Urinary excretion of histidine derivatives in megaloblastic anaemia and other conditions and a comparison with the folic acid clearance test

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SYNOPSIS Urinary excretion of urocanic acid and formimino-glutamic acid in abnormal amounts following oral doses of histidine has been observed not only in most cases of megaloblastic anaemia, but in iron-deficiency anaemia, haemolytic anaemia, and neoplastic disease.

The urinary excretion of these substances is increased in those disease states in which an abnormally rapid rate of removal from the plasma of intravenously injected folic acid has been demonstrated.

The excretion of a histidine derivative subsequently identified as formimino-glutamic acid in the urine of folic-acid-deficient rats was reported by Silverman, Bakerman, and Daft (1951). The urinary excretion of the same substance in man was reported by Broquist and Luhy (1957), Luhy, Cooperman, and Teller (1959), Chanarin (1959), Spray and Witts (1959), and Kohn, Mollin, and Rosenbach (1961). Urocanic acid, the precursor substance of formimino-glutamic acid, almost invariably appears in the urine with formimino-glutamic acid and sometimes may be the only histidine derivative present (Bennett and Chanarin, 1961): Urocanic acid has been identified in trace amounts in normal urine by Acheson, Paul, and Tomlinson (1958) and in kwashiorkor by Whitehead and Arnowit (1961).

All these reports have emphasized the excretion of formimino-glutamic acid in megaloblastic anaemia, particularly the group thought to be due to ‘folic-acid deficiency’. Urocanic acid and formimino-glutamic acid are excreted in a wide variety of disease states. This paper reports our results on the urinary excretion of these histidine derivatives in control subjects and in patients with megaloblastic anaemia and other conditions. The results have been compared with the results of the folic acid clearance test (Chanarin, Mollin, and Anderson, 1958a).

MATERIALS AND METHODS

Urocanic acid and formimino-glutamic acid were estimated spectrophotometrically (Chanarin and Bennett, 1962). The results are expressed as the urinary excretion of formimino-glutamic acid in the eight hours following an oral dose of 15 g. of histidine HCl. Significant amounts of histidine derivatives did not appear after eight hours. All the urocanic acid present is converted to formimino-glutamic acid by urocanase in the liver enzyme preparation used in the assay.

The folic acid clearance test was carried out by giving 15 μg. of folic acid per kilogram of body weight intravenously. Ten ml. blood was taken after 15 minutes and the serum folic acid level was measured microbiologically using Str. faecalis (ATCC 7083) as the test organism (Chanarin et al., 1958a). The serum folic acid level 15 minutes after the injection in normal subjects varies from 21 to 80 μμg./ml.

One hundred and fifty-four tests were carried out on 26 normal control subjects and 56 patients. Untreated megaloblastic anaemias accounted for 26 patients (Addisonian pernicious anaemia 11, idiopathic steatorrhoea 4, post-gastrectomy 3, Crohn’s disease 1, pregnancy 2, carcinoma 2, associated with D.L.E. 1, associated with rheumatoid arthritis 1, and ‘nutritional’ 1). The other 30 patients were suffering from conditions with normoblastic haemopoiesis (iron-deficiency anaemia 8, acute leukaemia 6, hereditary spherocytosis 3, chronic lymphocytic leukaemia 1, myeloma 1, osseous tuberculosis 1, chronic myelofibrosis 2, carcinomatosis 2, idiopathic steatorrhoea 1, intestinal resection 2, marrow aplasia due to chloramphenicol 2, and acanthocytosis 1).

UROCANIC ACID AND FORMIMINO-GLUTAMIC ACID EXCRETION

IN NORMAL SUBJECTS Following an oral dose of 15 g. histidine hydrochloride the urinary excretion of
urocanic acid and formimino-glutamic acid varied from 1 to 17 mg. per day with a mean of 9 mg. (Fig. 1).

**In megaloblastic anaemia** Figure 1 also illustrates the excretion in patients with various forms of megaloblastic anaemia.

**Vitamin B_{12} deficiency** Abnormal amounts of urocanic acid and formimino-glutamic acid were excreted by seven of 11 patients with Addisonian pernicious anaemia (range 4 to 87 mg., mean 27 mg.). There was no relationship to the degree of anaemia, the two most severely anaemic subjects with haemoglobin concentrations of 4·2 and 6·1 g. per 100 ml. respectively excreting between 6 and 16 and 15 to 17 mg. of histidine derivatives in serial tests respectively. On the other hand two patients with subacute combined degeneration of the cord and normal haemoglobin concentrations (13·0 and 13·2 g. per 100 ml.) excreted 27 to 33 and 87 mg. of urocanic acid and formimino-glutamic acid respectively.

The results in three patients with megaloblastic anaemia following partial gastrectomy were 7 to 12, 37 to 42, and 38 to 87 mg. respectively, and 32 to 59 mg. in one patient with megaloblastic anaemia following resection of gut for regional ileitis.

**Folic acid ‘deficiency’** Abnormal results were obtained in four patients with idiopathic steatorrhoea. The urinary excretion ranged from 130 to 1,500 mg. (mean 648 mg.). The results were abnormal in two patients with megaloblastic anaemia in pregnancy, the excretion being 120 mg. and 97 to 326 mg. respectively. Two patients with carcinoma of the bronchus and megaloblastic anaemia were studied. One patient excreted between 115 and 153 mg.; the excretion in the second was only 5 mg. Abnormal results were also found in three patients in whom none of the usual causes of megaloblastic anaemia were found. One had disseminated lupus erythematosus (histidine derivatives 440 mg.); a second had rheumatoid arthritis (histidine derivatives 360 mg.), and the third had no associated illness (‘nutritional’ megaloblastic anaemia) with an excretion varying between 87 and 178 mg. Patients with leukaemia and

**FIG. 1.** The combined excretion of urocanic acid and formimino-glutamic acid in the urine following oral doses of 15 g. of histidine hydrochloride in normal subjects and in patients with megaloblastic anaemia. All the observations in each group are shown; the number of patients studied is given in the text.
Urinary excretion of histidine derivatives in megaloblastic anaemia and other conditions

FIG. 2. The combined excretion of urocanic acid and formimino-glutamic acid in the urine, following oral doses of 15 g. of histidine hydrochloride in various groups of patients. All the observations in each group are shown; the number of patients studied is given in the text.

megaloblastic haemopoiesis are considered in the next section.

IN MISCELLANEOUS CONDITIONS Figure 2 shows the results obtained in various miscellaneous conditions.

Iron-deficiency anaemia Abnormal results were found in four of eight patients studied. The range of excretion was 3 to 57 mg. (mean 21 mg.).

Leukaemia Observations were made on six patients with acute myeloblastic leukaemia. None had received any form of specific therapy and three showed well-marked evidence of megaloblastic erythropoiesis. The initial diagnosis in two of these had been megaloblastic anaemia. The excretion of histidine derivatives was normal in five of the six patients, including three with megaloblastic haemopoiesis. The last patient excreted between 930 and 2,800 mg. of histidine derivatives of which only traces were formimino-glutamic acid and the rest urocanic acid.

One patient with chronic lymphocytic leukaemia excreted 41 mg. of histidine derivatives in the urine.

Hereditary spherocytosis Three patients were studied before splenectomy. The excretion was 33 to 62 mg., 20 to 36 mg., and 11 to 16 mg. in these three cases respectively.

Others Observations on the excretion of histidine derivatives were made on one patient with idiopathic steatorrhoea who had no haematological abnormality and was well clinically (28 and 29 mg.); one patient with tuberculosis of the spine and ribs (181 to 628 mg.); two patients with chronic myelofibrosis (9 and 60 to 91 mg.); three patients with neoplastic disease (carcinomatosis 13 to 14 mg., myeloma 2 mg., and Hodgkin's disease 55 mg.); two patients with small intestinal resection (16 and 90 mg.); two patients with marrow aplasia following chloramphenicol (15 to 29 mg. and 160 to 230 mg.) and one patient with acanthocytosis (46 mg.).

COMPARISON OF RESULTS OF THE FOLIC CLEARANCE TEST AND THE URINARY EXCRETION OF HISTIDINE DERIVATIVES

The urinary excretion of histidine derivatives was tested first and the folic acid clearance test was carried out one to three days later. The results of observations in four normal subjects, in 12 patients
with megaloblastic haemopoiesis, and in nine other patients are shown in Fig. 3.

With one exception, an abnormal excretion of histidine derivatives was always accompanied by an abnormally rapid folic acid clearance. The one exception was a woman of 70 with hereditary spherocytosis who excreted between 33 and 62 mg. of urocanic acid and formimino-glutamic acid. The serum folic acid level 15 minutes after the clearance dose, however, was 40 μg. per ml. which is well within the normal range. On the other hand, of five patients whose excretion of histidine derivatives was within the normal range, each had an abnormally rapid folic acid clearance. These were three patients with Addisonian pernicious anaemia, including two with very low haemoglobin concentrations, one patient with bronchial carcinoma and megaloblastic haemopoiesis, and one patient with chronic myelofibrosis.

**DISCUSSION**

The results confirm that following oral doses of histidine abnormal amounts of histidine derivatives appear in the urine in the great majority of patients with megaloblastic anaemia requiring treatment with folic acid. However, this is not confined to this group of megaloblastic anaemias. Abnormal amounts of histidine derivatives were also found in the urine in Addisonian pernicious anaemia (Chanarin, 1959) as well as in patients with B₁₂ deficiency due to gastrectomy and Crohn's disease.

Abnormal excretion of histidine derivatives was also noted in iron-deficiency anaemia, haemolytic anaemia, and neoplastic disease; these are all conditions in which an abnormally rapid folic acid clearance test has been observed (Chanarin et al., 1958a and b). In general these two tests give abnormal results in the same disease states. The results, however, do not parallel each other in all cases. The ability to metabolize single carbon units such as formimino groups depends on normally functioning folic acid coenzymes. These may function abnormally in folic acid deficiency, in B₁₂ deficiency, or in patients receiving folic acid antagonists. In the B₁₂-depleted experimental animal the excretion of formimino-glutamic acid is abolished by methionine supplements (Silverman and Pitney, 1958; Fox, Ludwig, and Baroody, 1961).
In most patients with megaloblastic anaemia there is probably depletion of folic acid stores as well as an inability to metabolize single carbon units in a normal manner, and both the rate of folic acid clearance and the urinary excretion of histidine derivatives are abnormal. In a significant proportion of patients, however, the rate of clearance of injected folic acid is abnormally rapid, but the excretion of histidine derivatives is within the normal range (Fig. 3). Subclinical folic acid deficiency is common, particularly in late pregnancy (Chanarin, M. C. G. Gibbon, O'Sullivan, and Mollin, 1959), and in many conditions associated with rapid cell turnover. In this group the folic acid clearance test is probably the most sensitive index of folic acid depletion available. Not only does the excretion of histidine derivatives appear to be a less sensitive index of folic acid deficiency but in the present state of our knowledge the results are often unpredictable. Thus a normal excretion of histidine derivatives was encountered in three patients with Addisonian pernicious anaemia, two of whom were severely anaemic, and in one patient with megaloblastic haemopoiesis associated with carcinomatosis and a normal serum $B_{12}$ level. All these patients cleared injected folic acid from the plasma very rapidly. Their failure to excrete urocanic acid and/or formimino-glutamic acid in abnormal amount is difficult to explain at the moment. Histidine in man is metabolized with a high degree of efficiency even when there is interference with folic acid function. The folic-acid-deficient rat excretes about 50% of isotopically labelled histidine given orally as formimino-glutamic acid (Tabor, Silverman, Mehler, Daft, and Bauer, 1953; Rabinowitz and Tabor, 1958). Results in man, however, show that even with oral doses of histidine as large as 15 g. (10 times the normal daily intake) the urinary excretion of histidine derivatives rarely exceeds 7% of the oral dose in megaloblastic anaemia due to folic acid deficiency, and is usually of the order of 0.5 to 2%.

Finally, one patient with hereditary spherocytosis excreted abnormal amounts of histidine derivatives but had a normal rate of folic acid clearance. Such a state can be anticipated in patients having methotrexate (Chanarin and Bennett, 1962), in patients with megaloblastic anaemia due to anticonvulsant drugs (Chanarin, Elmes, and Mollin, 1958; Chanarin, Laidlaw, Loughridge, and Mollin, 1960), and in some patients with megaloblastic anaemia requiring treatment with folic acid in whom the usual causes of folic acid deficiency are absent (Chanarin, 1959).

In all these patients it has been suggested that folic acid stores are normal, and the anaemia is the consequence of the action of a folic acid antagonist.

We wish to thank the physicians of St. Mary's Hospital for permission to study the patients admitted under their care, and Dr. E. Lester-Smith for a gift of formimino-glutamic acid.

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doi: 10.1136/jcp.15.3.269

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