

## 2,3 Diphosphoglycerate in Parkinson's disease

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**SYNOPSIS** The red cell 2,3 DPG, the most important factor for oxygen delivery in the tissues, was found to be increased in Parkinsonism patients compared with controls. The aging process seems not to be a factor in the increased 2,3 DPG concentration. Other factors relevant to raised 2,3 DPG level such as physical activity, increased oxygen requirements, and metabolic changes are discussed.

2,3 Diphosphoglycerate (2,3 DPG) is known to play an important role in the adaptive response of the red blood cell to hypoxia. Its increased concentration causes a shift of the oxygen dissociation curve to the right and this allows more oxygen to be released from the oxyhaemoglobin molecule at the tissue level (Miller *et al.*, 1970b).

High concentrations of red cell 2,3 DPG occur in patients with hypoxic diseases (Eaton and Brewer, 1968; Oski *et al.*, 1969), in high altitude residents (Lenfant *et al.*, 1968), during exercise (Eaton *et al.*, 1969b), after administration of androgens (Gorshein *et al.*, 1974), in thyrotoxicosis (Snyder and Reddy, 1970), and in red cell pyruvate kinase deficiency (Mourdjinis *et al.*, 1969).

On the other hand, Parkinson's disease is associated with biological factors which may affect the 2,3 DPG concentrations. The respiratory capacity is reduced in these patients because of postural abnormalities and rigidity (Mier, 1967). The associated hypoventilation may thus lead to changes of pH, which is another factor affecting the 2,3 DPG concentration (Bellingham *et al.*, 1971). Furthermore, tremor and rigidity may be accompanied by an increased metabolic rate, which, it is suggested, accounts for the increase of 2,3 DPG in hyperthyroidism and after the administration of androgens (Gorshein *et al.*, 1974). Finally, slight abnormalities in glucose metabolism have been reported in Parkinson's disease

(Lipman *et al.*, 1974) and 2,3 DPG is known to be involved in the regulation of the glycolytic process (Askari and Rao, 1968; Rose, 1968). As all these factors may be instrumental in changing 2,3 DPG and in the context of trends viewing Parkinson's disease as a biochemical disease not confined to the central nervous system (Barbeau *et al.*, 1975), we thought it of interest to investigate possible changes in 2,3 DPG concentration in patients with Parkinson's disease. Additionally, in some patients, adenosine triphosphate (ATP) concentration in red cells and the red cell glucose-6-phosphate dehydrogenase (G-6-PD) activity were determined. ATP is a less important factor in the red cell's affinity for oxygen and G-6-PD plays an important role in the anaerobic glycolysis (hexose monophosphate shunt).

### METHODS

Seventeen patients with Parkinson's disease (nine males, eight females) were selected for the study. They ranged in age from 37 to 75 years with a mean of 60.4 years  $\pm$  SD 11. Most were ambulatory and only four were confined to a wheelchair. Most of the patients were drug free and few were on anticholinergic drugs. Patients under treatment with levodopa were excluded.

The same number of healthy volunteers served as controls. Their ages ranged from 24 to 73 years with a mean of 38.6 years  $\pm$  SD 16 and differed significantly from the patient group ( $P < 0.001$ ).

Blood samples were taken immediately after admission from the drug free patients and one week after their previous medication was stopped from the patients on anticholinergic treatment.

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Haemoglobin and haematocrit were determined according to standard haematological techniques. Measurements of 2,3 DPG were carried out according to the chromatropic acid method of Bartlett (1959) with the modifications of Eaton *et al.* (1969a): one volume of packed red cells washed three times in isotonic saline was mixed with two volumes of cold 6.7% trichloroacetic acid. The mixture was stored frozen for an average of seven days and 0.1 ml of the protein free extract was added to 3.9 ml of 0.01% chromatropic acid in concentrated sulphuric acid. The mixture was incubated at 100°C for 135 minutes and the optical density was read in 695 nm in a Spectronic 88 spectrophotometer. 2,3 DPG concentration was estimated by standard curve and expressed in  $\mu\text{mol/ml}$  red blood cells. As the haemoglobin was known, the concentration per gram was calculated. Normal values were established on 17 healthy volunteers (nine males, eight females) and found to be 3.72–5.04  $\mu\text{mol/ml}$  red cells.

ATP was determined according to Dennemann's method (1961). The reagents used were supplied by Boehringer Mannheim in the form of a test kit for ATP determination (Cat. No 15979).

G-6-PD activity was determined by the method of Kornberg and Horecker (1955).

#### RESULTS

The results are shown in Tables 1, 2, and 3. Sex, age, and values of haematocrit (Ht), haemoglobin (Hb), 2,3 DPG, ATP, and G-6-PD for each con-

trol subject and each patient are tabulated in Tables 1 and 2 respectively. The comparative average values from the two groups for all these parameters are shown in Table 3.

The results showed that the 2,3 DPG, expressed in  $\mu\text{mol}$  per ml of red cells or  $\mu\text{mol}$  per g of haemoglobin, is raised in the patient group ( $P < 0.001$ ), while there is no significant difference between the two groups for Ht, Hb, ATP, and G-6-PD.

In correlating age and 2,3 DPG concentration in the control group (Fig. 1), it was found that 2,3 DPG levels do not increase with advancing age. By contrast with the control group, a negative correlation ( $r = -0.6$ ,  $P < 0.01$ ) has been found to exist between age and 2,3 DPG levels in the patient group (Fig. 2).

#### DISCUSSION

2,3 DPG is produced as an intermediate in glycolysis from 1,3 DPG in the presence of the enzyme 2,3 diphosphoglycerate mutase and is hydrolysed to 3-phosphoglycerate and inorganic phosphate by the enzyme 2,3 diphosphoglycerate phosphatase (Rapoport, 1965). Its concentration within the cell depends on a variety of factors such as pH, phosphate, pyruvate, triiodothyronine and the activity of 2,3 DPG mutase, while 2,3 DPG itself plays a regulatory role in controlling glycolysis (Askari and Rao, 1968; Rose, 1968). The more 2,3 DPG is bound by deoxyhaemoglobin the less are the inhibitory effects on hexokinase and 2,3 DPG

TABLE 1  
SEX, AGE, HT, HB, 2,3 DPG, ATP, AND G-6-PD IN HEALTHY VOLUNTEERS

No.	Sex	Age (yr)	Ht (%)	Hb (g/dl)	2,3 DPG ( $\mu\text{mol/mlRBC}$ )	2,3 DPG ( $\mu\text{mol/gHb}$ )	ATP (nmol/mlRBC)	G-6-PD (mU/mlRBC)
1	M	51	50.5	15.3	4.920	16.19	1230.89	1517.43
2	F	30	41.8	13.7	4.704	14.35	1455.21	1580.59
3	M	25	48	16.8	4.470	12.77	1396.75	1846.80
4	M	24	53.8	16.5	4.680	15.25	1303.94	1860.60
5	F	29	43.8	14.3	4.500	13.77	1955.30	1363.43
6	F	39	41.7	13.5	4.260	13.16	1276.18	1402.90
7	M	32	48	17.5	4.560	12.50	1206.20	2247
8	F	29	43	14.4	4.620	13.79	1424.93	1847
9	M	70	44	15.1	5.040	14.70	1533.82	2031.56
10	M	28	40	12.3	4.680	15.25	1365.30	2047
11	M	31	43.3	14.5	4.212	12.57	1374.04	1919.40
12	M	23	47.9	16	4.116	12.32	1418.14	1542.30
13	F	62	43	13.5	4.056	12.91		
14	M	39	47.4	15.9	3.756	11.19		
15	F	43	41	13.6	3.720	11.21		
16	M	29	43	15.6	4.320	11.90		
17	F	73	47.3	15.4	4.236	13.01		

TABLE 2  
SEX, AGE, HT, HB, 2,3 DPG, ATP, AND G-6-PD IN PATIENTS WITH PARKINSON'S DISEASE

No.	Sex	Age (yr)	Ht (%)	Hb (g/dl)	2,3 DPG ( $\mu\text{mol/mlRBC}$ )	2,3 DPG ( $\mu\text{mol/gHb}$ )	ATP (nmol/mlRBC)	G-6-PD (mU/mlRBC)
1	F	66	47	17.2	5.256	14.36	1450.08	1956.90
2	F	62	42	16	5.376	14.11	1956.28	
3	M	60	48	17.6	5.940	16.20	1143.30	1893.05
4	F	64	44.5	14.7	6.444	17.70	1287.10	1582.64
5	F	50	45.5	13.9	5.520	18.07	1005.01	1693.98
6	M	63	44.5	14.7	5.220	15.80	1237.21	1660.11
7	F	37	45	12.8	6.120	21.51	1262.93	1658.08
8	F	63	44	14.8	5.040	14.97	1654.91	2277.81
9	F	67	43	13.3	5.160	16.68	1208.09	1483.23
10	M	52	46	17.2	5.340	14.28	1313.69	
11	M	73	43	15.2	5.040	14.25	1517.86	2181.89
12	M	68	44	15	5.160	15.36	1584.27	2182.67
13	M	75	51	17.7	4.200	12.10		1752.72
14	F	36	43.5	16.3	6.168	16.56		
15	M	69	56.5	18	5.352	16.80		
16	M	59	44.8	17.6	6.180	15.55		
17	M	63	44	14.6	5.520	16.63		

TABLE 3  
VALUES OF AGE, HT, HB, 2,3 DPG, ATP, AND G-6-PD IN NORMAL SUBJECTS AND IN PATIENTS WITH PARKINSON'S DISEASE

	Age (yr)	Ht (%)	Hb (g/dl)	2,3 DPG ( $\mu\text{mol/mlRBC}$ )	2,3 DPG ( $\mu\text{mol/gHb}$ )	ATP (nmol/mlRBC)	G-6-PD (mU/mlRBC)
Normal cases (No.)	17	17	17	17	17	12	12
Mean	38.65	45.15	14.93	4.403	13.34	1411.72	1768
$\pm$ SD	16	3.74	1.39	0.370	1.43	195.68	280.07
Range	23-73	40-53.8	12.3-16.8	3.720-5.040	11.19-16.19	1206.2-1955.3	1363.43-2247
Patient cases (No.)	17	17	17	17	17	12	11
Mean	60.41	45.66	15.68	5.473	15.94	1355.07	1847.55
$\pm$ SD	11	3.52	1.63	0.551	2.07	203.11	270.14
Range	36-75	42-56.5	12.8-18	4.200-6.444	12.10-21.51	1005.01-1956.28	1483.23-2277.81
Significance	$P < 0.001$	NS	NS	$P < 0.001$	$P < 0.001$	NS	NS

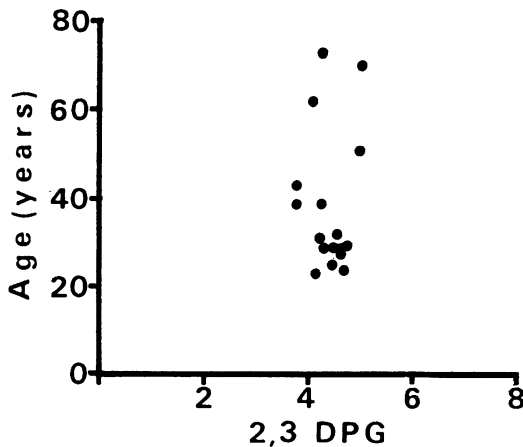


FIG. 1 2,3 DPG expressed as  $\mu\text{mol per ml}$  red cells, plotted against age, in normal subjects.

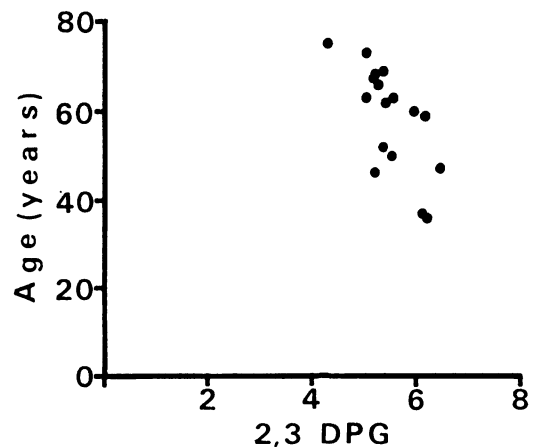


FIG. 2 2,3 DPG ( $\mu\text{mol/ml RBC}$ ), plotted against age, in Parkinsonism patients.

mutase promoting further glycolysis and 2,3 DPG production.

In our study, the red cell 2,3 DPG concentration was found to be significantly raised in Parkinsonism patients compared with control subjects. In view of these results and before any attempt is made to relate them to the biological process underlying Parkinson's disease, the following factors have to be considered:

#### AGE

Although the mean age of our patients was higher than that of our control subjects, no positive correlation was found between 2,3 DPG and age, suggesting that the aging process does not substantially affect 2,3 DPG levels. A significant negative correlation between age and 2,3 DPG has, however, been demonstrated in the patient group, which indicates that in younger patients the 2,3 DPG concentration tends to be higher. Assuming that the younger the patient the more uncontaminated the disease is by other physical symptoms, this finding may support the view that increased 2,3 DPG activity is more closely related to the biochemical disturbance underlying Parkinsonism itself than to secondary biological complication arising from it and from age.

#### PHYSICAL ACTIVITY

2,3 DPG levels may reflect the degree of physical activity since it is known that exercise results in raised 2,3 DPG levels. Physical activity was not assessed in our study groups. It is reasonable, however, to assume that, although our Parkinsonism patients were not confined to bed, by no means could they be considered as being physically overactive.

#### OXYGEN REQUIREMENTS

As the oxygen supply system is closely coupled with the oxygen requirements of physical activity, the muscular rigidity and tremor leading to an increased metabolic rate could account for the raised 2,3 DPG in our patients. This could be in agreement with the finding of raised 2,3 DPG levels in hyperthyroidism, although the rise in this case has been attributed to a specific action of thyroid hormone on glycolysis (Miller *et al.*, 1970a).

Postural abnormalities, rigidity, lack of exercise, and, possibly, other neurological deficits in patients with Parkinsonism may result in a limitation of respiratory excursion and diminution of respiratory function. Paulson and Tafra (1970)

who measured the pulmonary function in Parkinsonism patients before and after L-dopa treatment, found a marked increase in minute ventilatory volume with a less marked change in vital capacity. The hypoventilation consequent to these changes may lead to hypoxia and this could well explain the raised red cell concentration of 2,3 DPG. In disorders of oxygen loading as in pulmonary disease, in which there is severe restriction in ventilation, the arterial oxygen desaturation evokes a discharge of erythropoietin and polycythaemia with raised haematocrit (Krantz and Jacobson, 1970). 2,3 DPG is increased and the associated decrease in haemoglobin affinity improves release of oxygen. The simultaneously increased carbon dioxide retention leads to acidosis, which could decrease the 2,3 DPG concentration (Bellingham *et al.*, 1971). Our patients' haematocrit was found not to differ from that of our control subjects. Should hypoxia be held responsible for the raised 2,3 DPG, it is to be assumed that the carbon dioxide retention and the associated acidosis with opposite effect on oxygen release account for the observed variable response in red cell mass and 2,3 DPG concentration in our material.

Bearing in mind the possibility that the raised 2,3 DPG reflects a primary biochemical lesion in Parkinson's disease the following factors could be mentioned:

2,3 DPG is generated by the anaerobic glycolytic pathway. When deoxyhaemoglobin increases the red cell glycolysis is activated and this leads to an increase in 2,3 DPG. Inhibition of 2,3 DPG phosphatase or activation of 2,3 DPG mutase could also lead to increase of 2,3 DPG. Congenital abnormalities in red cell glycolytic enzymes can alter the 2,3 DPG concentration and the oxygen affinity of haemoglobin. It is not known if an abnormality exists in Parkinsonism patients.

Parkinson's disease is characterised by a generalised defect of the dopamine system (Barbeau, 1969; Barbeau *et al.*, 1975). Dopamine administration has been shown to cause hyperglycaemia in animals, probably by interference with the insulin-releasing mechanism in pancreatic beta cells (Hakanson *et al.*, 1967), and an impaired glucose tolerance has been demonstrated in Parkinson's disease (Lipman *et al.*, 1974). Dopamine also plays a part in physiological release of luteinizing hormone, follicle stimulating hormone, prolactin and growth hormone (Cavagnini *et al.*, 1972) probably by hypothalamic involvement, as it is known that the hypothalamus is affected in Par-

kinson's disease (Forno, 1966). It is not known, to our knowledge, if dopamine insufficiency and/or hypothalamic involvement may lead to increase of red cell 2,3 DPG. It might be pertinent that androgens as well as thyroxine administration cause an increase of 2,3 DPG.

According to the hypothesis that the syndrome of Parkinsonism results from an imbalance between cholinergic-excitatory and dopaminergic-inhibitory actions in favour of cholinergic dominance in the extrapyramidal system, because of dopamine insufficiency (Klawans, 1968, Calne, 1970), the question arises if the raised 2,3 DPG results from an interaction with the red cell acetylcholinesterase. It is possible that a significant portion of the red cell 2,3 DPG and/or ATP is bound to the red cell membrane (Bunn *et al.*, 1971). Acetylcholinesterase is also localised in the red cell membrane. If acetylcholinesterase interacts with 2,3 DPG it could keep it away from the haemoglobin due to an imidazole group in the active site (Egger and Rapoport, 1963). In nucleated red cells (birds) the 2,3 DPG is 'replaced' by phosphoinositol, which is found inside the neurone as well as the acetylcholinesterase. Furthermore, acetylcholine is shown to interact with phosphoinositol turnover (Durell *et al.*, 1969).

It is worth noting that anticholinergic drugs alleviate the Parkinsonism symptomatology and drugs such as chlorpromazine, which prevent the removal of acetylcholinesterase from the red cell, also prevent deterioration of the red cell in storage due to permeability changes (Greig and Gibbons, 1955). In neonatal hypothyroidism (rats) a significant reduction in acetylcholinesterase activity in the cerebral cortex and the hypothalamus has been shown. The administration of thyroxine restored brain growth and enzyme activity to normal (Geel and Timiras, 1967). A similar effect of hypothyroidism and thyroxine on red cell 2,3 DPG has been demonstrated (Grosz and Farmer, 1969, Miller *et al.*, 1970a).

Whether the observed rise in 2,3 DPG level in patients with Parkinsonism is connected with the pathogenesis of the disease or reflects a secondary compensatory mechanism remains to be elucidated. However, further work is needed to investigate the role of 2,3 DPG in relation to different forms of Parkinsonism, specific features and effectiveness of treatment.

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