine did not increase excretion of phenylketones in the urine which is in agreement with the slightly elevated plasma phenylalanine values. No consistently increased excretion of urinary phenylketones was observed in rats fed 5% L-phenylalanine diet. However, phenylketones as high as 4 mg phenylpyruvic acid in the 24-hour urine sample were always observed in all animals fed 7% L-phenylalanine diet. Psychological and behavior tests of rats fed the 5 or 7% L-phenylalanine diet kept in light-dark rooms are now being correlated with plasma phenylalanine and urinary phenylpyruvic acid levels.

Summary. Experimental phenylketonuria has been produced in young rats by feeding excessive quantities of L-phenylalanine. Rats fed 7% L-phenylalanine diet showed consistently high phenylalanine values and increased excretion of urinary phenylketones. When fed in a light-dark room, rats fed 3.5%, 5%, or 7% L-phenylalanine showed reduction in liver phenylalanine hydroxylase as compared with rats fed a normal diet.

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