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].

1 Acknowledgments. The authors are grateful to the Mrs James A. Richardson Foundation and the Medical Research Council of Canada (grant No. M.A. 5447) for providing financial assistance. The authors also wish to thank Dr L. Lowther, J. Crabtree and Dolores Suga for their assistance in this study.

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]

R. Novák[2], B. Matkovics, M. Marik and J. Fachet

Biological Isotops Laboratory, 'A.J.' University of Szeged, and Institute of Genetics, Biological Research Center of the Hungarian Academy of Sciences, H-6701 Szeged (Hungary), 17 October 1977

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].

Table 1. Liver homogenate lipid peroxidation data for mice strains B10 and B10.A of various ages in terms of nM MDA/mg protein. X±S (n=10)

	Age (days)	LP values in μM MDA/mg protein and μM MDA/g wet tissue wt B10		B10.A	
Embryo	10	0.65±0.04	0.021±0.001	0.64±0.05	0.022±0.001
	20	0.80±0.07	0.008±0.001	0.70±0.06	0.008±0.001
Postpartum	1	0.85±0.08	0.008±0.003	0.98±0.08	0.010±0.001
	30	1.40±0.12	0.007±0.003	2.65±0.19	0.014±0.001
	90	3.15±0.30	0.021±0.001	4.90±0.38	0.029±0.002
	120	3.40±0.25	0.020±0.002	7.10±0.70	0.044±0.003
	150	3.30±0.35	0.016±0.001	6.40±0.55	0.035±0.003
	200	3.20±0.27	0.014±0.001	5.20±0.43	0.025±0.002
	250	4.00±0.38	0.022±0.001	3.00±0.27	0.018±0.001

Table 2. SOD activity values in mice liver homogenates of various ages in units/g wet tissue wt and units/mg protein, respectively. X±S (n=10)

	Age (days)	Liver SOD values in units/g wet tissue wt and units/mg protein B10		B10.A	
Embryo	10	230± 22.1	5.0±0.4	195± 18.6	3.5±0.2
	20	1760±150	24.5±2.3	1370±125.5	18.5±1.8
Postpartum	1	2260±220	22.4±2.0	940± 93.6	9.2±0.8
	30	3950±300.5	30.5±2.8	1800±170	12.0±1.0
	90	5400±535.5	42.1±4.1	2800±268.5	23.2±2.1
	120	7500±735	53.0±5.2	4460±428	29.0±2.6
	150	9407±935	54.0±5.1	4500±440	28.5±2.2
	200	8300±828.5	43.9±4.3	6650±645.5	36.9±3.1
	250	6000±580	31.9±3.1	9300±900	48.8±3.6

All of the chemicals used were of the highest purity. All of the data reported are the averages of 10 objects and 10 measurements in each case (n=10). The SE of the SOD assays is about ±10%, and that of the LP about ±5%.

Results and discussion. Table 1 presents the LP values for the 2 inbred mouse strains, expressed in μM malondialdehyde (MDA)/mg protein.

2 tendencies can be observed from the data of table 1: a) after a certain stagnation, the LP values calculated in terms of μM MDA/mg protein display an increasing tendency with age; b) when our results are compared with the rat liver mitochondrial LP decrease during the embryonic period as described by Utsumi et al.[12], the difference can be perceived only if the MDA values are referred to mg protein and not to g wet tissue wt. Analogous observations relating to rat liver homogenates are now awaiting publication[13].

Table 2 compares the SOD values of the liver homogenates of the 2 mouse strains. The data show well that the SOD activities of the liver homogenates progressively increase during foetal development. This increase comes to a stop, and for strain BlO.A even changes to a significant decrease, in the first period of postpartum life. Following this, however, the increase again becomes progressive, and by the age of 4–5 months the value reaches a constant level. It stagnates here until the obvious signs of aging appear (see the values measured at the postpartum age of 9 months). It is also very important to emphasize that the above tendency relating to aging refers to the BlO strain. At the same time, a continuous increase with age can be detected in the SOD activity values of the BlO.A strain crude liver homogenate. However, significant differences can be demonstrated between the 2 strains as regards their immune response also[8]. It emerges from our data, therefore, that, from the embryonic age on, including the extrauterine life, the LP progressively increases in the liver tissue. The LP increase in the liver tissue is not hindered by extrauterine life. The liver LP reaches a new constant level in extrauterine life, which rises further only when aging begins.

From a comparison of the data of tables 1 and 2, it appears that, similarly to the rat liver data of Utsumi et al.[12], there is an inverse relation between the liver SOD and the LP, in so far as higher liver homogenate SOD values are accompanied by lower LP values.

It is also unambiguously shown by our examinations that the blood oxygen increase occurring as a consequence of the extrauterine pulmonary respiration is one of the inductors of the synthesis of SOD.

1 Part XVIII of the series 'Properties of enzymes'.
2 To whom reprint request and correspondence should be addressed: Középfasor 52, H-6726 Szeged, Hungary.
3 I. Fridovich, A. Rev. Biochem. *44*, 149 (1975).
4 E. Frieden, Chem. Engng News, March 25, p. 42 (1974).
5 B. Matkovics, R. Novák, Hoang Duc Hanh, L. Szabó, Sz. I. Varga and G. Zalesna, Comp. Biochem. Physiol. *56B*, 31 (1977).
6 B. Matkovics, R. Novák, Hoang Duc Hanh, L. Szabó and Sz. I. Varga: Comp. Biochem. Physiol. *56B*, 397 (1977).
7 B. Matkovics, in: Superoxide and superoxide dismutase. Ed. A.M. Michelson, J.M. McCord and I. Fridovich. Academic Press, London 1977.
8 R. Novák, Sz. I. Varga, B. Matkovics and J. Fachet, in preparation.
9 O.H. Lowry, N.J. Rosenbrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
10 Z.A. Placer, L.L. Cushman and B.C. Johnson, Analyt. Biochem. *16*, 359 (1966).
11 H.P. Misra and I. Fridovich, J. biol. Chem. *247*, 3170 (1972).
12 K. Utsumi, T. Yoshioka, N. Yamanaka and T. Nakazawa, FEBS Letters *79*, 1 (1977).
13 L. Mindszenty, J. Iván, M. Marik, R. Novák, L. Szabó and B. Matkovics, Comp. Pharmac., in press.