Haem biosynthesis studied in patients with rheumatoid arthritis

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SYNOPSIS In a study of the urinary excretion of haem precursors in patients with rheumatoid arthritis, iron-deficiency anaemia, and in healthy controls, certain differences were found.

In iron-deficiency anaemia the excretion of both porphobilinogen and δ -aminolevulinic acid was increased, whereas in patients with rheumatoid arthritis only the porphobilinogen excretion was increased.

A further study on the erythrocyte activity of δ -aminolevulinic acid dehydrase showed a higher activity in the erythrocytes from patients with rheumatoid arthritis compared with healthy controls.

In most of the literature concerning inflammatory anaemias a distinction is made from uncomplicated iron-deficiency anaemia (Nilsson, 1948; Jeffrey, 1953; Cartwright, 1968). The anaemia is characterized as a normochromic normocytic anaemia of varying degree. Our own studies on patients with active rheumatoid arthritis did not display any findings suggestive of a more prominent iron-deficiency component in these patients. Thus the erythrocyte indices were only slightly decreased, and the mean corpuscular haemoglobin did not vary with the clinical activity of the disease. The total iron-binding capacity was subnormal and impressively decreased in comparison with the TIBC values from patients with uncomplicated iron deficiency. The gastrointestinal uptake of ^{59}Fe was subnormal in arthritic patients, although signs of unimpaired erythrocyte iron utilization were found expressed as a percentage of the absorbed dose (Strandberg, 1966). We found empty iron bone marrow stores in only 20% of 65 arthritic patients (unpublished study) determined using histochemical and chemical methods (Johansson, Plantin, Strandberg, and Uusma, 1970). According to the literature (Richmond, Gardner, Roy, and Duthie, 1956; McCrea, 1958; Weinstein, 1959; Jeffrey, 1961), the frequency of empty bone marrow iron stores lies between 0 and 50% in patients with rheumatoid arthritis.

Heilmeyer (1964) reported increased urinary excretion of δ -aminolevulinic acid (ALA) and

porphobilinogen (PBG) in patients with uncomplicated iron-deficiency anaemia. We first performed a comparative study of the urinary excretion of these haem precursors in patients with uncomplicated iron deficiency, in patients with active rheumatoid arthritis without signs of iron deficiency, and in healthy controls.

As we found significant differences between the groups the study was extended to a comparison of the erythrocyte activities of the enzymatic steps of haem synthesis with special reference to the δ -aminolevulinic acid dehydrase activity.

Subjects

Patients with rheumatoid arthritis in this study fulfilled the diagnostic criteria of the American Rheumatism Association (Kellgren, 1962). All were inpatients in the department of rheumatology of this hospital and all had active disease. Patients with iron deficiency were diagnosed according to the tests of Bainton and Finch (1964). The controls were healthy laboratory staff and blood donors without signs of infection or iron deficiency.

Methods

Haemoglobin concentration was determined in capillary blood as cyan methaemoglobin (van Kampen and Zijlstra, 1961) (100% = 15.3 g/100). The volume of packed red cells was determined

with an International haematocrit centrifuge, type MB.

Reticulocytes were counted with a supravital staining method. One thousand cells were counted. No corrections for decreased numbers of red cells were made.

The serum iron concentration was determined in an AutoAnalyzer according to a method described by Zak and Epstein (1965).

To estimate total iron-binding capacity, serum was saturated with $\text{Fe SO}_4 \cdot (\text{NH}_4)_2 \text{SO}_4$ at pH 7.5 and surplus iron was eliminated with an ion exchange resin (Amberlite IRA 400). The iron content in the saturated serum was then determined by the same method as for serum iron.

Porphobilinogen and δ -aminolevulinic acid in urine were determined according to the method of Mauzerall and Granick (1956). Initially the results were expressed as mg/g creatinine, depending on the uncertainty in collecting 24-hour volumes of urine. However, as the excretion of creatinine in arthritic patients was subnormal (arthritic patients: $n = 15$, mean 90.5 mg/100 ml, SD 63.5; iron-deficient patients: $n = 9$, mean 162.8 mg/100 ml, SD 35.0; controls: $n = 8$, mean 143.8 mg/100 ml, SD 99.2), we decided to express the figures as mg precursor/100 ml urine.

The δ -aminolevulinic acid dehydrase activity was determined in nitrogen atmosphere according to Nakao, Wada, and Yano (1968). The 'total enzymatic activity' was determined in the same way but in air atmosphere. After incubation we extracted the copro- and protoporphyrin and determined them fluorometrically against a coproporphyrin standard and expressed the activity in arbitrary units.

Copro- and protoporphyrin in erythrocytes were determined according to Wranne (1960). Our values are not corrected for recovery, and are thus about 30% lower than with correction.

Results

Table I demonstrates that the degree of anaemia is the same in the two groups of patients. There is a clear-cut difference in the TIBC values among patients with rheumatoid arthritis and uncomplicated sideropenia.

In the first part of our study we compared the excretion values for ALA and PBG in urine for the three groups described in Table I. For reasons mentioned above and discussed later we expressed the values as mg per 100 ml of urine. It is obvious from Table II that patients with rheumatoid arthritis differ both from healthy controls and iron-deficient patients in their excretion pattern. The quotient δ -ALA/PBG for iron deficiency was 3.7, for healthy

	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>Range</i>
<i>Hb (g/100 ml)</i>				
Rheumatoid arthritis	21	11.5	1.47	9.1-14.0
Sideropenia	11	11.0	2.46	5.7-13.1
Controls	9	13.4	0.90	12.4-14.8
<i>Haematocrit (%)</i>				
Rheumatoid arthritis	17	38.5	4.27	29.6-44.4
Sideropenia	11	33.8	5.46	22.7-40.5
Controls	8	40.3	2.18	37.5-43.4
<i>MCHC</i>				
Rheumatoid arthritis	17	30.3	2.12	25-34
Sideropenia	11	32.3	3.77	25-37
Controls	8	33.5	1.20	32-35
<i>Reticulocytes (per 1,000)</i>				
Rheumatoid arthritis	17	24.1	10.12	8-46
Sideropenia	8	20.6	15.10	6-50
Controls	8	12.8	4.83	8-21
<i>Serum Iron ($\mu\text{g}/100 \text{ ml}$)</i>				
Rheumatoid arthritis	21	42.9	30.58	10-114
Sideropenia	11	61.3	41.34	18-162
Controls	9	96.4	25.43	52-140
<i>TIBC ($\mu\text{g}/100 \text{ ml}$)</i>				
Rheumatoid arthritis	21	269.2	46.21	198-360
Sideropenia	11	438.5	56.84	366-525
Controls	9	319.3	37.20	258-360
<i>Iron Saturation (%)</i>				
Rheumatoid arthritis	21	15.8	10.47	3-41
Sideropenia	11	13.8	9.07	4-38
Controls	9	30.9	9.83	14-47

Table I Haematological values for patients with rheumatoid arthritis and sideropenia and healthy controls

	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>Significance of Difference between Patients and Controls</i>	
				<i>t</i>	<i>P</i>
<i>ALA (mg/100 ml)</i>					
Rheumatoid arthritis	21	0.227	0.098	1.5	$P < 0.05$
Control	8	0.271	0.058		
Iron deficiency	11	0.439	0.151	3.4	$P > 0.001$
<i>PBG (mg/100 ml)</i>					
Rheumatoid arthritis	20	0.119	0.055	4.1	$P < 0.001$
Control	8	0.061	0.018		
Iron deficiency	11	0.119	0.053	3.4	$P > 0.001$

Table II The urine concentrations of δ -aminolevulinic acid (ALA) and porphobilinogen (PBG) in patients with rheumatoid arthritis, with iron deficiency, and in healthy controls

controls 4.4, and for patients with rheumatoid arthritis 1.9. In an attempt to evaluate further this increase in PBG but not in δ -ALA excretion we decided to study the concentrations of copro- and protoporphyrin in red blood cells as well as ALA dehydrase and 'total enzymatic activity' in a group of patients with rheumatoid arthritis and healthy controls. Table III displays an elevated protoporphyrin concentration and δ -ALA-dehydrase activity in the erythrocytes from patients with rheumatoid arthritis.

	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>Significance of Difference between Patients and Controls</i>	
				<i>t</i>	<i>P</i>
<i>Photoporphyrin (μg/100 ml erythrocytes)</i>					
Rheumatoid arthritis	45	22.18	12.75	6.57	< 0.001
Control	50	8.99	4.61		
<i>Coproporphyrin (μg/100 ml erythrocytes)</i>					
Rheumatoid arthritis	44	0.169	0.139	1.19	> 0.05
Control	50	0.141	0.075		
<i>ALA-dehydrase Activity (μmol/min/g Hb)</i>					
Rheumatoid arthritis	32	0.397	0.137	7.6	< 0.001
Control	83	0.206	0.087		
<i>Total Enzymatic Activity (arbitrary units)</i>					
Rheumatoid arthritis	46	0.714	0.489	1.2	> 0.05
Control	49	0.802	0.182		

Table III The concentration of proto- and coproporphyrin and the activity of δ -aminolevulinic acid dehydrase (ALA-dehydrase) and 'total enzymatic activity' in erythrocytes from patients with rheumatoid arthritis and healthy controls

Discussion

According to our present knowledge, there exists a feedback control of the biosynthesis of haem with an inhibition, particularly of δ -LA synthetase activity, exerted from haem and to a lesser degree from protoporphyrin (Burnham and Lascelles, 1963). The mechanism is certainly the same for the synthesis of haemoproteins in the liver. When there exists a defect in the synthesis with an accumulation of haem or protoporphyrin one would expect a more or less pronounced rise in the excretion of both δ -ALA and PBG as described by Heilmeyer (1964) and found by us in the patients with iron deficiency. One should also expect a slightly lower quotient between δ -ALA and PBG than in healthy controls. That is what we found for patients with iron deficiency, but for rheumatoid arthritis the quotient was extremely low. The reason why we decided to relate the values for δ -ALA and PBG to creatinine is the well known fact that δ -ALA is well stabilized in acid but poorly in alkaline urine, whereas the stability for PBG is in the opposite direction. If one performs the determinations on fresh urinary samples and relates the values to creatinine it is possible to avoid the error exerted by a poor stability in precursors. As all patients had normal renal function the concentration values will give a representative picture of the excretion of haem precursors.

The clear-cut and significant elevation of δ -ALA dehydrase activity in the patients with rheumatoid arthritis could possibly be the effect arising from a pronounced elevation of the reticulocyte mass, as reticulocytes have a higher activity of δ -ALA dehydrase activity than mature red cells (Gibson,

Neuberger, and Scott, 1955). In our material there was no difference between patients with rheumatoid arthritis who had as a mean 17.1 per 1,000 reticulocytes and the controls with 11.6 per 1,000 reticulocytes. The elevated δ -ALA dehydrase activity might be the result of factors not directly involved in the biosynthesis of haem. We have not determined the δ -ALA dehydrase activity in patients with uncomplicated iron-deficiency anaemia, but one might assume that the excretion values of the precursors mirror the concentration of precursors in haem-synthesizing cells. From the clear-cut differences in the excretion pattern between the two groups of patients there is no reason to believe that iron-deficient patients should have the same elevation of dehydrase activity. In fact, Nakao, Wada, and Yano (1968) have described three cases of iron-deficiency anaemia with δ -ALA dehydrase activity within or in the neighbourhood of the normal range.

Of the other parameters tested there was a moderate elevation of the concentration of protoporphyrin in red blood cells. The elevation of protoporphyrin concentration in red blood cells of patients with iron-deficiency anaemia is usually much more pronounced.

Our results are different from those described by Gutniak, Kopeć, and Nieczaj (1971). Their patients with rheumatoid arthritis seem to have a high degree of iron deficiency which is the most reasonable explanation of the different results.

This as well as our previous studies indicates that the anaemia of rheumatoid arthritis differs markedly from iron-deficiency anaemia.

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