Comment. Triiodothyronine acts to augment erythropoiesis in the rat and dog. This accelerated erythropoiesis has been attributed to the calorigenic properties of the hormone and, thus, the imbalance between total tissue oxygen requirements and red blood cell mass has been postulated as the mechanism by which increased erythropoiesis occurs. The present results demonstrate that triiodothyronine produces increased erythrocyte Fe^{59} incorporation which is not diminished by administration of reserpine, and therefore, presumably is not dependent upon the autonomic hyperactivity produced by thyroid hormone. In the animals given DT^3 and LT^3 the increase in Fe^{59} RBC incorporation was comparable; although oxygen utilization of the LT^3 treated animals was markedly increased in comparison to the DT^3 treated animals (Table II). These results suggest that the mechanism by which triiodothyronine produces increased erythropoiesis is not dependent upon the autonomic effects of the hormone and is not completely dependent upon changes in oxygen utilization.

Summary. LT^3 administration produced an increase in erythrocyte radioiron incorporation in rats. This response was not modified by administration of reserpine. Animals given LT^3 or DT^3 showed comparable increases in erythrocyte radioiron incorporation, however rats given LT^3 demonstrated a marked increase in oxygen utilization when compared to DT^3 treated rats.

1. Evans, E. S., Rosenberg, L. L., Simpson, M. E., *Endocrinology,* 1961, v68, 517.

2. Waldmann, T. A., Weissman, S. M., Levin, E. H., *J. Lab. Clin. Med.,* 1962, v59, 926.

3. Jacobson, L. O., Goldwasser, E., *Brookhaven Symposium on Biology,* 1958, v10, 110.

4. Gallagher, N. I., Hagan, E. Q., McCarthy, J. M., Lange, R. D., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 127.

5. Fried, W., Pizak, L. F., Jacobson, L. O., Goldwasser, E., *ibid.,* 1957, v94, 237.

Received September 16, 1963. P.S.E.B.M., 1964, v115.

Influence of Excess Dietary Phenylalanine on Pregnant Rats and Their Fetuses.* (28926)

DALLAS E. BOGGS AND HARRY A. WAISMAN

Department of Pediatrics, Joseph P. Kennedy, Jr. Laboratory, University of Wisconsin Medical School, Madison

Our earlier publication(1) provided evidence that female rats fed excess quantities of phenylalanine during pregnancy and lactation had normal litter size with no gross abnormalities. Some of the suckling rats died early and this was found to be due to decreased milk flow. The surviving offspring showed slightly elevated plasma phenylalanine levels but no phenylketonuria. This report will provide data on plasma phenylalanine and tyrosine levels of the fetuses and the role of maternal phenylalanine hydroxylase in regulating these plasma values.

Experimental. Pregnant albino rats, 3-5 months old (Holtzman Rat Co.) were fed 5 or 7% L-phenylalanine from about the tenth day of timed gestation.† Comparable pregnant females fed the unsupplemented diet served as controls. The animals were housed in individual cages in a dark-light room previously described(2). On the eighteenth to twenty-first day of pregnancy the rats were anesthetized with ether and blood was obtained directly from the heart. The fetal young were removed by cesarean section and then bled directly from the chest into heparinized tubes. The maternal livers were excised, weighed, and homogenized and a soluble fraction was prepared and assayed for phenylalanine hydroxylase activity according

* Supported in part by grant from Nat. Inst. Mental Health, U.S.P.H.S.

† Onset of pregnancy determined by presence of sperm in vaginal smear.

TABLE I. Phenylalanine Hydroxylase Activity and Phenylalanine and Tyrosine Plasma Levels in Pregnant Rats Fed Excess Phenylalanine. (Means and Standard Deviations.)

Diet	Wt (g)	Phenylalanine hydroxylase activity* /g liver	/100 g body wt	/g protein	Phe mg/100 ml Plasma	Tyr mg/100 ml Plasma
Control (5)	426 ± 21	12.2 ± 2.3	41.6 ± 7.5	152 ± 17	2.7 ± 2.1	1.3 ± 2.2
5% L-phenylalanine (11)	353 ± 53	5.7 ± 2.0	20.3 ± 8.1	71 ± 23	8.7 ± 8.1	3.4 ± 1.5
7% L-phenylalanine (15)	306 ± 69	6.3 ± 3.0	19.5 ± 12.5	72 ± 32	14.5 ± 10.5	6.2 ± 3.2

* Activity is expressed in μmoles tyrosine formed per hour per gram of liver, per 100 g body weight, and per gram of protein.
No. in parentheses represents No. of determinations.

to the method of Freedland, Krakowski, and Waisman(3). This fraction was also analyzed for its protein content according to the method of Lowry *et al*(4). Blood plasma phenylalanine and tyrosine were determined by the method of La Du and Michael(5).

Results. Table I shows blood phenylalanine and tyrosine levels and crude liver phenylalanine hydroxylase activity in the pregnant rats. Blood levels were strikingly elevated in some of the females, but a wide range of values was observed. Phenylalanine hydroxylase activity was lower in the pregnant rats fed phenylalanine than in the control females and, as expected from previous studies in this laboratory(3), these values were consistently lower than those found in male rats.

Phenylalanine and tyrosine levels of fetal rats from some of the pregnant females shown in Table I, along with fetal-maternal ratios for these amino acids, are listed in Table II. The fetal rats from females fed 5 or 7% L-phenylalanine showed plasma phenylalanine and tyrosine levels averaging approximately twice the maternal levels. When maternal levels were high, fetal levels were also high so that the ratios were less variable than the actual plasma levels. The control fetal rats had similar fetal-maternal plasma phenylalanine ratios, but the plasma tyrosine levels were almost 5 times as high as the maternal levels.

Discussion. On the basis of the data presented, feeding L-phenylalanine to pregnant females will not always increase blood phenylalanine levels. Some females exhibit extremely high plasma phenylalanine and others show almost normal levels. It is not known whether this variation is due to fluctuations in blood level of the individual rat or to differences among rats. These factors were minimized by keeping the animals in a dark-light room to encourage uniform eating habits and by taking blood at the same time of day. It seems clear from the data that when the maternal level is high, the fetal plasma phenylalanine is also elevated. Our data also show that when young rats are to be used to study the effects of high fetal phenylalanine levels, it is only necessary to determine the plasma phenylalanine levels of the pregnant female,

TABLE II. Phenylalanine and Tyrosine Plasma Levels in Fetal Rats and Fetal-Maternal Ratios for Pregnant Rats Fed Excess Dietary Phenylalanine. (Means and Standard Deviations.)

Maternal diet	Fetal wt	Plasma phenylalanine Fetal (mg/100 ml)	Fetal-maternal ratio†	Plasma tyrosine Fetal (mg/100 ml)	Fetal-maternal ratio
Control (5)	4.6 ± .9	4.9 ± 1.0	2.2 ± 1.3	5.9 ± 1.5	4.9 ± 1.7
5% L-phenylalanine (5)	4.8 ± 1.3	17.0 ± 15.9	2.3 ± .6	7.7 ± 2.3	2.4 ± 1.1
7% L-phenylalanine (9)*	4.9 ± 1.0	30.5 ± 28.3	2.4 ± .7	10.7 ± 4.5	2.0 ± .6

* No. in parentheses indicates No. of pooled plasma samples analyzed, each sample representing one litter.
† Fetal-maternal ratios were determined for each mother rat and her own litter.

before selecting the animals for the experiment. It is well known that fetal plasma constituents are usually higher than in maternal plasma, but it is noteworthy that the fetal-maternal phenylalanine ratio remains the same when the actual plasma values are raised by feeding L-phenylalanine to the pregnant female.

Tyrosine levels were also raised when phenylalanine was fed, but the fetal-maternal ratio was not so high as in the controls. The high tyrosine levels were expected because considerable phenylalanine hydroxylase activity is present in maternal liver and in fetal livers near the end of gestation(3). When more substrate is provided by the dietary phenylalanine, additional tyrosine accumulates because of the hydroxylase action, but the fetal-maternal ratio of 5 falls to about 2 since the rise in maternal tyrosine was proportionally greater than in the fetus. This change in ratios may indicate that the fetal rats have metabolized some tyrosine through an inducable tyrosine oxidizing system. The activity of the tyrosine oxidizing system is usually very low in fetal rats(6). It is interesting that suckling rats from females fed L-phenylalanine had lower tyrosine levels than normal(1) which could also be explained by induced development of the enzymes of the tyrosine oxidizing system which would reduce plasma tyrosine.

The phenylalanine hydroxylase activity is lowest in the pregnant female rat, followed by higher activity in the non-pregnant female rat. The highest phenylalanine hydroxylase activity observed is in the adult male rat (3). When these groups of animals receive diets containing excess phenylalanine, the activities are about 50% of the unsupplemented rats. It is not surprising therefore, that the pregnant female rats fed phenylalanine have high plasma phenylalanine levels. The tyrosine level is lower than the phenylalanine level, in contrast to the adult male rat, in which the tyrosine level is nearly always greater than the phenylalanine level(7). The tyrosine-phenylalanine ratio is probably a better indication of the difference in activity of the phenylalanine hydroxylase in males and pregnant females than is the plasma phenylalanine level.

From the data it is clear that the fetal plasma phenylalanine levels can be increased, but this did not cause gross abnormalities in surviving newborn pups. It is still likely that *in utero* brain damage resulted from the excess phenylalanine and its abnormal metabolites. Our knowledge of *in utero* brain damage is limited to a few observations on the intelligence of offspring of phenylketonuric patients. There have been reports of apparently normal children born to phenylketonuric women. Jervis(8) described a phenylketonuric mother who delivered 2 normal children. Plasma phenylalanine levels were not reported. Woolf *et al*(9) reported that 2 phenylketonuric sisters had 6 children, all of whom were normal. However, the plasma phenylalanine levels in these 2 sisters were much lower than those usually found in patients with phenylketonuria and both sisters had normal intelligence. Retarded children born to phenylketonuric women have also been reported. Dent(10) reported that Richards had knowledge of a phenylketonuric woman who had 3 grossly retarded children. According to Denniston(11), 5 retarded children were born to a phenylketonuric woman. Three of the children had normal phenylalanine plasma levels. The fourth sibling had left the institution and the fifth had died. Mabry *et al*(12) provided a preliminary report of the family described by Denniston together with another phenylketonuric mother who had a retarded child whose serum phenylalanine was normal. All authors suggest that the retardation is most likely on the basis of *in utero* brain damage from high phenylalanine or its metabolites. While no data are available on the learning ability of rats who were brain damaged *in utero* by high circulating phenylalanine, it has been possible to study rats made phenylketonuric from weaning. Performance by such animals showed deficits in higher-order behavior(13). This is especially important now that some adequately treated phenylketonuric children will be able to lead normal lives and in adult life to have children.

Summary. High blood phenylalanine levels and low phenylalanine hydroxylase activity were found in pregnant female rats fed excess phenylalanine. Fetal plasma phenylalanine levels averaged twice as high as the maternal levels. Tyrosine levels were elevated in pregnant females and in their fetuses. The ratio of fetal tyrosine to maternal tyrosine was lower in phenylalanine fed pregnant rats than in normally fed pregnant rats.

The valuable help of Robert Colwell and Daren McLay is gratefully acknowledged.

1. Boggs, D. E., Waisman, H. A., *Life Sciences,* 1962, v8, 373.

2. Wang, H. L., Waisman, H. A., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 332.

3. Freedland, R. A., Krakowski, M. C., Waisman, H. A., *Am. J. Physiol.,* 1962, v202, 145.

4. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.,* 1951, v193, 265.

5. La Du, B., Michael, P. J., *J. Lab. Clin. Med.,* 1960, v55, 491.

6. Kretchmer, N., McNamara, H., *J. Clin. Invest.,* 1956, v35, 1089.

7. Boggs, D. E., Waisman, H. A., submitted for publication.

8. Jervis, G. A., In report of 23rd Ross Pediatric Research Conf., Nov. 8-9, 1956, p33.

9. Woolf, L. I., Ounsted, C., Lee, D., Humphrey, M., Cheshire, N. M., Steed, G. R., *Lancet,* 1961, v2, 464.

10. Dent, C. E., In report of 23rd Ross Pediatric Research Conf., Nov. 8-9, 1956, p32.

11. Denniston, J. C., *J. Pediatrics,* 1963, v63, 461.

12. Mabry, C. C., Nelson, T. L., Denniston, J. C., Abst. of 73rd Annual Meeting of Am. Pediat. Soc., 1963, p92.

13. Polidora, V. J., Boggs, D. E., Waisman, H. A., PROC. SOC. EXP. BIOL. AND MED., 1963, v113, 817.

Received September 18, 1963. P.S.E.B.M., 1964, v115.

Antimalarials and Other Anticonvulsant Drugs: Predictive Validity of Laboratory Tests. (28927)

FRANKLIN J. ROSENBERG AND JOHN A. BOTTIROLI (Introduced by A. M. Lands)
Department of Pharmacology, Sterling-Winthrop Research Institute, Rensselaer, N. Y.

A convincing number of reports have attested to the efficacy of quinacrine in juvenile petit mal seizures(1-11). Quinacrine has been effective in cases resistant to other treatment (2,9,11) where it has controlled seizures for up to 4½ years(9,11). Pathological EEG's were often normalized(8,11). While petit mal episodes were controlled, major attacks were sometimes precipitated in patients showing no previous major convulsive seizures (2,6). Twenty-four to 72 hours were needed for the drug to exert its effect(1,9,11) and seizure control was sometimes preceded by a period of excitation(1).

The clinical profile of quinacrine then, as an anticonvulsant has been fairly well established and it was considered pertinent to assay quinacrine for its effects on several of the standard laboratory anticonvulsant procedures. Of specific interest was the performance of this compound on the maximal electroshock seizure test (MES), maximal Metrazol (Pentylenetetrazol) seizure test (MMS), and the minimal Metrazol seizure test (mMS). Jenney and Pfeiffer(12) on the basis of their modifications of these tests suggest that pentylenetetrazol provides qualitatively different information from electroshock. According to their hypothesis, compounds exhibiting high anti-pentylenetetrazol activity correlate with clinical effectiveness in juvenile petit mal. High anti-electroshock activity is correlated with grand mal efficacy, and broad spectrum laboratory effectiveness suggests clinical trial for psychomotor and adult petit mal as well as other seizure syndromes. Mitchell and Keasling(13) similarly concluded that there are qualitative differences between electroshock and pentylenetetrazol as convulsant agents. To the contrary, Chen(14) suggests that petit mal drugs be assayed against minimal clonic seizures induced either by pentylenetetrazol or electroshock while grand mal drugs should be tested