

Plasma erythropoietin assay in patients with chronic renal failure

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SYNOPSIS Using the post-hypoxic mouse method of assay, values for erythropoietin in the plasma of patients with chronic renal failure were equal to, or greater than, normal values. Results suggest that the source of erythropoietin may be primarily extrarenal. Normal renal tissue, provided in the assay by the intact mouse, is required for activation of the hormone. There still remains to be explained the enhanced erythropoietic response to haemorrhage or hypoxia that can occur in anephric man.

For some years it was believed that the plasma of patients with chronic renal failure contained no measurable erythropoietin (Penington, 1961; Brown, 1965). There have been some reports to the contrary (Gallagher *et al*, 1959; Naets and Wittek, 1968; Mirand and Murphy, 1970). Results similar to our own, though lacking normal control values, have been obtained by Gral *et al* (1972). The following report presents values for erythropoietin in the plasma of normal subjects and in the plasma of patients with chronic renal failure who were receiving regular haemodialysis.

Material and Methods

Samples of blood for estimation of normal values were taken from laboratory volunteers and collected into commercial lithium heparin containers (Searle Diagnostic Ltd, High Wycombe). The plasma was separated and used immediately or stored at -20°C until it was required for assay when it was thawed at room temperature before use. Plasma from patients with chronic renal failure was collected similarly just before dialysis.

The blood haemoglobin and the packed cell volume were measured in normal volunteers. In patients with chronic renal failure the blood pressure was recorded to correlate with the erythropoietin assay, and an account was made of all drugs that were given at the time. A full peripheral blood

picture was obtained with relevant biochemical parameters.

Plasma erythropoietin was measured by the post-hypoxic mouse method (Camiscoli and Gordon, 1970; Cotes, 1971). Standard human urinary erythropoietin B was obtained from the National Institute for Biological Standards. Values were expressed as percentage uptake of ^{59}Fe per millilitre dose of plasma in the test animals.

Results

NORMAL VALUES

Values for erythropoietin in 38 samples of normal plasma were: mean $7.11 \pm 6.73\%$ ^{59}Fe uptake per ml. The figures were equivalent to 0.32 ± 0.28 units of standard human urinary erythropoietin B. Values for 21 samples of plasma from normal males were $7.43 \pm 6.73\%$ ^{59}Fe uptake per ml, and for 17 samples from normal females $6.79 \pm 4.42\%$ ^{59}Fe uptake per ml; males: females = $P > 0.70$.

CHRONIC RENAL FAILURE

Values for erythropoietin in 30 samples of plasma from 26 patients with chronic renal failure were: mean $7.62 \pm 6.74\%$ ^{59}Fe uptake per ml; the figures were equivalent to 0.34 ± 0.28 units of standard human urinary erythropoietin B (table). There was no significant difference between values for normal plasma and plasma from patients with chronic renal failure ($P > 0.70$).

No correlation was found between the values for

No.	Sex	Age	Blood Pressure (kPa)	Blood Urea (mmol/l)	Hb (g/dl)	PCV (%)	EPO	Drugs
1	M	41	17.3/10.6	29.9	6.6	21	3.7	Valium
Chronic pyelonephritis								
2	M	28	20.0/13.3	16.6	6.6	18	22.6	Methyl dopa, propranolol chlorothiazide
Malignant hypertension								
3	M	40	20.0/11.3	26.9	8.2	25	2.6	Oxprenolol, clonidine, allopurinol, Pabrinex
Glomerular nephritis								
4	M	40	17.3/ 8.6	12.6	5.9	18	0.5	Allopurinol
'End stage kidney'								
5	M	24	21.3/13.3	23.2	6.2	18	10.1	Chlorothiazide, propranolol, methyl testosterone
Post-malarial nephrosis								
6	M	36	18.6/10.6	23.4	7.0	20	3.1	Nil
Chronic pyelonephritis								
7	M	37	18.6/12.0	26.5	12.5	38	5.5	Nil
Polycystic kidneys								
8	M	59	17.3/12.0	16.6	9.5	27	19.8 14.9	Nil
Obstructive uropathy								
9	M	25	10.6/ 5.3	11.8	13.9	42	6.2	Nil
'End stage kidney': red cell volume 21 ml/kg; plasma volume 44 ml/kg								
10	M	33	21.3/10.6	28.7	9.0	26	14.3	Methyl testosterone
'End stage kidney'								
11	M	21	16.0/ 9.3	19.9	5.8	17	0.3	Methyl testosterone, Decadurabolin
Fanconi syndrome								
12	M	56	16.6/11.4	14.9	4.3	14	21.3 22.9	Methyl testosterone
Obstructive uropathy: intramuscular haemorrhage								
13	M	42	16.0/11.4	19.9	9.1	27	9.3	Carbimazole, allopurinol propranolol, Decadurabolin
Post-malarial nephrosis: thyrotoxicosis								
14	M	22	21.3/11.3	16.6	5.9	18	4.7	Propranolol
Alport's syndrome								
15	M	22	18.6/12.8	12.6	6.6	19	6.0	Propranolol, Decadurabolin Methyl testosterone
Alport's syndrome: twin to No. 14								
16	M	45	21.3/15.7	29.9	9.7	29	5.2	Propranolol, allopurinol
Membrano-proliferative nephritis								
17	M	16	16.0/ 8.0	21.6	7.5	23	6.0	Propranolol, allopurinol
Haemolytic-uraemic syndrome: anephric								
18	M	56	18.6/12.0	16.6	8.8	24	1.2	Methyl testosterone
Polycystic kidneys								
19	M	47	24.0/13.3	29.9	7.4	20	2.2	Prednisolone, cyclophosphamide bethanidine
Proliferative nephritis								
20	F	23	20.0/12.0	13.3	7.2	21	6.6	Cyclophosphamide, propranolol
Proliferative nephritis								
21	F	30	20.0/13.5	10.3	5.8	17	5.3	Valium
Analgesic nephropathy								
22	F	30	18.6/11.4	8.3	5.6	18	9.7	Nil
Post-irradiation: unilateral kidney								
23	F	37	17.3/13.3	29.9	6.4	18	1.6	Prednisolone, imuran, Decadurabolin
Lupus erythematosus								
24	F	53(a)	22.0/13.3	11.6	11.8	39	3.1	Bethanidine
Red cell volume 29 ml/kg; plasma volume 56 ml/kg; arterial oxygen 9.3 kPa								
		53(b)	24.6/11.3	33.2	8.3	19	10.7	
Malignant hypertension								
25	F	36	18.6/12.8	23.2	7.8	22	0.0	Nil
'End stage kidney'								
26	F	38	16.0/ 6.6	14.9	4.3	12	6.2	Nil
Malignant hypertension: pre-nephrectomy								

Table Investigation of plasma from 26 patients with chronic renal failure

EPO = plasma erythropoietin % ⁵⁹Fe uptake per ml

All patients received oral iron and folic acid and injections of Neocytamen.

Normal values for blood haemoglobin and packed cell volume:

males Hb 15.3 ± 0.8 g/dl; PCV 45.3 ± 3.2%

females Hb 14.0 ± 1.5 g/dl; PCV 41.2 ± 4.0%

the blood haemoglobin ($r = 0.10$), packed cell volume ($r = 0.12$) or blood urea ($r = 0.13$) and the plasma erythropoietin. There was no demonstrable difference in values for the blood haemoglobin or plasma erythropoietin values in patients who were treated with methyl testosterone compared with others who did not receive methyl testosterone ($P > 0.2$; > 0.5). Some of the patients showed unusual features and these are described below.

Patient 9

This man suffered an accidental intramuscular haemorrhage and his blood haemoglobin fell to 4.3 g/dl. At the same time the plasma erythropoietin measured 21.3 and 22.9% ^{59}Fe uptake per ml, in two separate assays.

Patient 24

Eighteen months after bilateral nephrectomy for the management of malignant hypertension, this woman had a blood haemoglobin of 11.8 g/dl, and packed cell volume 39%. Blood volume studies showed a red cell volume of 24.8 ml/kg (normal 20-30 ml/kg) with plasma volume 56 ml/kg (normal 40-50 ml/kg). The plasma erythropoietin measured 3.2 and 3.0% ^{59}Fe uptake per ml in two separate assays. There was no evidence of pulmonary insufficiency but the arterial oxygen saturation was 9.3 kPa (normal 13.3-16.6 kPa). Twelve weeks later the blood haemoglobin was 8.3 g/dl; plasma erythropoietin then measured 10.7% ^{59}Fe uptake per ml.

No clinical evidence of haemorrhage or hypoxia was found in patients 2 and 8 to account for the high values for the plasma erythropoietin. Patient 9 had relatively high values for the blood haemoglobin, packed cell volume, and red cell volume with a normal plasma erythropoietin level; again, no reason could be found for the results.

Discussion

NORMAL VALUES

Although there are many reports of values for plasma erythropoietin in patients with various haematological disorders, few series include normal values. Those using the post-hypoxic mouse method of assay include Bourgoignie *et al* (1968) with values in 12 normal subjects of $0.60 \pm 0.05\%$ ^{59}Fe uptake per ml, and Whitcomb *et al* (1970) with values in four normal subjects of $0.4-1.2\%$ ^{59}Fe uptake per ml. Hasewaga *et al* (1968), using the starved rat method of assay, obtained levels of $9.6 \pm 2.0\%$ ^{59}Fe uptake per ml. It is difficult to correlate the results of haemagglutination-inhibition assay with biological assay. However, Lange *et al* (1969) measured

serum erythropoietin levels in a total of 141 normal blood donors and obtained values of 0.0035 to 0.60 units of erythropoiesis stimulating factor (ESF) per ml.

CHRONIC RENAL FAILURE

Only one of our patients had no measurable erythropoietin in the plasma; in 25, the range of values was 0.5-22.9% ^{59}Fe uptake per ml.

Adamson *et al* (1968) studied 24 patients with chronic renal failure and reported plasma erythropoietin values less than normal in 17, normal in four, and increased above normal in three. Naets and Wittek (1968) reported a value for plasma erythropoietin of 19.3% ^{59}Fe uptake per ml in one of 11 patients with chronic renal failure. In five anephric subjects who were investigated by Mirand and Murphy (1970) values for plasma erythropoietin showed a range of 0.78-25.2% ^{59}Fe uptake per ml.

Blood transfusions were avoided in the patients in our series. It was shown that plasma erythropoietin levels were greater in non-transfused patients with chronic renal failure (Gral and Schroth, 1970). The same workers (Gral *et al*, 1972) measured plasma erythropoietin in 73 patients with chronic renal failure; their results were shown in the form of a graph and expressed in Napierian logarithms with a range of 0.4-2.6% ^{59}Fe uptake per ml. When our values were converted to the same terms, the figures were similar to those of Gral *et al*.

In our patients, the administration of methyl testosterone produced no remarkable effect on values for the blood haemoglobin, packed cell volume, or plasma erythropoietin levels. Moreover, when equivalent amounts of testosterone to normal male physiological levels were added to normal female plasma and included in a standard assay, no increase in % ^{59}Fe uptake was found although other workers have reported stimulating effects upon erythropoiesis by androgens in experimental animals (Meinecke and Crafts, 1968). DeGowin *et al* (1970) described two patients with chronic renal failure who were treated with testosterone and reported increased values for the plasma erythropoietin. However, blood transfusions were discontinued during the test period so that the results could be compared with those of Gral and Schroth in non-transfused patients who had not received testosterone. However, clinical response to testosterone therapy in patients with chronic renal failure has been reported by Shaldon *et al* (1970).

The effects of hypoxia and haemorrhage in two patients in our series confirmed the results of experimental studies by Fried and Gurney (1968), showing that erythropoietic response can occur in these conditions in the anephric state.

Conclusion

We have been able to obtain values for plasma erythropoietin in normal subjects and in patients with chronic renal failure. That we have done so may be due to the fact that our biological material is more sensitive to erythropoietin than that of other workers since our method of assay follows accepted procedures.

Zanjani *et al* (1972) outlined the function of renal erythropoietic factor (REF) as activator of erythropoietin. So far, REF has not been identified. If, as we have shown, patients with renal failure do have erythropoietin in their plasma in normal amounts, it would seem important for the future to isolate and identify renal erythropoietic factor as a possible therapeutic agent in the management of the anaemia of renal failure.

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