Abnormal Phenylalanine Loading Tests in Mothers of Children with Cleft Defects

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The genetic endowment, the nutritional status, and the reaction to stress in the mother have all been proposed as separate etiological factors in producing clefts. The interaction of these factors on the developing fetus is itself probably the relevant factor. However, no biochemical differences to support this thesis have been previously demonstrated in women who produce affected children.

The purpose of this study was to investigate the absorption of phenylalanine by women who had borne children with cleft defects. The amino acid phenylalanine was chosen because of the consistency of excretion of phenolic acids, metabolic end products of this amino acid, in the urine of the women in question, especially p-hydroxyphenylacetic acid. These findings were reported earlier (*7*). Figure 1 shows the metabolic pathway from the phenylalanine to the phenolic acids.

Methods

Water solutions of L-phenylalanine and L-tyrosine, in the concentration of 0.1 g/kg, were given on different days to the subjects after they were on overnight fast.

Samples of blood and urine were taken at 0, 1, 2, and 4 hours. The blood levels of phenylalanine and tyrosine were determined fluorometrically by the methods of McCaman and Robins (*4*) and Waalkes and Udenfriend (*8*), respectively.

Women with no defective children were used as controls. They were chosen to match as closely as possible the age of the mothers of cleft defect children (hereafter abbreviated as MCD).

Results

Table 1 shows the results of the phenylalanine loading tests of fifteen of the MCD and of fourteen controls. The control group had fasting

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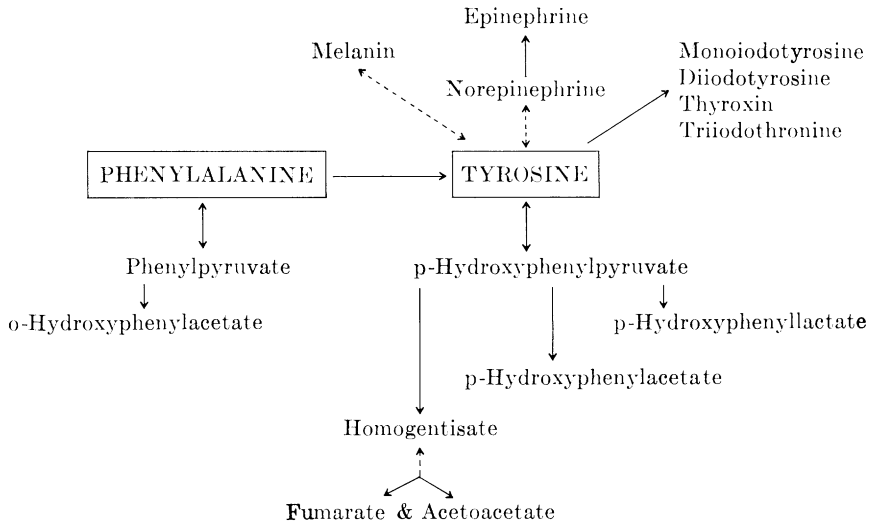

FIGURE 1. The metabolic pathway from phenylalanine to the phenolic acids.

phenylalanine plasma levels that were between 0.6 and 2.2 mg%. The level rose to a peak in either the first or second hour and declined to between 3.5 and 8.7 mg% in the fourth hour. The means of the study group (MCD) are very similar to the controls. One can see that there are some very low flat curves (MCD cases 2, 8, 10, and 12). These women were separated according to the shape of the curve and the data rearranged on Table 2. Group I is comprised of women who did not achieve the arbitrarily chosen value of 6.5 mg% phenylalanine plasma one hour after the dose. Group II is those women who achieved this level after one hour. Group I has four of the fifteen MCD, while none of the control women fell in this category. Table 2 compares the means of the three categories for the phenylalanine load. The tyrosine responses after the phenylalanine load are shown in Table 3. Group I exhibits a low tyrosine throughout the four hours, increasing slightly up to four hours. Group II is not very different from the controls.

Discussion

The concept of a maternal biochemical problem causing a defect in the infant is not new (*1*). McCarrison (*5*), in 1921, postulated that an endocrine disturbance due to nutritional deficiencies may cause malformations of the fetus. In 1943, Warkany and associates (*9*) showed that it was possible to produce cleft palate in rats through controlled dietary deficiencies.

Strean and Peer (*6*) studied 228 mothers of children born with cleft defects and concluded that stressful situations contribute to the etiology of clefts. They wrote:

TABLE 1. Plasma phenylalanine levels after phenylalanine loading (0.1 g/kg L-phenylalanine). Entries are 0, 1, 2, 4 hours after dosage.

case	*0*	*1*	*2*	*4*
controls				
1*	0.6	7.5	5.2	5.2
2	1.2	13.6	8.5	3.5
3	1.7	7.5	9.6	5.5
4	1.3	6.6	7.4	3.8
5	1.5	19.5	16.3	8.7
6†	1.4	13.3	10.5	5.2
7	1.5	13.2	11.6	6.7
8	0.8	10.5	6.5	4.9
9	0.9	14.5	8.5	7.7
10	1.0	11.1	7.7	4.6
11	1.7	14.0	14.1	7.4
12	1.4	13.3	9.3	5.5
13	—	20.9	14.9	7.6
14	2.2	21.6	12.5	8.2
mean	1.3	13.3	10.1	6.0
MCD				
1	1.8	15.3	17.8	8.9
2	1.7	4.0	2.8	2.5
3	1.5	13.9	10.6	4.5
4	1.7	17.6	17.6	10.1
5	1.5	9.4	8.0	7.2
6	1.0	16.9	13.4	5.0
7	1.3	17.7	12.3	7.8
8*	1.0	4.2	5.8	5.2
9	0.9	10.3	5.9	2.7
10	0.5	6.4	7.0	2.9
11†	1.3	13.1	9.4	5.6
12	1.7	3.2	2.4	7.4
13	1.4	15.4	9.5	8.1
14	1.0	13.0	8.0	4.6
15	2.0	7.6	7.1	4.1
mean	1.3	11.2	9.2	5.7

* Average of two tests.
† Average of three tests.

Stressor agents are known to influence adrenal cortical activity via the anterior pituitary gland. The resulting stress is reflected in the quantity of circulating hydrocortisone. Since excess of this hormone inhibits fibroblastic proliferation and produces histochemical changes in the collagen fibers, it is possible that the threshold for such catabolic effects in the developing embryo is lower for pregnant women in whom a predisposition to cleft palate exists. In the absence of such genic influence, severe stress between the eighth and eleventh weeks of pregnancy may produce an abundance of hydrocortisone capable of initiating teratogenic effects.

TABLE 2. Comparison of plasma phenylalanine responses to phenylalanine loading (0.1 g/kg L-phenylalanine). Entries for controls show mean plus or minus 1 SD.

category	*number*	*hours after dosage*			
		0	*1*	*2*	*4*
controls	14	1.3 ± 0.4	13.3 ± 4.6	10.1 ± 3.2	6.0 ± 1.6
Group I	4	1.2	4.4	4.5	4.5
Group II	11	1.4	13.9	10.9	6.2

TABLE 3. Blood tyrosine levels after phenylalanine loading (0.1 g/kg L-phenylalanine).

category	*number*	*hours after dosage*			
		0	*1*	*2*	*4*
controls	14				
mean		1.6	2.5	3.0	3.1
range		(0.9–2.3)	(2.0–2.9)	(2.2–3.5)	(1.9–3.8)
MCD group I	4				
mean		1.1	1.7	1.8	2.1
range		(0.7–1.6)	(1.2–2.5)	(1.4–2.5)	(1.4–3.1)
MCD group II	11				
mean		1.6	2.3	2.7	2.9
range		(1.0–2.2)	(1.5–4.1)	(2.0–3.6)	(1.8–5.1)

This thesis has been supported by the work of Harris and others (*3*).

It may be that the response to the loads reflects a more basic defect. For example, the appearance of phenylalanine and tyrosine in the plasma after a phenylalanine load is very slow in four of the women tested. It is not sufficiently impaired to cause any outward clinical signs. One can speculate, however, that in the period of rapid growth and development of the fetus, a transient requirement of phenylalanine-tyrosine stores in the bodies of the women may seriously deprive the fetus of these amino acids. As can be seen in Figure 1, tyrosine is a precursor of epinephrine, norepinephrine, melanin, and thyroxin.

The occurrence of cleft palate or cleft lip with Waardenburg's syndrome has been reported by many authors and most recently by Giacoia and Klein (*2*). The incidence of cleft problems in Waardenburg's syndrome is approximately ten per cent and often occurs with heterochromic iridium and white forelock. The latter are probably due to faulty deposition of melanin. Melanin is another product of tyrosine metabolism, as seen in Figure 1. This is an intriguing syndrome in that it exhibits a visible link of cleft defects and an abnormality of tyrosine metabolism

TABLE 4. Patient data arranged according to phenylalanine response.

case	*race, sex of propositus*	*rank of birth*	*age of mother at birth of propositus*	*age of father at birth of propositus*	*type of defect*
			Group I		
2	W/F	1	23	23	complete cleft lip & palate
8	W/F	2	22	25	complete bilateral cleft lip & palate
10	W/F	2	21	23	complete bilateral cleft lip & palate
12	W/F	3	21	21	complete bilateral cleft lip & palate
			Group II		
1	W/M	1	18	19	bifid uvula & higharch
3	W/M	4	36	35	complete lip & palate
4	W/M	10	34	38	partial paralysis & higharch, mentally retarded
5	W/M	1	21	24	left lip & alveolus
6	N/M	2	27	37	soft palate only
7	W/M	2	23	31	soft palate only
9	W/M	1	23	—	micotia-unilateral
11	W/M	3	38	38	left lip & palate
13	W/F	4	31	32	soft palate only
14	W/M	3	26	28	left lip & palate
15	W/F	2	22	27	palate only

in the midline of the skull. It is interesting to note the arrangement of the types of cleft defects with the different responses to phenylalanine loads as seen in Table 4.

Further speculation is imprudent. More investigation is needed on the genetic, biochemical, and environmental factors which seem to act in concert to produce defective children.

Summary

Oral phenylalanine loads were given to fifteen mothers of children with cleft defects and fourteen control women. The plasma was examined at 0, 1, 2, and 4 hours for phenylalanine and tyrosine. Four of the fifteen mothers exhibited unusual tolerance curves; that is, the phenylalanine levels did not rise appreciably over the four hours nor did the tyrosine increase commensurate with the controls or the other eleven mothers. The possible implications of these findings are discussed.

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