URINARY IMIDAZOLES IN RHEUMATOID ARTHRITIS

BY

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This is a study of a small group of patients with rheumatoid arthritis, in whom the urine excretion of imidazole compounds was examined by Figlu assay and chromatography of 12-hour urine collections following 15 g. histidine load, and by assay of 4-amino-imidazole-5-carboxamide (AIC) in 12-hour urines with and without histidine load.

Gough, McCarthy, Read, Mollin, and Waters (1964) found low serum folate in one-third of cases with rheumatoid arthritis and a raised Figlu excretion in three quarters of these. This, they thought, was biochemical evidence of folic acid deficiency. 33 per cent. of their cases had serum folates of less than 4 ng. per ml., but many other patients with folates between 3 and 7 ng. per ml. had a positive Figlu result.

Abnormalities of liver function in rheumatoid arthritis are known but perhaps not well recognized. Darby (1956) found abnormal bromosulphophthalein tests in 23 per cent. cases of rheumatoid disease and urine coproporphyrin was elevated in 31 per cent. cases. Proliferation of reticulo-endothelial tissue is recognized in rheumatoid disease and accounts for lymph gland enlargement, splenic enlargement, Kupffer cell hyperplasia, and sometimes reticulum cell hyperplasia in the bone marrow (Wardle and Attan, 1967).

Methods

The patients examined were hospitalized with active rheumatoid arthritis. Ten patients were given a Figlu test and had a serum folate estimation; they were then given folic acid 10 mg. daily for at least 3 days and the Figlu test was repeated. Four other patients missed the repeat test. Amino-imidazole-carboxamide (AIC) was determined in control 12-hour urine collections and again after histidine load on these same rheumatoid patients. AIC was also estimated on 12-hour urine collections without histidine load in a group of cases of megaloblastic anaemia due to vitamin B₁₂ or folic acid deficiency and in a miscellaneous group of patients with reticuloses and leukaemias.

Serum Folate was assayed with *Lactobacillus casei*, using "Difco folic acid casei" medium, and was regarded as low when less than 3 ng. per ml.

Excretion of Figlu was assayed as the combined assay of Chanarin and Bennett (1962) for urocanic acid and Figlu combined. Values greater than 40 mg. Figlu in 12 hours. (Noeypatimanond, Watson-Williams, and Israels, 1966) were considered abnormal.

Chromatograms of Imidazole Compounds were run after desalting of urine in n-butanol/acetic acid water, followed by n-butanol/pyridine water and stained with Pauly's reagent, and the R_f values were compared with a standard table (Smith, 1958). Excretion was assessed semi-quantitatively by the size and intensity of the spots. For urocanic acid excretion a positive grading was given when the urinary level approximated to at least 5 µg./ml. urine.

Imidazole Pyruvic Acid in the same urines was detected qualitatively by Phenistix testing and quantitatively by an arsenate-borate technique (Lin, Pitt, Civen, and Knox, 1958).

4-amino-imidazole-5-carboxamide was estimated by the method of Coward and Smith (1965) by absorption from urine on Dowex 50, elution with aqueous pyridine, and analysis of the extract for non-acetylatable diazotisable amines using α-naphthyl-ethylene diamine reagent. The normal values obtained had a mean value of 284 µg. in 12 hours (S.D. ± 150).

Results

Table I gives the results of the Figlu assay together with the serum folate results and the basic haematological data for fourteen rheumatoid cases, including ten pairs which were studied. Cases 1, 2, and 3 have raised Figlu values that are unchanged after folic acid, therapy, and are indeed accompanied by normal serum folate levels, but all have marked iron deficiency anaemia, as shown by a microcytic hypochromic blood film and very low serum iron. Cases 4, 5, 6, and 7 have low serum folate results and yet normal Figlu tests, but are only mildly anaemic, as is usual in active rheumatoid disease. Cases 8 to 14 have normal Figlu values. Hence, in this small series, iron deficiency and not folate deficiency was correlated with impaired Figlu metabolism. Moreover, this was true iron deficiency and not the normochromic anaemia that accompanies active rheumatoid disease.

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TABLE I
COMPARISON OF FIGLU EXCRETION WITH SERUM
FOLATE AND HAEMATOLOGICAL STATUS

Case No.	Urinary Figlu Excretion (mg./12 hrs)	Serum Folate (ng./ml.)	Haemoglobin (g./100 ml.)	Red Cells
1	47.7 52.0	4.25	9.3	Hypochromic iron deficient
2	38.0 44.0	3.62	8.7	Hypochromic iron deficient
3	191.0 150.0	3.5	4.3	Hypochromic iron deficient
4	9.55 8.05	2.6	11.0	Normochromic
5	15.1	2.78	12.0	Normochromic
6	31.3	2.72	12.0	Normochromic
7	7.8	1.49	10.0	Normochromic
8	13.9 17.1	3.62	14.0	Normochromic
9	30.8 18.6	3.57	11.4	Normochromic
10	5.75 2.64	4.0	10.2	Hypochromic
11	8.0 8.6	3.47	15.8	Normochromic
12	20.0 12.0	3.15	16.9	Normochromic
13	24.4 12.0	3.0	12.0	Hypochromic
14	3.5	2.69	12.0	Normochromic

Table II indicates that in seven of the fourteen cases urocanic acid, as detected by paper chromatography, was the principal imidazole metabolite in the urine. Half of the cases also showed diversion of some of the histidine load to imidazole-lactic acid, but no imidazole-pyruvic acid was found and there is no reason to suspect an acquired histidase deficiency. However, as indicated, a relative deficiency of urocanase may be present. This does not seem to depend upon the folate status.

Table III gives the results of the assay for AIC. The values for the rheumatoid cases are clearly higher than those for the normal group, but are

TABLE II
PAPER CHROMATOGRAPHY OF URINARY
IMIDAZOLES

Folate	Urocanic Acid	Imidazole Propionic Acid	Imidazole-lactic Acid	Imidazole-pyruvic Acid
Normal	5/9	4/9	4/9	0/9
Low	2/5	0/5	4/5	0/5

The numbers indicate the proportion of cases with positive values.

unchanged after histidine load, and they are of the same order as the values for the cases of megaloblastic anaemia. However, the rheumatoid group contained no cases of frank folic acid deficiency. For comparison the results on a large group of cases of reticuloses and leukaemias are also given.

Discussion

A deficiency of the enzyme Figlu transferase has been postulated in cirrhosis of the liver and in rheumatoid disease (Luhby and Cooperman, 1964) and (Vitale, Streiff, and Hellerstein, 1965) have produced experimental evidence of impairment of this enzyme in iron deficiency in rats. Such a deficiency could explain the three cases of raised Figlu with iron deficiency anaemia. Furthermore, Hoffbrand, Neale, Hines, and Mollin (1966) have noted that patients with pure folate deficiency excrete Figlu but not urocanic acid, whereas patients with hypoproteinaemia or other forms of liver damage excrete urocanic acid because of urocanase deficiency. This would explain the chromatographic finding of urocanic acid in half of the patients.

The high values for amino-imidazole-carboxamide excretion found in some cases of rheumatoid disease must reflect an increased purine turnover, as do elevated values in some cases of folic acid or of vitamin B₁₂ deficiency. This may indicate that patients with rheumatoid disease are under continuous stress, as were the cases of Coward and Smith (1965), but may also be due to the increased activity of the reticulo-endothelial tissues. The

TABLE III
URINARY AMINO-IMIDAZOLE-CARBOXAMIDE (AIC) EXCRETION

Category		No. of Patients	AIC Excretion (μg./12 hrs)	
			Mean and Standard Deviation	Range
Normal		15	284 ± 150	60 — 580
Megaloblastic	(B ₁₂ or folate deficiency)	11	1,110 ± 620	560 — 2,640
Rheumatoid Arthritis	Before histidine	9	1,240 ± 1,060	80 — 3,120
	With low folate	3	1,027 ± 232	860, 860, 1,360
	After histidine	14	1,430 ± 910	320 — 3,500
Reticuloses and Leukaemias	Normal B ₁₂ or folate	24	336 ± 198	90 — 850
	Low B ₁₂ or folate	24	1,180 ± 280	270 — 1,240

serum uric acid levels in cases of rheumatoid arthritis are no higher than in the general population.

AIC is synthesized from glycine and ultimately forms the purine inosine. Histidine loading would, therefore, not be expected to have any influence and the results in these rheumatoid patients bear this out.

Summary

In a group of patients with rheumatoid disease raised urinary Figlu excretion after histidine load was associated, not with folic acid deficiency, but with iron deficiency anaemia. Half the patients

excreted a significant amount of urocanic acid in the urine. 4 amino-imidazole-5-carboxamide (AIC) excretion was high in some rheumatoid patients and this may be due either to stress or to enhanced turnover of purines by a hyperplastic reticulo-endothelial system, as the levels are of the same order as those found in megaloblastic anaemias. AIC excretion is not affected by histidine load.

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Les imidazoles urinaires dans la polyarthrite rhumatoïde

RÉSUMÉ

Chez un groupe de malades atteints de maladie rhumatoïde, une excrétion de l'acide formiminoglutamique urinaire élevée après une forte dose d'histidine était associée, non pas à une déficience d'acide folique, mais à une anémie causée par un manque de fer. La moitié des malades avait excrété une quantité importante d'acide urocanique dans l'urine.

L'excrétion de 4 amino-imidazole-5-carboxamide était élevée chez certains patients rhumatoïdes et cela peut être due ou au stress ou à un métabolisme accru des purines par un système réticulo-endothélial hyperplastique, comme ces taux sont du même ordre que ceux trouvés dans les anémies mégalo-blastiques. L'excrétion de 4 amino-imidazole-5-carboxamide n'est pas affectée par la forte dose d'histidine.

Imidazolas urinarias en la poliartritis reumatoide

SUMARIO

En un grupo de pacientes con enfermedad reumatoide, el aumento de la excreción Figlu en la orina al cabo de una carga de histidina se asoció, no con deficiencia de ácido fólico, sino con anemia producida por deficiencia de hierro. La mitad de los pacientes excretaron una cantidad significativa de ácido urocánico en la orina. La excreción de 4 aminoimidazola-5-carboxamida (AIC) fue alta en algunos pacientes reumatoides y esto quizá se deba a *stress* o a mayor producción de purinas mediante un sistema reticuloendotelial hiperplásico, ya que los niveles son del mismo orden que aquellos hallados en anemias megaloblasticas. La excreción de AIC no es afectada por la carga de histidina.