ERYTHROCYTIC ENZYMES DECOMPOSING REACTIVE OXYGEN SPECIES AND GLUCOSE 6-PHOSPHATE DEHYDROGENASE DEFICIENCY

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SYNOPSIS

Superoxide dismutase (SOD) (EC 1.15.1.1), glutathione peroxidase (GPx) (EC 1.11.1.9) and catalase (EC 1.11.1.6) were assayed in cord blood samples designated by a screening procedure as glucose 6-phosphate dehydrogenase (G6PD) deficient, mosaic, or normal. Mean SOD activity was increased, although not significantly, in full G6PD deficiency, whereas catalase and GPx activities were significantly decreased. Mosaic subjects exhibited no changes. SOD activity correlated well with catalase activity suggesting that synthesis of these two enzymes is interdependent in the inherited lack of reduced glutathione (GSH) necessary for the GPx reaction; however the lack of an overall pattern for the results makes it unlikely that there is any pattern of adaptation among the enzymes removing toxic oxygen species.

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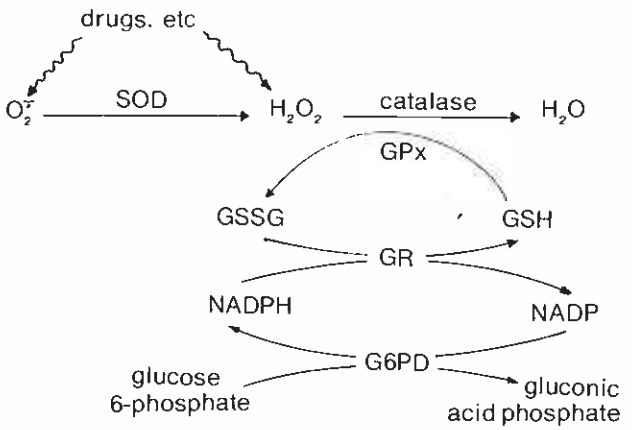
INTRODUCTION

In G6PD deficiency haemolysis is presumed to be the result of a chain of events consquent on the primary lack of this enzyme. Thus drugs and other agents (aromatic hydrocarbons food toxins) appear to generate superoxide ions(1), which can be dismutated to peroxide by the enzyme SOD. Peroxide, which in turn is markedly noxious to the cell membrane, would normally be detoxicated by either or both of the enzymes GPx and catalase. GPx however requires GSH, which is generated by the glutathione reductase (GR) reaction, in itself requiring oxidised glutathione (GSSG) and NADPH generated by the G6PD reaction, thus:



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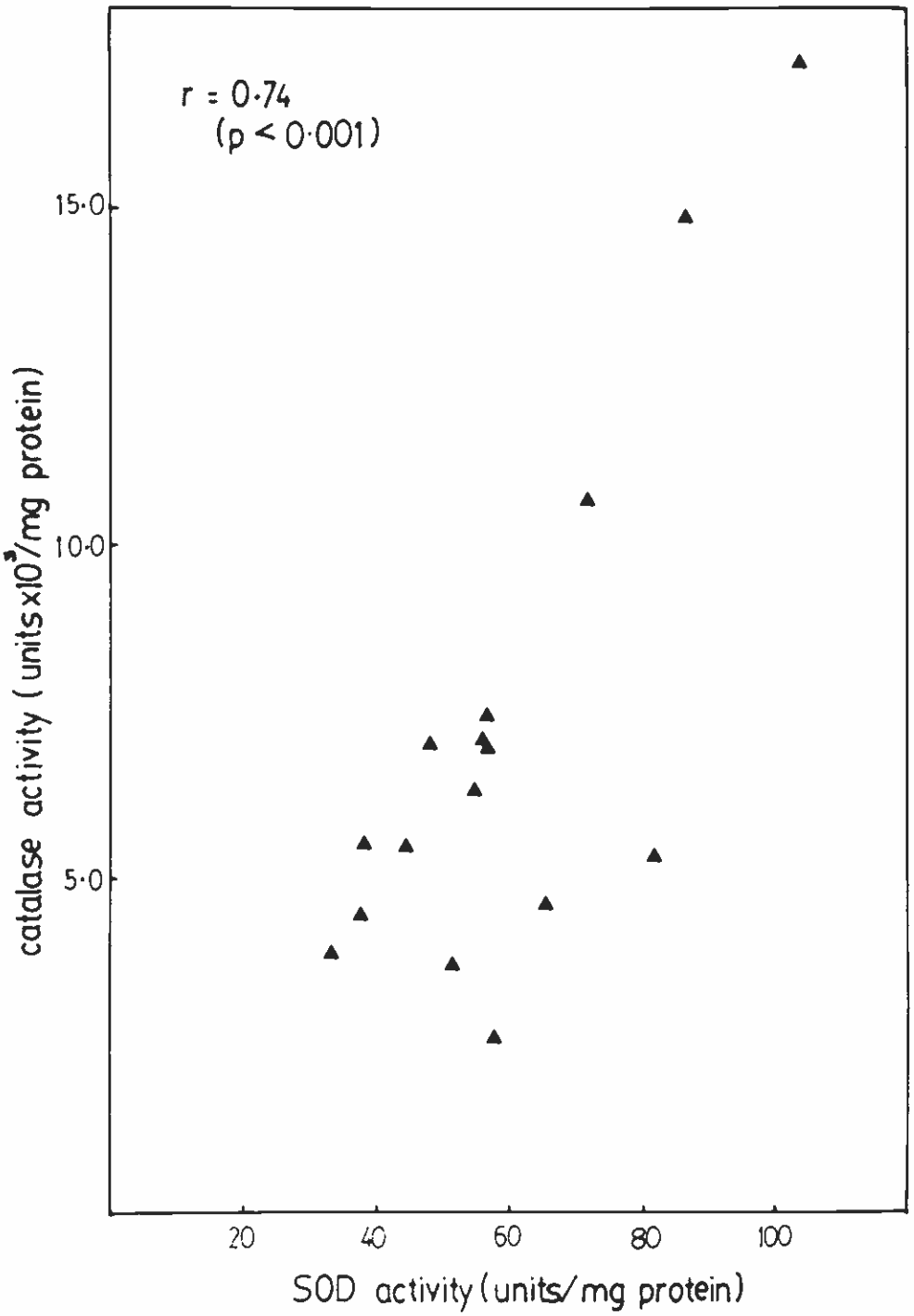
We are interested in the roles of the enzymes detoxicating reactive oxygen species like O_2^- and peroxides and their adaptive synthesis or repression in various pathophysiological states. In cases of G6PD deficiency, the lack of one enzyme to detoxicate peroxide might be compensated for by enhanced activity of catalase, although it has been suggested that NADPH as a product of the G6PD reaction is necessary for optimal activity of catalase(2). Thus if there is any adaptation to the lack of G6PD, one would expect it to be manifested by greater synthesis of catalase to bind the peroxide produced by SOD, the activity of which would perhaps not be affected. At the same time, since GPx is essentially non-functional in the absence of GSH, its synthesis might be expected to be minimised. By studying the activities of the enzymes in the fully deficient and mosaic subjects, we hoped to cast some light on this possibility. There do not appear to have been any studies of the role of the three enzymes in glucose 6-phosphate deficiency, although sickle cells have been examined. Thus Schachter et al(3) found that sickle cells had less SOD the more severe the symptoms of the patients whence they were derived; Das and Nair (4) suggested that membrane lipid peroxidation occurs in sickled erythrocytes although they found in them increased activities of SOD (as well as reduced activities of GPx and catalase) Yenchitsomanus and Wasi(5) showed that SOD is higher in the erythrocytes of patients with β-thalassaemia and haemoglobin H disease. In addition Mavelli et al(6) showed that erythrocytes from patients in the acute haemolyic crisis of favism have enhanced activities of SOD (but less GPx) than erythrocytes from normal or G6PD deficient subjects. Thus the various findings, in so far as they relate to the activity of SOD in erythrocytes at risk of haemolysis, appear to be somewhat inconsistent.

MATERIALS AND METHODS

Cord blood samples were obtained from the Population Genetics Laboratory of the National University Hospital, Singapore subsequent to screening for G6PD deficiency by the method of Bernstein(7). Thus the samples could be classified as normal, deficient, or mosaic. The gestational ages and birthweights were also noted. The



Plot of activity of superoxide dismutase against catalase activity in the red cells of subjects with full G6PD deficiency (both in units/mg soluble cell protein)

TABLE 1
SOD, G6PD AND CATALASE ACTIVITIES IN G6PD DEFICIENCY (MEANS ± STANDARD DEVIATIONS)

	Normal n = 58	Full Deficiency n = 16	Mosaic n = 8
SOD (units/mg protein)	57.6 ± 10.4	64.0 ± 16.6	58.0 ± 20.4
GPx (units/mg protein)	0.258 ± 0.069	0.195* ± 0.055	0.230 ± 0.082
Catalase (units × 10^3 /mg protein)	6.83 ± 1.48	5.21 ± 1.93 **	6.17 ± 1.37

Significantly different from normals * $p < 0.005$
** $p < 0.001$

whole blood was centrifuged immediately at low speed for 30 min, after which the packed erythrocytes were washed three times with saline and lysed by hypotonic shock with water(8) Haemoglobin was precipitated with a mixture (5/3) of ethanol and chloroform and removed by centrifugation at 2500 rpm for 10 min. The colourless haemolysate was assayed, using standard methods, for SOD(9), GPx(10), and catalase(11) using a Shimadzu UV 260 spectrophoto meter, all at 37°C. Protein in the haemolysates was estimated by the Lowry method.

Differences between means were assessed by Student's t-test, and simple regression analysis was used to calculate correlation coefficients.

RESULTS AND DISCUSSION

In mosaics and G6PD deficient samples, both GPx and catalase were lower than in the normal samples. although significantly so ($p < 0.005$) only in the former. SOD was higher in the G6PD deficient samples than in the normal samples, although not significantly so ($0.05 < p < 0.1$) (Table 1). There seems to be no discernable pattern in terms of the expectations outlined in the introduction.

Scatter diagrams however were constructed and correlation coefficients calculated, and there is an apparent relationship over the whole range of values between SOD and catalase in both full G6PD deficiency ($r = 0.74$) and in mosaics ($r = 0.67$), a phenomenon of course not demonstrated by comparing means. Since the mosaic group however consisted of only eight samples, only the diagram for the fully deficient group ($n = 16$) is reproduced here (Fig 1).

It is thus concluded that although a general picture of adaptation of the enzymes detoxicating reactive oxygen species in cases of G6PD deficiency cannot readily be demonstrated, there is a distinct possibility that an adaptive synthesis of catalase takes place in line with the peroxide producing ability of SOD.

Of course the present observations do not reveal the situation in the intact erythrocyte, wherein catalase may be partially inhibited by the lack of NADPH(2) and the relative excess of SOD (Table 1) may generate enough peroxide to contribute to the haemolysis when it occurs in presence of drugs and other precipitating agents. Further work will be directed towards attempts to assay enzyme activities in media which resemble as far as possible the stroma of the intact red cells.

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