

# AN ASSESSMENT OF THE HISTIDINE-LOADING (FIGLU) TEST IN INFANCY

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In 1956 Broquist<sup>1</sup> first used the formiminoglutamic-acid (Figlu) test in clinical medicine as a parameter of folic acid deficiency. To improve the sensitivity of the test, Luhby *et al.*<sup>2</sup> suggested the use of histidine loading. Within 6 years, reports were readily available on the value of the Figlu test in the assessment of folic-acid deficiency,<sup>3-9</sup> but little had been written concerning its use following histidine loading in infancy.<sup>10, 11</sup>

In December 1962 we investigated the haemopoietic deficiencies existing in non-White infants with gastro-enteritis. The histidine-loading (Figlu) test was used as one parameter in the assessment of folic-acid deficiency. At the commencement of the study, as the publications on this test in infancy were not available to us, a load of 2.5 G of histidine was decided upon by inference from the adult dose of 15 G.<sup>12</sup> Since this load could well have been excessive, especially in infants where enzyme function might be suspect, it was considered necessary to evaluate the test in asymptomatic infants.

## MATERIAL AND METHODS

The Figlu test was performed on 34 asymptomatic non-White infants attending welfare clinics, day creches or admitted to the surgical wards for minor surgical procedures. Using the classification of malnutrition of Gomez *et al.*<sup>13</sup> applied to the Boston 50th percentile, 21 of these infants were well-nourished, 10 suffered from first-degree malnutrition and 3 from second-degree malnutrition.

Urinary Figlu was estimated by the method of Kohn *et al.*<sup>12</sup> using conventional voltage electrophoresis on cellulose acetate strips. The Figlu spot was graded from a trace to 4+. Anything above a trace was regarded as positive.

18 infants were given a histidine load of 1 G and 16 a load of 2.5 G before the determination of Figlu excretion (Table I).

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Where the urinary Figlu test was negative, those who were initially loaded with 1 G of histidine received a subsequent

TABLE I. INCIDENCE OF POSITIVE FIGLU'S IN WELL BABIES UNDER ONE YEAR OF AGE

Age months	Group A Initial load of histidine 1 G		Group B Initial load of histidine 2.5 G	
	No. of cases	No. of positives	No. of cases	No. of positives
Under 3	5	1	5	4
3-6 ..	5	4	4	4
6-9 ..	5	1	4	2
9-12 ..	3	1	3	2
Total ..	18	7	16	12

TABLE II. EFFECT OF AN INCREASED HISTIDINE LOAD ON CHILDREN WITH INITIALLY NEGATIVE FIGLU'S

Age months	Group A		Group B	
	Initial load 1 G No. of negative Figlu's	Increased load 2.5 G Resultant positives	Initial load 2.5 G No. of negative Figlu's	Increased load 4-5 G Resultant positives
Under 3	4	1	—	—
3-6 ..	1	1	—	—
6-9 ..	4	1	2	0
9-12 ..	2	0	1	0
Total ..	11	3	3	0

One infant under three months of age (group B, Table I) who had a negative Figlu test did not receive an increased histidine load.

load of 2.5 G and those who had previously received 2.5 G of histidine were given a 4 G or 5 G load (Table II). All infants who at any stage showed a positive Figlu test received 0.4 mg. of folic acid daily for 14-28 days, when the urinary Figlu test was repeated using a 2.5 G load of histidine in all cases (Table III).



TABLE III. THERAPEUTIC TRIAL OF FOLIC ACID 0.4 MG. DAILY FOR 14-28 DAYS\*

Age (months)	Complete follow-up		Incomplete follow-up	
	No. of positive Figlu's	Resulting negative Figlu's	No. of cases	Result
Under 3	3	3	3	Figlu test less positive in all three
3-6 ..	9	8		
6-9 ..	4	4		
9-12 ..	3	3		

\*One case received 5 mg. daily for 10 days.

TABLE IV. RE-ASSESSED INCIDENCE OF POSITIVE FIGLU'S IN EACH AGE GROUP ON A LOAD OF 2.5 G OF HISTIDINE

Age (months)	Incidence %
Under 3	60%
3-6	100%
6-9	44%
9-12	50%
Over-all incidence	64.7%

#### RESULTS

The Figlu test was positive in 64.7% of cases.

The incidence of positive Figlu tests found at 3-monthly age intervals up to 1 year is recorded (Table IV). Three infants negative on a loading dose of 1 G of histidine became positive on increasing the loading dose to 2.5 G. In the group given an initial load of 2.5 G of histidine, 3 showing negative results, who were subsequently loaded with 4 G and/or 5 G of histidine, remained negative (Table II).

All except 1 of the 19 infants who were given a therapeutic trial of folic acid showed negative Figlu tests when retested after 14-28 days. In a further 3 cases where follow-up was incomplete, there was a reduction in the amount of Figlu excreted (Table III).

In the 21 well-nourished infants, 71% had positive Figlu tests and in 13 of the infants who were first- or second-degree malnourished, 54% were positive.

#### DISCUSSIONS

It is apparent from Table II that the size of the histidine load to some extent influenced the number of positive results obtained in the Figlu test, since increasing the load (group A) to 2.5 G resulted in a further 3 positive results. What is not clear is whether the loading dose of 2.5 G of histidine was excessive or whether it is in fact an optimum dose in infants of this age for the demonstration of folic-acid deficiency. The almost complete reversal of positive tests to negative following a therapeutic trial with folic acid, when a 2.5 G histidine load was used in the repeat test, would seem to suggest that the original loading dose even at the 2.5 G level was not too great (Table III).

The therapeutic dose of 0.4 mg. of folic acid daily was selected on the basis of the work of Marshall and Jandl.<sup>14</sup> Subsequently several authors have claimed that 0.4 mg. may be an excessive dose in the treatment of folic-acid deficiency and have recommended doses of the order of 0.1-0.2 mg. a day.<sup>15,16</sup> Even smaller dosage has been recommended by others.<sup>17</sup> On the other hand, Hansen and Weinfeld<sup>16</sup> have reported that patients with a positive Figlu test owing to folic-acid deficiency sometimes required fairly large doses of folic acid to convert a positive Figlu test to a negative one. On the whole it seems unlikely that

the Figlu test in these cases was converted to negative on the basis of a folic-acid overload.

In one instance the Figlu test remained positive despite large doses of folic acid. This case is being further investigated and may well be similar to cases described by Chanarin.<sup>18</sup>

The specificity of a positive Figlu test as an indication of folic-acid deficiency has been questioned.<sup>6,12,19</sup> Using the method of Kohn *et al.*<sup>12</sup> a positive Figlu test might have resulted from the presence of either iron deficiency or B<sub>12</sub> deficiency in these infants. In view of the effectiveness of the therapeutic trial it is unlikely that these deficiencies were responsible for the positive Figlu tests.

It is not clear whether the Figlu test indicated significant folic-acid deficiency in these asymptomatic infants as no other parameters of folic-acid deficiency were investigated. In a series of infants with gastroenteritis, 70% agreement was found when the results of bone-marrow examinations and Figlu tests were compared.<sup>20</sup>

Dormandy *et al.*<sup>21</sup> found a 75% incidence (12 out of 16 cases) of positive Figlu tests in control infants under 1 year of age following a histidine load of 3 G. In 5 out of 12 cases the serum-folate level was low. The evidence from the study on infants with gastroenteritis and that of Dormandy *et al.*<sup>21</sup> suggests that the Figlu tests following histidine-loading in infancy may be a significant criterion of folic-acid deficiency.

The development of folic-acid deficiency in non-White infants under 1 year of age is not difficult to understand. Invariably the infants are on a diet containing very little folic acid. Cow's milk or breast milk with a folic-acid content of 2 µg./l.<sup>22</sup> was the only source of folic acid in most of these infants. Proprietary milks contain even less folic acid, thereby further reducing the intake of this vitamin, when this form of feeding is used.

Ascorbic-acid deficiency is thought to contribute to the development of megaloblastic anaemia.<sup>23</sup> In South Africa, proprietary milk products (with the exception of a few brands which are too expensive to be popular with low-income groups) are not supplemented with vitamin C.<sup>24</sup> No clear differentiation could be drawn between breast-fed and artificially-fed infants in this trial.

It may be argued that, because 13 of these infants were underweight for age, the results obtained could be attributed to generalized undernutrition and not to a simple dietary deficiency of folic acid. That this is not so is suggested by the finding of positive Figlu tests in 71% of the well-nourished group and only 54% in the less well-nourished group.

It is interesting to note that the maximal incidence of positive Figlu tests was obtained in the 3-6 months age group (Table IV). At this stage enzyme function should be approaching normal, maternal supplies have been utilized, growth has increased the demand for folic acid and locally the milk diet is usually still not supplemented.

The interpretation of the histidine-loading (Figlu) test and the significance to be attached to it when done in very young infants must be approached with caution. Obviously much more experience of the test in a variety of circumstances and under critical survey must be built up before a positive test is acceptable by itself as an indication of folic-acid deficiency.



If it can subsequently be proven that a positive Figlu test in infants under 1 year of age has significance, it would mean that 64.7% of asymptomatic non-White infants are folic-acid deficient. This is an unexpected finding and requires further investigation before folic-acid supplementation is generally advised.

## SUMMARY

In 64.7% of asymptomatic non-White infants under 1 year of age, positive urinary-Figlu tests were obtained following histidine loading. These became negative following a therapeutic trial of folic acid, suggesting that the histidine load of 2.5 G was not excessive. The implications of a positive Figlu test in infancy are discussed.

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