# Adenosine triphosphate and diphosphoglycerate levels in red blood cells from patients with Down's syndrome

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*Summary.* The levels of ATP and ATP plus DPG were significantly elevated in erythrocytes from Down's syndrome patients when compared to erythrocytes from age matched controls. The hemoglobin content and hematocrit values were significantly reduced. The resultant tendency towards anemia probably explains the elevation in metabolite levels.

Recent studies on growth and development of trisomics utilizing cephalometric analysis have shown considerable reduction in the nasal airway of trisomics. The reduction causes changes in both soft and hard oral tissues which facilitate mouth breathing[2,3]. These results led to speculating that trisomics may be subject to hypoxia. Other reports tend to substantiate this contention[4,5,9-12]. Arteriovenous differences in oxygen across the brain have been reported to be less in trisomics than in age matched controls and the authors suggested that there may be decreased oxygen consumption by the central nervous system in Down's syndrome[4,5]. The impairment appeared to be worse in infants than adults. Using halothane anesthetized adults, arteriovenous oxygen differences were not observed[6]. However, halothane may have complicated the study because in animal experiments, anesthetics inhibit carbohydrate metabolism[7,8]. $NAD^+$ is reduced to 50% and NADH to 65% in cerebrospinal fluid from Down's syndrome individuals, also suggesting a deficiency in neural energy metabolism[9]. Several reports indicate a deficiency in platelet energy metabolism in trisomics[10-12]. There are, however, conflicting reports about red blood cell energy metabolism. 2 metabolites, adenosine 5'triphosphate (ATP) and 2,3-diphosphoglycerate (DPG), are directly related to glycolysis, the primary pathway involved in energy production in erythrocytes. These metabolites are also very important in regulating the affinity of hemoglobin for oxygen. If these metabolites are high, hemoglobin readily releases oxygen to the tissues. If the metabolites are low, hemoglobin affinity for oxygen is increased and tissue hypoxia may occur[13-15]. DPG is more significant than ATP in regulation of hemoglobin affinity for oxygen since its concentration is 3-4 times greater than that of ATP[16]. Further in vitro estimations suggest that 60-80% of the ATP is present in a MgATP complex and is not free to interact with hemoglobin[16] thus further decreasing the role played by ATP in oxygen binding by hemoglobin. Nonetheless the small increment of free ATP likely plays an important role in determining the final position of oxygen dissociation curves[17,18].

In a study of 20 trisomic subjects, 12 male and 8 female ranging from 9 to 26 years of age and 20 corresponding controls, the respective DPG values were 5.37 and 4.32 µmoles/ml of red blood cells[19]. In another study, ATP levels were decreased from 1.47 mM in red cells from controls to 1.19 mM in red cells from 15 Down's syndrome children ranging from 1.5 to 6.5 years of age[20]. A 3rd study showed ATP and DPG were both lower in erythrocytes from 7 11-17-year-old Down's syndrome patients. ATP and DPG were 1.33 mM and 7.33 mM respectively and corresponding levels for Down's syndrome erythrocytes were 0.79 and 3.70 mM[21].

*Materials and methods.* Blood was collected in vacutainer tubes containing EDTA from 16-20-year-old trisomic males and 18-22-year-old control males. The metabolites, ATP and DPG were extracted by addition of 1 ml of cold 0.6 M perchloric acid to each of 2 duplicate 0.5 ml samples of whole blood immediately after sample collection. Each sample was immediately mixed thoroughly by vortex action and then placed in ice. Before 2 h had elapsed the samples were centrifuged at $2000 \times g$, 10 min. Aliquots of the supernatant solutions were adjusted to pH 6.5-7.5 by addition of pre-determined amount of 2.0 M potassium carbonate. The potassium carbonate precipitates were removed by centrifugation. Aliquots were assayed for ATP, using a hexokinase, glucose-6-phosphate dehydrogenase coupled assay[22]. Quantitation of 2,3-diphosphoglycerate was performed using 2 phosphoglycolic acid as a stimulator of phosphoglycerate mutase[23]. The methodology is adequately described in the Technical Bulletin No. 35 UV, Sigma Chemical Company, St. Louis, Mo., USA, 63178. Aliquots of whole blood were analyzed for hematocrit and hemoglobin at the Health Sciences Center at the University of Manitoba. All supplies were obtained from Sigma Chemical Company.

*Results.* The values obtained for hematocrit, hemoglobin g%, DPG and ATP are shown in the table. Statistically significant decreases in Down's syndrome were observed for hematocrit and hemoglobin g% as was previously observed[24]. In contrast to the earlier data[7,21], we did not observe decreased levels of ATP in Down's syndrome red blood cells, rather we observed ATP levels to be significantly increased above the levels observed in control samples. DPG was also observed to be higher in Down's syndrome than in controls as was observed by others[19], however, in our study the increase was not statistically significant ($p > 0.1$). observed in the study of a large number of individuals[25].

Control experiments were performed to check lability of ATP and DPG. Whole blood was allowed to remain on ice 3 h after addition of perchloric acid and before neutralization with potassium carbonate. The ATP and DPG values did not change significantly, e.g. $1.55 \pm 0.11$ and $4.55 \pm 0.48$ mM respectively for immediately precipitated control samples and $1.57 \pm 0.03$ and $4.64 \pm 0.35$ mM for samples precipitated 3 h later ($n = 20$). Nor did ATP or DPG changes occur when neutralized samples were stored at $-80\,^{\circ}C$ and assayed 24 or 48 h later as has been previously reported[8]. The data reported herein was obtained from samples neutralized between 0.5-2 h after

Levels of ATP and 2,3-diphosphoglycerate in red blood cells of trisomic mongoloids and healthy controls

| | n | ATP (mM) | DPG (mM) | Hematocrit (%) | Hemoglobin (g%) |
|---|---|---|---|---|---|
| Control | 22 | $1.549 \pm 0.142$ | $4.505 \pm 0.481$ | $44.28 \pm 2.34$ | $15.54 \pm 0.940$ |
| Trisomics | 19 | $1.882 \pm 0.278$* | $4.794 \pm 0.556$ | $41.61 \pm 2.90$* | $14.44 \pm 1.165$* |

Data represent mean ± SD. * $p > 0.01$.

addition of perchloric acid. ATP and DPG were determined within 24 h.

*Discussion.* Several studies have shown that DPG and ATP (DPG more so than ATP[16]) are important physiological regulators of oxygen affinity of hemoglobin. DPG and ATP combine reversibly with deoxyhemoglobin[14,15]. If the levels of these metabolites increase above normal, the hemoglobin affinity for oxygen is decreased. The result is a right shifted oxygen dissociation curve which permits tissue demands for oxygen to be met when oxygen tension is reduced. ATP and DPG levels are increased in patients with long standing hypoxias such as anemia and pulmonary dysfunction[25]. This change allows these patients increased oxygen delivery. The cause of the altered ATP plus DPG levels observed in our study has not been definitely determined. Most likely, however, the explanation for the small alteration in metabolite levels reported herein is related to the tendency towards anemia observed in our study as is indicated by the statistically significant decrease in hemoglobin level or hematocrit (table). This interpretation is consistent with several others in which DPG and ATP are increased when hemoglobin levels are decreased[25,26].

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2 P. Porter, Thesis, University of Manitoba, Faculty of Dentistry, 1976.
3 R. Queen, Thesis, University of Manitoba, Faculty of Dentistry, 1975.
4 E. Himwich and J.F. Fazekas, Archs Neurol. Psychiat. *44*, 1213 (1940).
5 E. Himwich, J.F. Fazekas and S. Nesin, Am. J. ment. Defic. *64*, 37 (1940).
6 N.A. Lassen, S. Christensen, K. Hoedt-Rasmussen and B.M. Stewart, Archs Neurol. *15*, 595 (1966).
7 E.A. Brunner, J.V. Passonneau and C. Mostad, J. Neurochem. *18*, 2301 (1971).
8 O.H. Lowry, J.V. Passonneau, F.X. Hasselberger and D.W. Schultz, J. biol. Chem. *239*, 18 (1964).
9 M. Mino, Modern Med., Japan *24*, 322 (1969).
10 D.J. Boullin, M. Colma and R.A. O'Brien, J. Physiol. *204*, 128P (1969).
11 D.J. Boullin and R.A. O'Brien, J. Physiol. *212*, 287 (1971).
12 S.M. Bayer and E.E. McCoy, Biochem. Med. *9*, 225 (1974).
13 R. Benesch and R.E. Benesch, Biochem. biophys. Res. Commun. *26*, 162 (1967).
14 R. Benesch, R.E. Benesch and C.I. Yu, Proc. natl Acad. Sci. *59*, 526 (1968).
15 A. Chanutin and R.R. Curnish, Archs Biochem. Biophys. *121*, 96 (1967).
16 H.F. Bunn, B.J. Ransil and A. Chao, J. biol. Chem. *246*, 5273 (1971).
17 R.T. Card and M.C. Brain, New Engl. J. Med. *288*, 388 (1973).
18 H.F. Bunn, New Engl. J. Med. *288*, 416 (1973).
19 E. Nelson and P.F. Benson, Archs Dis. Childh. *47*, 672 (1972).
20 H. Bartels, Blut *22*, 216 (1971).
21 J. Kedziora, H. Hubner, M. Kanski, J. Jeske and W. Leyko, Ped. Res. *6*, 10 (1972b).
22 S.E. Granett and W.W. Wells, J. Neurochem. *19*, 1089 (1972).
23 Z.B. Rose and J. Liebowitz, J. analyt. Biochem. *35*, 177 (1970).
24 J. Kedziora, A.J. Hlynczak, J. Jeske and M. Kanski, Polish Endocr. *23*, 63 (1972a).
25 J.W. Eaton, G.J. Brewer, J.S. Schultz and C.F. Sing, Adv. exp. Med. Biol. *6*, 21 (1970).
26 J.W. Eaton and G.J. Brewer, Proc. natl Acad. Sci. *61*, 756 (1968).

## Changes in mouse liver superoxide dismutase activity and lipid peroxidation during embryonic and postpartum development[1]

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*Summary.* In inbred mice possessing 'high' and 'low' tissue superoxide dismutase (SOD) activity, it was observed that the difference in the SOD activities of the liver homogenates during development attains the maximum characteristic of the strain by about the 150th day. Subsequently, the SOD activity change displays a tendency in contrast with the age and the basic state. In the course of the development, a difference was also observed between the 2 mouse strains in the lipid peroxidation variation.

In the view of many authors, superoxide dismutase (EC 1.15.1.1) is one of the most important enzymes of aerobic life[3,4]. Our own examinations were primarily aimed at clarifying the roles of the peroxide metabolism enzymes, i.e. SOD, peroxidase (EC 1.11.1.6) and catalase (EC 1.11.1.7) in cell respiration and in cell defence against external damage[7]. We were also interested in the changes in the liver SOD and the lipid peroxidation (LP) in the course of development, and we therefore made a study of these in 2 inbred mouse strains, 1 possessing a 'high', and the other a 'low' SOD level.

*Materials and methods.* The mice used in the examinations were the inbred strains BlO and BlO.A provided by the Institute of Genetics of the B.R.C. Szeged[8]. The mice consumed normal rat pellets, and received water ad libitum. On the 10th day of intrauterine life, the SOD activity of the total embryo homogenate was determined. In the 20-day embryo, and subsequently, the liver could be well separated, and thus only the SOD activity and LP of the liver were examined. In general the crude liver homogenates were prepared in 10 volumes of 0.05 M $K_2HPO_4$ solution (pH 7.8). The homogenates were centrifuged at $8000 \times g$ for 15 min, and aliquots of the supernatant, or appropriate dilutions of this with the above phosphate buffer, were used for SOD measurement. Protein concentration was determined by Lowry's method[9].

Lipid peroxidation was measured via the thiobarbituric acid colour reaction on the whole homogenizates at 548 nm by the method of Placer et al.[10]. The SOD activity was measured by the method of Misra and Fridovich[11], using the epinephrine adrenochrome reaction. Under these conditions, the rate of increase of $A_{480}$ due to adrenochrome was about 0.01 units/min. 1 unit of SOD activity was defined as the amount of the enzyme required for 50% inhibition[5,6].