Effect of Histidine on the Urinary Excretion of 4-Aminoimidazole-5-carboxamide

We have recently shown[1] that patients with folic acid and/or vitamin B_{12} ($FA–B_{12}$) deficiency have an increased excretion of 4-aminoimidazole-5-carboxamide (AIC) compared with anaemic patients who are not so deficient, or with non-anaemic hospital patients. A FIGLU test, using an oral load of 15 g L-histidine hydrochloride, was performed on many of these patients as a diagnostic procedure; since a haematological response has been observed in such patients following histidine dosage[2,3], which would imply a partial correction at least of the disordered nucleoprotein metabolism present in these deficiencies, we have taken the opportunity to investigate its effect on the urinary excretion of AIC, itself an intermediary in purine synthesis. For this purpose urine passed between 4 and 8 h after histidine dosage was examined (Period 3); urines collected during one hour in the morning immediately before the test (Period 2) and in some cases for a similar 5-h period the previous afternoon (Period 1) served as controls. For comparison, similar collections were made from 8 normal adult volunteers and 3 patients who had anaemia not associated with $FA–B_{12}$ deficiency. No dietary restrictions were imposed during the test. Urines were preserved with 1 ml. 6 N HCl per h of the save and stored at 4° C. AIC was determined by an ion-exchange chromatographic technique[4]. In order to minimize any errors in the timing of the urine collections all results were expressed as AIC μg/mg creatinine.

Table 1 shows the mean AIC excretion in the two groups. In the 11 subjects without $FA–B_{12}$ deficiency the excretion was significantly less in Period 2 than in either Periods 1 or 3 ($P < 0.01$ in each case), but Periods 1 and 3 did not differ significantly. We therefore conclude that histidine does not normally affect AIC excretion and regard the low values in Period 2 as resulting from the diurnal variation reported elsewhere[4]. In the 11 patients with $FA–B_{12}$ deficiency where excretion in all three periods was measured, no statistically significant difference in excretion was found between these periods. In these subjects, however, values were slightly lower rather than higher in Period 3 as compared to Period 2. Inclusion of an additional number of subjects from whom urine was collected only over Periods 2 and 3 raised the total to 26, and the fall in AIC excretion during Period 3 was shown to be significant at the 2 per cent level. We therefore provisionally conclude that histidine does produce a fall in AIC excretion in subjects with $FA–B_{12}$ deficiency. However, the study of larger and more sharply defined subgroups within this broad classification would be desirable, as would further information on diurnal variations in the excretion of AIC and creatinine.

All the above subjects with $FA–B_{12}$ deficiency showed, by virtue of an increased excretion of formiminoglutamic acid and/or urocanic acid following histidine, evidence of a defect in the metabolism of C_1 units. It is difficult to visualize any role for AIC in purine synthesis in man which does not involve C_1 units, and we consider that this metabolic defect must account at least in part for the high excretion of AIC in such subjects, reported previously[1]. The provision of an increased number of potential C_1 units in the form of histidine might well, as a mass action effect, promote a more efficient utilization of AIC, accompanied by a decrease in its excretion. The resulting increase in purine synthesis might provide a basis for explaining the haematological response noted in megaloblastic anaemia following histidine dosage.

Table 1. Urinary AIC Excretion
(μg/mg creatinine; mean + S.E.)

	No. subjects tested	Pre-histidine Period 1 (afternoon)	Pre-histidine Period 2 (morning)	Post-histidine Period 3 (afternoon)
Normals and non-deficient anaemic patients	11	0·62 ± 0·029	0·50 ± 0·029	0·65 ± 0·029
Folic-acid and/or vitamin-B_{12}-deficient patients	11	2·09 ± 0·120	1·84 ± 0·120	1·77 ± 0·120
	26	—	2·20 ± 0·164	1·59 ± 0·164

If the high rate of excretion of AIC in anaemia is regarded as due to an impairment in the formylation of AIC ribotide in the absence of adequate quantities of folic acid, as occurs in sulphonamide inhibited micro-organisms, our experimental findings would all appear to be adequately accommodated. If this were the only abnormality, however, an overall depression of purine synthesis would be implied, whereas the exact opposite appears to be the case as judged from recent studies. The finding that patients with vitamin B_{12} deficiency, for example, excrete a smaller than normal percentage of orally administered AIC has led to the suggestion that interference with feedback control of purine synthesis may mean that the shunt mechanism of by-passing nucleic acids in purine biosynthesis is functioning at a rate well above normal in these subjects[5]. Interpretation of AIC excretion is further complicated by the possibility that AIC might be a product of purine degradation. If such degradation were inhibited by histidine, as has been shown to occur in micro-organisms[6], the effects of the latter on the haematological status and on the excretion of AIC could be explained.

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Lathyrism and 7,12-Dimethylbenzanthracene-induced Carcinoma in the Rat

The lathyritic agent, β-amino-poprionitrile (BAPN), reduces the tensile strength of mesenchymal tissue and modifies the collagen content[1,2]. Since local tissue factors are believed to be of importance in tumour growth, BAPN was administered to rats in which tumours were induced by a polycyclic hydrocarbon. 7,12-Dimethylbenzanthracene (DMBA) was used because it regularly induces mammary carcinoma in young female Sprague–Dawley rats. The strain of rat, the age, and the hormonal status are important factors in the induction of mammary carcinoma[3].

50-day-old female Sprague–Dawley rats were given a purified diet with or without addition of 0·5 per cent BAPN. 20 mg 7,12-dimethylbenzanthracene in sesame oil was administered intragastrically at the beginning of the experiment in a single dose[3]. The time of induction, rate of growth and number of tumours were noted for 150 days. Urine samples were collected at 60–70 days after the beginning of the experiment. Oestrogenic substance was extracted, chromatographed on a 3·5 per cent hydrated alumina, and measured by a micro-Kober reaction[5,6].

The animals were divided into three groups: Group I, intact female rats; Group II, DMBA; Group III, DMBA and BAPN. The lathyritic rats (Group III) developed tumours at an earlier stage than the control rats (Group II). The tumours in the lathyritic group grew faster and the total weight of tumour mass was significantly greater than in the Group II rats. Lathyritic rats did not show any significant increase in the number of tumours. The results are summarized in Table 1.