Protective effects of sodium orthovanadate in diabetic reticulocytes and ageing red blood cells of Wistar rats

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The reticulocytes and the ageing red blood cells (RBCs) namely young (Y), middle-aged (M) and old RBCs (O) of female Wistar rats from different groups such as control animals (C), controls treated with vanadate (C + V), alloxan-induced diabetic (D), diabetic-treated with insulin (D + I) and vanadate (D + V), were fractionated on a percoll/BSA gradient. The following enzymes were measured – hexokinase (HK), glutathione peroxidase (GSH-Px), glutathione reductase (GSSG-R), glutathione-s-transferase (GST), alanine aminotransferase (AlaAT), aspartate aminotransferase (AsAT) and arginase in the hemolysates of all the RBCs fractions. Decreases in the activity of HK and AsAT by about 70%, arginase and GSH-Px by 30% in old RBCs were observed in comparison to reticulocytes of control animals. Increases in the activity of GSSG-R by 86%, AlaAT by more than 400% and GST by 70% were observed in old RBCs in comparison to reticulocytes of control animals.

Alloxan diabetic animals showed a further decrease in the activities of HK in Y RBCs by 37%, M RBCs by 39% and O RBCs by 32%, GSH-Px activity in Y RBCs by 13%, M RBCs by 20% and O RBCs by 33% and GST activity in Y RBCs by 14%, M RBCs by 42% and O RBCs by 60% in comparison to their corresponding cells of control animals. An increase in the activity of all the enzymes studied was also observed in reticulocytes of diabetic animals in comparison to reticulocytes of controls. The GSSG-R activity was found to be increased in Y RBCs by 49%, M RBCs by 67% and O RBCs by 64% as compared to the corresponding age-matched cells of control animals. The activity of arginase also decreased in Y RBCs by about10%, M RBCs by 20% and O RBCs by 30% in comparison to the age-matched cells of control animals. A decrease in the activity of AsAT in Y and M RBCs by 30%, and O RBCs by 25% was observed in diabetic animals in comparison to the age-matched cells of control animals. The activity of AlaAT was found to be decreased by more than 10% in Y and M RBCs and 25% in O RBCs of diabetic animals in comparison to the age-matched cells of control animals.

Insulin administration to diabetic animals reversed the altered enzyme activity to control values. Vanadate treatment also reversed the enzyme levels except for that of GST in old cells.

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1. Introduction

The ageing of an organism leads to slowing down of metabolic pathways, antioxidant capacity, hormone secretion and to an increase in protein degrading activity (Clark and Sohet 1985). Diabetes is accompanied by an increase in free radicals and their products thereby accelerating process of ageing in animals. Hyperglycemia in its due

Keywords. Ageing; alloxan diabetes; antioxidants; arginase; vanadate; insulin; red blood cells; transaminases

Abbreviations used: AlaAT, Alanine aminotransferase; AsAT, aspartate aminotransferase; C, controls; C + V, controls treated with vanadate; D, diabetic; D + I, diabetic treated with insulin; D + V, diabetic treated with vanadate; G6PDH, glucose-6-phosphate dehydrogenase; GSH, glutathione; GSH-Px, glutathione peroxidase; GSSG-R, glutathione reductase; GST, glutathione-s-transferase; HK, hexokinase; SOV, sodium orthovanadate.

course leads to generation of free radicals and their other oxidative products (Bonnefont-Rousselot 2002). Hexokinase being the rate-limiting enzyme of glycolysis plays an important role in the metabolism of glucose. Glucose-6 phosphate dehydrogenase (G6PDH) the primary enzyme of pentose phosphate pathway generates NADPH, which is also utilized in the formation of reduced glutathione (GSH) by glutathione reductase (GSSG-R) (Ames and Shigenaga 1992). GSH, an important free radical scavenger, also acts as a coenzyme in the activity of glutathione-s-transferase (GST) to detoxify the xenobiotic and toxic compounds. Glutathione peroxidase (GSH-Px) detoxifies low levels of hydrogen peroxide with the help of GSH causing its oxidation.

The major protein-degrading pathway includes aspartate aminotransferase and alanine aminotransferase which transfer the amino groups from aspartate and alanine respectively to glutamate which is finally metabolised to generate ammonium ions. Excess ammonium ions are excreted via the urea cycle – the enzyme arginase being a key enzyme of this cycle (Salimuddin *et al* 1996). The status of these enzymes in red blood cell metabolism is not yet well understood in diabetes.

Insulin administration improves the diabetic state by normalizing the blood glucose levels in type-1 diabetes and restores the altered metabolic pathways (Baquer *et al* 1995). However, sodium orthovanadate (SOV), an insulin mimetic agent, has been shown to be effective in controlling the blood glucose levels in both type-1 and type-2 diabetes (Goldfine *et al* 1995). The efficacy of SOV in reversing the diabetic complications in different tissues was also widely studied in the 90s. However, its effect on ageing has not yet been studied. The present work undertakes to study the changes in the activities of enzymes like hexokinase (HK), GSH-Px, GSSG-R, GST, alanine aminotransferase and aspartate aminotransferase and arginase in different age fractions of red blood cells (RBCs) of diabetic animals and those treated with insulin and SOV.

2. Materials and methods

2.1 *Animals*

A group of 40–50 female rats of Wistar strain, weighing between 180–200 g and 3–4 months of age, were used in all the experiments. Animals were maintained in the climatically controlled animal house facility of Jawaharlal Nehru University, New Delhi. The animals were fed *ad libitum* with rat feed (Hindustan Lever, India) and tap water.

2.2 *Induction of diabetes and experimental groups*

Diabetes was induced in rats by subcutaneous injection of alloxan monohydrate (15 mg/100 g body wt.). After six days of alloxan injection, rats were divided into three groups: diabetic (D); diabetic treated with insulin (D + I) and with vanadate (D + V). Two IU of protamine-zinc insulin/rat/day were injected to rats of D + I group for three weeks. SOV was given in 0·5% saline in the drinking water as follows: 0·2 mg/ml for initial three days, 0·4 mg/ml for another three days, and 0·6 mg/ml for rest of the duration of treatment to (D + V). Similar vanadate treatment was also given to control animals (C + V). Control animals were treated with vehicle only (Gupta and Baquer 1998). The blood was collected by heart puncture after 21 days, i.e. at the end of above treatment. Throughout the experiment, the urine glucose was monitored using glucose kits (Glaxo) so that the level of reversal from diabetic state to normal may be observed.

2.3 *Isolation of reticulocytes and fractionation of red blood cells into young, middle-aged and old cells*

The blood was passed through a column of *a*-cellulose and microcrystalline cellulose (1 : 1) as described by Beutler *et al* (1976). The eluate was centrifuged at 3000 rpm in a Sorvall RC 5C refrigerated centrifuge for the separation (97–98%) of RBCs. Isolation of reticulocytes, young, middle-aged and old RBCs was carried out by using percoll/BSA gradient as described by Rennie *et al* (1979). RBCs (0·75 ml) suspension (1 : 1) was layered on a 13·5 ml of gradient and centrifuged in a vertical rotor at 1500 rpm for 2 min. The upper-most layer comprising reticulocytes and few young red blood cells was again layered on percoll/BSA gradient and the reticulocytes were separated with high degree of purity. In the previous gradient the three layers from top to bottom were collected. The bottom layer comprised of old cells, the top layer of the young cells and middle layer of the middle-aged cells. These four types of RBCs were separated according to the density of cells. These cells were washed three times with cold phosphate buffered saline and then a hemolysate was made by forceful lysis with phosphate buffer (5 mM, pH 8·0). The hemolysates were kept at – 70°C for 4 h and then thawed at room temperature so that the hemoglobin may settle at the bottom of the test tube (Beutler *et al* 1976). The upper layers of the hemolysate were used for the assay of the enzymes.

2.4 *Determination of enzyme activities*

All enzyme measurements were carried out in a Beckman DU-68 spectrophotometer. Hexokinase (EC 2·7·1·1) was measured using a coupled enzyme assay as described earlier (Gupta *et al* 1997). The reaction mixture of 1 ml contained the following in the final concentration: 100 mM Tris-HCl, pH 7·4; 5 mM EDTA, pH 8·0; 10 mM $MgCl_2$;

0·2 mM NADP; 10 mM ATP, pH 7·2; 5 mM glucose; and 1U of G6PD. The sample was incubated at 37°C for 10 min and the reaction was started by adding 0·1 ml of 1 : 40 hemolysate and the change in absorbance was measured at 340 nm.

GSH-Px peroxidase EC (1·11·1·9) and GSSG-R (EC 1·6·4·2) were measured as described by Beutler (1988). For measuring the activity of GSH-Px the following ingradients were taken in 1 ml of cuvette with final concentration of potassium phosphate buffer, 0·1 M, pH 7·0; GSH 1 mM; EDTA 4 mM; GSSG-R 3U per assay, sodium azide 0·4 mM; NADPH 0·2 mM; and 0·1 ml of diluted hemolysate (1 : 40). The reaction was started by adding 0·5 ml of tert-butyl hydroperoxide 0·1 mM and the change in absorbance was measured at 340 nm.

For measuring GSSG-R activity the following assay mixture was taken in 1 ml of cuvette in a final concentration of Tris/HCl 4·1 mM, pH 7·5; $MgCl_2$ 15 mM; EDTA 5·1 mM; KCl 60 mM; saponin 0·017%; GSSG 2·6 mM; and NADPH 0·2 mM. The reaction was started by adding 0·1 ml of hemolysate (1 : 40) and the change in absorbance was taken at 340 nm. GST (EC 2·5·1·18) was essentially measured by the method of Beutler (1988). One ml of assay mixture contained the following final concentration: potassium phosphate buffer, 0·5 M, pH 6·5; CDNB 10 mM and the reaction was started by adding GSH 20 mM and 0·1 ml of 1 : 20 hemolysate. The change in absorbance was read at 412 nm.

Aspartate aminotransferase (AsAT) (EC 2·6·1·1) and alanine aminotransferase (AlaAT) (EC 2·6·1·2.) were measured by the method of Bergmeyer and Bernt (1974). For AsAT assay the reaction mixture in 1·5 ml in a final concentration contained potassium phosphate buffer 80 mM, pH 7·4; L-aspartate 66 mM, pH 7·4; malate dehydrogenase (dialysed) one unit; NADH 0·18 mM; and 10 µl of 1 : 40 diluted hemolysate. The reaction was started by adding 2-oxoglutarate 12 mM, pH 7·4 and the change in absorbance was measured at 340 nm. AlaAT was measured by taking the following in the final concentration of assay mixture: potassium phosphate buffer 80 mM, pH 7·4; L-alanine 200 mM, pH 7·4, lactate dehydrogenase (LDH) (dialysed) 2 units; NADH 0·18 mM; and 50 µl of 1 : 40 diluted hemolysate. The reaction was started by adding 2-oxoglutarate 0·18 mM, pH 7·0 and the change in absorbance was measured at 340 nm. Arginase (EC 3·5·3·1) was measured by the method of Murthy *et al* (1980). The reaction mixture contained the following in a total volume of 2·35 ml: 0·6 ml of 1·25 M glycine buffer, pH 9·5; 1·25 ml of a 0·46 M arginine, pH 9·5; and the reaction was started by adding 0·5 ml of 1 : 40 hemolysate (the hemolysate was activated by incubating with 0·01 M $MnCl_2$ at 55°C prior to assay). The samples were incubated at 37°C for 15 min in a shaking water bath. The reaction was stopped by adding 2 ml of 1 M perchloric acid. The samples were centrifuged and urea was estimated in the supernatant by the method of Schimke (1970). Isonitrosopropiophenone (0·5 ml) and 10 ml of H_2SO_4/H_3PO_4 mixture (1 : 3) were added to 1·5 ml of supernatant and heated in a boiling water bath for 1 h in dark and cooled to room temperature. The optical density was measured at 540 nm and the concentration of urea was calculated against the standard curve made by using urea in the range of 1–10 µmol.

2.5 *Enzyme units*

Enzyme units were defined as the oxidation/reduction of 1 µmol of NADH/NADPH per ml hemolysate/min and is converted to µmol/g of hemoglobin in the case of all enzymes using NADH/NADPH. In the case of other enzymes like GST, the unit was expressed as µmol/g of hemoglobin and for arginase was expressed as µmol urea formed per g of hemoglobin.

2.6 *Other determinations*

The glucose oxidase kits were used to measure the levels of blood glucose taking 10 µl of plasma. The percentage of glycosylated hemoglobin was measured by Raheja *et al* (1981) using carbon tetrachloride and thiobarbituric acid. The optical density was measured at 443 nm. GHb percent was calculated on assumption that 1% corresponds to an absorbance of 0·029 at 443 nm. Insulin was measured by radioimmunoassay as described (Wilson and Miles 1985). Bound and free insulin were separated by the second antibody and polyethylene glycol precipitation for quantitation in the sample and standards. Porcine insulin was used as standards.

2.7 *Chemicals*

All the chemicals used were of analytical grade and were obtained from Sigma Chemicals (St. Louis, MO, USA). Polyethylene glycol was purchased from BDH, UK. Protamine-zinc insulin was purchased from Boots India Ltd., India.

2.8 *Statistical analysis*

The significance of differences between the data pairs was evaluated by the analysis of variance (ANOVA) followed by Mann-Whitney *U* test.

3. Results

3.1 *General parameters*

The general parameters like the blood glucose levels showed a four-fold increase in the diabetic animals, which

was normalized by the treatment of insulin or vanadate. Glycosylated hemoglobin levels were higher in diabetic groups, which were not reversed either by insulin or vanadate. A low insulin level as seen in diabetic animals was also observed in diabetic animals treated with SOV. The results are presented in table 1.

3.2 *Changes in enzyme activities*

3.2a *Effect of SOV and insulin on hexokinase activity*: Hexokinase activity was found to be decreasing from reticulocytes to old RBCs in control animals. There was no significant change in HK activity in reticulocytes and young RBCs but a decrease by 50% in middle-aged and 30% in old RBCs in comparison to reticulocytes was observed in control animals. The diabetic animals showed a decrease of 36% in young cells, 38% in middle-aged cells and 32% in old RBCs in comparison to their corresponding cell type of control animals. Insulin reversed the enzyme activity to control values in all the ageing cell type. Vanadate increased the enzyme activity in diabetics by 200% in reticulocytes and old RBCs in comparison to their respective cells of control animals. The results are presented in table 2.

3.2b *Effect of SOV and insulin on GSH-Px activity*: The GSH-Px activity also decreased from reticulocytes to old RBCs in control animals. The enzyme activity showed an increase of 180% in reticulocytes, a decrease of 13% in young, 20% in middle-aged and 32% in old RBCs in diabetic animals in comparison to the respective cell type of control animals. Insulin and vanadate almost reversed the enzyme activity in diabetics to control values.

3.2c *Effect of SOV and insulin on GSSG-R activity*: The GSSG-R activity was found to be increasing from reticulocytes to old RBCs in control animals. The enzyme activity increased in diabetic animals by 360% in reticulocytes, almost more than 150% in young, middle and old RBCs in comparison to their respective cell type in controls. Insulin normalized the enzyme change to control values in all ageing cell type. Vanadate showed more than 200% increase in the enzyme activity in all cell types.

3.2d *Effect of SOV and insulin on GST activity*: The GST activity also showed an increasing pattern from reticulocytes to old RBCs cells. The diabetic animals showed an increase in the levels of the enzyme by 300% in reticulocyte, a decrease by 14% in young, 40% in middle and 59% in old RBCs in comparison to their cell type of control animals. Insulin reversed the enzyme activity only in reticulocytes. Vanadate could not reverse the enzyme activity in old cells.

3.2e *Effect of SOV and insulin on AsAT*: AsAT activity was observed to be decreasing from reticulocytes to old RBCs in control animals with a 50% decrease in middle-aged and a 30% decrease in old RBCs in comparison to reticulocytes. The diabetic animals showed more than 30% decrease in young, middle and old RBCs in comparison to their respective cell type of control animals. Insulin recovered the enzyme activity only in reticulocytes and young RBCs. Vanadate also reversed the enzyme activity. The results are presented in table 3.

3.2f *Effect of SOV and insulin on AlaAT activity*: AlaAT showed an increase in activity from reticulocytes to old RBCs of control animals by 200% in young, > 300% in middle-aged and > 500% in old RBCs in comparison to reticulocytes. Diabetes further increased its activity by > 300% in reticulocytes, > 200% in young and around 40% in middle-aged and old cells. Insulin recovered the enzyme activity only in reticulocytes whereas vanadate recovered the enzyme activity in middle-aged as well as old RBCs.

3.2g *Effect of SOV and insulin on arginase activity*. The activity of arginase was found to be decreasing from reticulocytes to old RBCs by 20% in middle-aged and 23% in old RBCs. Diabetes showed an increase of the enzyme

Table 1. Changes in the levels of glucose, glycosylated hemoglobin (GHb), insulin and body weight in different experimental groups.

General parameters	Controls	Controls + vanadate	Diabetes	Diabetes + insulin	Diabetes + vanadate
Glucose (mg/dl of blood)	91 ± 6	94 ± 8	352 ± 64[a]	163 ± 16[a]	136 ± 18[b]
GHb (%)	2·8 ± 0·4	2·6 ± 0·5	4·4 ± 0·3[a]	4·0 ± 0·04[a]	4·1 ± 0·3[a]
Insulin (μU/ml)	15·8 ± 1·8	17·9 ± 1·9[b]	4·8 ± 0·8[a]	12·6 ± 2·1[b]	5·4 ± 1·1[a]
Body weight (g)	220 ± 20	210 ± 15	145 ± 25[a]	165 ± 22[a]	170 ± 26[c]

Values are mean ± SEM of four or more separate experiments with four to six animals in each group.
Fisher's P values are shown as [a]$P < 0{\cdot}001$, [b]$P < 0{\cdot}005$, [c]$P < 0{\cdot}01$.The groups like controls + vanadate, diabetes, diabetes insulin and diabetes + vanadate were compared with controls.

activity by > 200% in reticulocytes and young RBCs, 87% in middle-aged and 79% in old RBCs in comparison to their corresponding cell type of control animals. Insulin and vanadate showed the recovery of enzyme in all the cell types.

4. Discussion

Ageing of cells leads to production of free radicals that generate lipid radicals, hydrogen peroxides and other toxins, which gradually accumulate and play a major role in death of cells (Kirkwood 1992). The living cells have antioxidant enzymes and certain metabolites, which try to repair the damage caused by free radicals and their products. Normally the antioxidant capacity and the metabolic potential of cells decrease in due course of ageing with a decrease in most of the glucose metabolizing enzymes (Clark and Sohet 1985; Wei 1998).

In normal ageing process, the levels of hexokinase are higher in reticulocytes and lower in older cells, therefore metabolism of glucose is more active in reticulocytes than in older RBCs (Rapoport *et al* 1974). The metabolism of glucose in these older cells generate more GSH by increasing the activity of GSSG-R as observed in the present study, which may be a compensatory mechanism for the survival of cells. Excess GSH may be used in protecting cells from oxidative injury. GSH may be utilized for other biological reactions such as in detoxification of toxins and other xenobiotic compounds in combination with GST. The decreased activity of GSH-Px observed in older cells may be responsible for the decreasing antioxidant capacity of older cells. The antioxidant capacity of reticulocytes may be higher than other cell types, as GSH-Px activity in reticulocytes was found to be higher in the present study. The GSSG/GSH ratio in RBCs regulate the hexose monophosphate shunt pathway when RBCs are subjected to oxidative stress. In older cells the availability of NADPH is more due to decrease in biosynthetic pathway like lipogenesis (Gupta *et al* 1997). This may be due to increased GSH-Px and a decrease in the oxidative pathway, leading to an increase in the production of GSSG and NADPH. The excess GSSG may be transported out from the RBCs (LaBelle *et al* 1986), because if it remains inside the cell it may combine with

Table 2. Enzyme activities of HK, GSH-Px, GSSG-R and GST in reticulocytes (Ret), young red cells (Y), middle-aged red cells (M) and old red cells (O) of control and diabetic rats and their treatment with insulin and vanadate.

Enzymes/cells	Controls	Controls + vanadate	Diabetes	Diabetes + insulin	Diabetes + vanadate
HK					
Ret	1·30 ± 0·30	1·70 ± 0·3^{c}	1·60 ± 0·20	1·40 ± 0·30	2·6 ± 0·40^{b}
Y	1·35 ± 0·15	1·61 ± 0·5^{c}	0·86 ± 0·04^{a}	1·25 ± 0·28	1·42 ± 0·3
M	0·60 ± 0·07^{a}	1·21 ± 0·3^{b}	0·37 ± 0·03^{b}	0·58 ± 0·05	1·10 ± 0·2^{b}
O	0·38 ± 0·04^{a}	1·02 ± 0·4^{b}	0·26 ± 0·03^{c}	0·40 ± 0·08	0·80 ± 0·08^{b}
GSH-Px					
Ret	1·28 ± 0·41	3·12 ± 0·78^{b}	2·32 ± 0·59^{b}	1·25 ± 0·22	1·29 ± 0·26
Y	1·24 ± 0·36	3·29 ± 0·60^{b}	1·08 ± 0·14^{a}	1·08 ± 0·16^{a}	1·29 ± 0·23
M	1·01 ± 0·22^{b}	1·89 ± 0·40^{c}	0·81 ± 0·08^{c}	1·18 ± 0·18^{a}	1·35 ± 0·04^{a}
O	0·86 ± 0·11^{a}	1·54 ± 0·26^{b}	0·58 ± 0·06^{c}	1·24 ± 0·22^{c}	0·82 ± 0·02
GSSG-R					
Ret	0·71 ± 0·04	1·65 ± 1·30^{b}	2·61 ± 0·34^{b}	0·68 ± 0·05	1·93 ± 1·48^{b}
Y	0·81 ± 0·03^{b}	1·85 ± 2·42^{b}	1·21 ± 0·14^{b}	0·75 ± 0·12	1·90 ± 0·80^{b}
M	1·05 ± 0·05^{a}	2·42 ± 0·72^{b}	1·76 ± 0·42^{b}	0·92 ± 0·16	2·98 ± 0·33^{b}
O	1·18 ± 0·05^{a}	2·25 ± 0·38^{b}	1·94 ± 0·29^{b}	0·98 ± 0·18	3·37 ± 0·41^{b}
GST					
Ret	129 ± 33	258 ± 45^{b}	394 ± 48^{b}	98 ± 31^{a}	243 ± 38^{b}
Y	152 ± 21^{b}	215 ± 198^{a}	131 ± 18	231 ± 19^{b}	243 ± 41^{b}
M	185 ± 36^{a}	134 ± 23^{a}	108 ± 15^{c}	271 ± 18^{b}	141 ± 38^{a}
O	218 ± 58^{a}	49 ± 8^{b}	89 ± 10^{b}	236 ± 23^{a}	88 ± 12^{b}

The enzyme activity is expressed as μmol/min/g Hb.
Values are mean ± SEM of four or more separate experiments each with four to six animals in each group.
Fisher's *P* values are shown as $^{a}P < 0.001$, $^{b}P < 0.05$, $^{c}P < 0.01$.
The groups like controls + vanadate, diabetes, diabetes + insulin and diabetes + vanadate were compared with controls with their corresponding age fractionated cells. In control group Y, M and O were compared with reticulocytes.

other proteins to inactivate them. NADP generated during glutathione reduction as observed in older cells, may be stimulating G6PDH which may lead to an increase in the utilization of glucose via the shunt pathway (Gonzalez *et al.* 1986; Gupta *et al* 1997).

Aspartate aminotransferase and arginase showing a decreasing activity with the age of cells may indicate that aspartate metabolism is higher in reticulocytes in comparison to other cell types. AlaAT showed an increasing pattern from reticulocytes to old cells. Diabetes is associated with excess generation of free radicals and their products. Most of the enzymes like HK, G6PDH, GSH-Px and GST showed a further decrease from young to old cells when compared to corresponding control values. The enzyme GSSG-R, however, showed a higher activity in diabetes; this could be to maintain the GSH level, which may be to augment its role in defense against oxidants. Not only GSSG-R activity was increased in reticulocytes in diabetes, but other enzymes like HK, G6PDH, GSH-Px and GST also increased in these cells. When freshly synthesized reticulocytes come out into the circulation they have to face an increased concentration of glucose which may produce glucose toxicity (Rapoport *et al* 1974). As senescence of cells progresses, the observed decrease in the levels of glucose metabolizing enzymes (Gupta and Baquer 1998) may be due to a lower stimulation of glucose transporter in older RBCs.

Vanadate stimulates the phosphatases which leads to dephosphorylation of insulin receptors (Ezaki 1990; Tamura 1984) thus stimulating the glucose transporters (Brichard *et al* 1993). In the present investigation diabetic animals treated with vanadate died more in the initial stage of treatment in comparison to untreated diabetic animals. It may be because vanadate decreases food and water intake by diabetic animals due to the bitter taste of vanadate (data not shown), which may lead to dehydration causing their initial death. The short-term effects such as gastrointestinal discomfort and decreased body weight gain have been reported (Srivastava 2000). The glycolytic and antioxidant status of aged cells and their recovery by insulin has already been reported from our laboratory (Gupta *et al* 1993). Visualizing the overall effects seen in the present study, it may be proposed that vanadate is potentially active in enhancing the antioxidant status in aged cells.

The protein degradation in diabetes was more as the diabetic animals use carbon skeleton of the proteins as the source of energy (Atchley and Richards 1953), as indicated by the increased activity of AsAT and arginase in diabetic reticulocytes which is restored near to control levels by treatment with vanadate in all the cell types. On the other hand vanadate stimulated protein breakdown in control animals. Insulin was found to restore the increased activity of protein degrading enzymes in diabetic

Table 3. Enzyme activities of AsAT, AlaAT and arginase in reticulocytes (Ret), young red cells (Y), middle-aged red cells (M) and old red cells (O) of control and diabetic rats and their treatment with insulin and vanadate.

Enzymes/cells	Controls	Controls + vanadate	Diabetes	Diabetes + insulin	Diabetes + vanadate
AsAT					
Ret	1·33 ± 0·34	1·86 ± 0·6^a	1·72 ± 0·26^a	0·99 ± 0·38	2·71 ± 0·7^b
Y	1·38 ± 0·17	1·71 ± 0·9^a	0·92 ± 0·07^a	1·17 ± 0·21	1·47 ± 0·6
M	0·70 ± 0·11^a	1·29 ± 0·4^b	0·46 ± 0·06^a	1·39 ± 0·20^b	1·21 ± 0·4^b
O	0·46 ± 0·08^a	1·14 ± 0·7^b	0·32 ± 0·08	1·41 ± 0·61^b	0·93 ± 0·1^b
AlaAT					
Ret	0·45 ± 0·06	1·53 ± 0·36^b	3·07 ± 1·02^b	0·46 ± 0·08	0·88 ± 1·23
Y	0·95 ± 0·31^a	2·21 ± 0·71^b	0·63 ± 0·12^a	0·84 ± 0·19	1·68 ± 1·18^a
M	1·71 ± 0·13^a	2·40 ± 0·65^a	1·57 ± 0·25	1·49 ± 0·48	2·15 ± 0·32^a
O	2·54 ± 0·74^a	3·34 ± 0·34^a	2·80 ± 0·22^c	1·89 ± 0·66^c	3·08 ± 0·80^a
Arginase					
Ret	1·32 ± 0·39	3·26 ± 0·81^b	2·46 ± 0·58^b	1·66 ± 0·98^c	1·34 ± 0·31
Y	1·29 ± 0·42	3·31 ± 0·68^b	1·18 ± 0·19	1·46 ± 0·49	1·33 ± 0·29
M	1·11 ± 0·26^a	1·95 ± 0·49^b	0·90 ± 0·11^a	0·93 ± 0·39	1·39 ± 0·06
O	0·92 ± 0·17^a	1·63 ± 0·28^b	0·59 ± 0·08^a	0·82 ± 0·35	0·88 ± 0·04

The enzyme activity is expressed as μmol/min/g Hb.
Values are mean ± SEM of four or more separate experiments each with four to six animals in each group.
Fisher's *P* values are shown as $^aP < 0.001$, $^bP < 0.05$, $^cP < 0.01$.
The groups like controls + vanadate, diabetes, diabetes + insulin and diabetes + vanadate were compared with controls with their respective age fractionated cells. In control group Y, M and O were compared with reticulocytes.

animals and has also been reported to show protein anabolic action in the human body (Bonnet and Rennie 1991).

The present study shows that as the cell ages, metabolism of glucose is lowered, antioxidant capacity of the cell is decreased and these effects are accentuated further in the diabetic state. It may be suggested that vanadate may be an additional antidiabetic agent in preventing diabetic complications in RBCs.

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