tested by concentrations of the metals up to 10^{-4} M seems unlikely. No quantitative correlation between the extent of hemolysis and the degree of enzyme inactivation was found. This is probably due to the significant differences in the properties of the hemolytic heavy metals. Some of them may change the permeability of the erythrocyte membrane[17], thus leading to increased osmotic pressure which accelerates the hemolysis. Nevertheless, it is clear that the peroxidation and eventually lysis are associated with a significant inactivation of the enzymes protecting erythrocytes against peroxidative damage, as well with a decrease of the GSH content.

1 Ribarov, S.R., and Benov, L.C., Biochim. biophys. Acta *640* (1980) 721.
2 Sree Kumar, K., Rowse, C., and Hochstein, P., Biochem. biophys. Res. Commun. *83* (1978) 587.
3 Ribarov, S.R., Bochev, P.G., and Juravlev, A.I., Studia biophys. *88* (1982) 171.
4 Ribarov, S.R., Benov, L.C., and Benchev, I.C., Biochim. biophys. Acta *664* (1981) 453.
5 Carrell, R.W., Winterbourn, C.C., and Rachmilewitz, E.A., Br. J. Haemat. *30* (1975) 259.
6 Ribarov, S.R., and Bochev, P.G., Archs Biochem. Biophys. *213* (1982) 288.
7 Kellog, E.W., and Fridovich, I., J. biol. Chem. *252* (1977) 6721.
8 Kong, S., and Davison, A.J., Archs Biochem. Biophys. *204* (1980) 18.
9 Czapski, G., and Han, Y.A., Photochem. Photobiol. *28* (1978) 651.
10 Kolthoff, I.M., Stricks, W., and Morren, L., Analyt. Chem. *26* (1954) 366.
11 Maral, J., Puget, K., and Michelson, A.M., Biochem. biophys. Res. Commun. *77* (1977) 1525.
12 Snyder, L.M., Liu, S.C., Palek, J., Bulat, P., Edelstein, L., Srivastava, S.K., and Forbier, N.L., Biochim. biophys. Acta *470* (1977) 290.
13 Utno, L.Y., Lipsberga, Z.A., Silova, A.A., and Korzan, V.A., Appl. Biochem. Microbiol. *14* (1978) 910.
14 Awasthi, Y.C., Beutler, E., and Srivastawa, S.K., J. biol. Chem. *250* (1975) 5144.
15 Rosen, J.F., and Sorell, M., in: Topics of environmental health, vol. 13, p. 151. Ed. J.O. Nriago. Elsevier/North-Holland Biomedical Press, Amsterdam, New York, Oxford 1978.
16 Ribarov, S.R., and Benchev, I.C., C. r. Acad. bulg. Sci. *35* (1982) 233.
17 Passow, H., Rothstein, A., and Clarkson, T.W., Pharmac. Rev. *13* (1961) 185.

Increase in superoxide dismutase activity induced by thyroid hormones in the brains of neonate and adult rats

V.M. Petrović, M. Spasić, Z. Saičić, B. Milić and R. Radojičić

Department of Endocrinology, Institute for Biological Research and Institute of Physiology and Biochemistry, Faculty of Sciences, University of Belgrade, 29. Novembra 142, Belgrade (Yugoslavia), 29 September 1981

Summary. The activity of cytoplasmic superoxide dismutase (SOD) in the liver was about twice as high in adult rats as it was in neonates. In the brain and in the interscapular brown adipose tissue (IBAT), SOD activity was not changed during postnatal development, although it was slightly higher in the brain than in the IBAT ($p > 0.1$). Thyroid hormones produced an increase in SOD activity in the brain of newborn rats, as well as in those animals 30 and 60 days old. The same quantity of hormones did not produce any significant changes in the liver or in the IBAT.

Superoxide dismutase (SOD), prepared mainly from the liver, erythrocytes and brain, has been intensively studied[1]. Results obtained in the last few years showed that this enzyme may have a marked radioprotective influence and may be an important factor in the antiinflammatory action[2,3]. We have recently shown that noradrenaline (1.6 mg/kg/b.wt, i.p.) produced an increase in SOD activity in the interscapular brown adipose tissue (IBAT), but not in the liver of the rat[4]. However, higher doses of this neurohormone did produce a marked increase of SOD activity in both tissues[5]. The aim of present experiment was to test the possible influence of thyroid hormones on SOD activity in some tissues, with a special attention to the brain during postnatal development.

Material and methods. 8 groups of male Mill Hill hooded rats, aged 10, 30, 60, 90, 120 and 180 days, were used for the experiment. Animals were kept at room temperature (22 °C). In the preliminary experiments, 6 groups (each consisting of 8–10 animals of the 6 different ages) were used for testing SOD activity during postnatal development. 3 other groups of 10-, 30- and 60-day-old rats were treated intragastrally once daily, 3 days prior to sacrifice, with novothyral (Lek) which contains 10 µg of L-3,5,3′-triiodothyronine and 50 µg of L-3,5,3′,5′-tetraiodothyronine. The liver, IBAT and brain were removed within 3 min, the liver being perfused prior to the removal.

Tissues were minced and then dispersed with a loosely-fitting pestle in a Potter-Elvehjem homogenizer in 9 vols of the buffer, containing 0.05 M KH_2PO_4 and 10^{-4} M EDTA, pH 7.8. All the operations were performed at 4 °C. The homogenate was centrifuged for 15 min at 6000 × g in a Sorvall centrifuge. The supernatant was centrifuged for 90 min at 85,000 × g and used for the determination of SOD activity, as described by Misra and Fridovich[6]. This method is based on the capacity of SOD to inhibit autooxidation of adrenaline to adrenochrome. 1 unit of SOD activity was

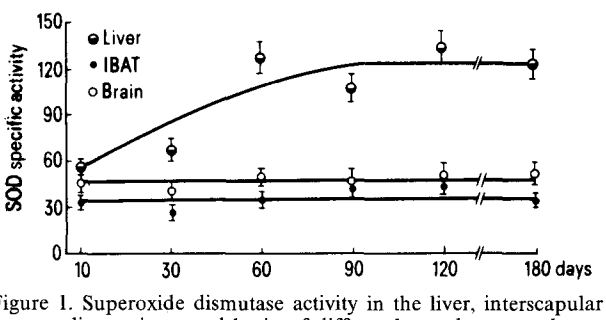

Figure 1. Superoxide dismutase activity in the liver, interscapular brown adipose tissue and brain of differently aged untreated rats. Mean ± SEM of 8 or 10 animals.

defined as the amount of protein causing 50% inhibition of the autooxidation of adrenaline in a volume of 3.2 ml reaction mixture, containing 3×10^{-4} M adrenaline, 1×10^{-4} M EDTA and 0.05 M Na_2CO_3 at pH 10.2. The procedure was performed at 26 °C. Protein was determined according to the method of Lowry et al.[7]. Spectrophotometric assays were performed in a Gilford model 250. All reagents used were p.a.

Results and discussion. Since the developmental period of the rat brain is considered to be completed in the 1st weeks after birth[8], we examined cytoplasmic SOD in neonate, young and adult rats. The liver and IBAT were analyzed as well. As shown in figure 1, SOD activity in the livers of untreated animals was found to increase progressively from 10 to 60 days after birth. It was twice as high in adults as in immature animals ($p < 0.001$). However, the activity of this enzyme in the brain and in IBAT remained approximately constant during postnatal development; the specific activity was slightly higher in the brain than in IBAT (fig. 1). Our results are in agreement with those of Bohnekamp and Weser[9], who analyzed the SOD activity within different segments of the rat brain and did not find age-dependent differences. They reported that numerical values remained constant from 3 weeks to 3 months of age. Similar results were obtained by Van Balgooy and Roberts[10], who observed no quantitative differences in SOD activities of mice aged between 1 and 23 days. Contrary to that, Mavelli et al.[11] did find changes in SOD activity in the rat brain in the period between the 10th and 180th day of postnatal development. This apparent discrepancy may be due, at least partly, to the difference in the procedure used by Mavelli et al.[11]. (The sonication allow them to extract cytoplasmic as well as mitochondrial SOD). Our results concerning SOD activity in the liver during postnatal development are in agreement with the findings of Yoshioka et al.[12], who found a rapid increase in the activity level from about the 10th day after birth. The higher enzyme activity found in the present experiment in the liver of untreated animals, as compared with other tissues studied, is probably due to the high rate of metabolic activity of this organ.

Until now the SOD activity in the IBAT has not been studied. Our present findings show that this activity was very slightly lower in the IBAT than in the brain in both neonate and adult rats, but the difference was not statistically significant. In addition SOD activity did not change during postnatal development.

The main interest in the present study was to examine the effect of thyroid hormones on cytoplasmic SOD activity in the brain during postnatal development. As shown in figure 2, thyroid hormones, given intragastrally once daily during a period of 3 days, produced an increase in the cytoplasmic SOD activity in the whole brain of 10-, 30- or 60-day-old rats ($p < 0.02$, $p < 0.05$ and $p < 0.01$, respectively). In the liver and IBAT the same amount of thyroid hormones did not produce significant changes in SOD activity. The increase in SOD activity in the brains of the animals treated with thyroid hormones may be the consequence of the increased level of superoxide radicals in the tissue studied. Taking into consideration that the brain was unresponsive to thyroid hormones in vitro, and that binding molecules for these hormones were not found in the adult brain[13,14], our present results suggest the possibility of producing superoxide radicals in the brain under the influence of thyroid hormones outside the electro-transport sequential process. The increased SOD activity found in the brains of hormone-treated animals may be particulary important in protecting them against the toxicity of free oxygen radicals.

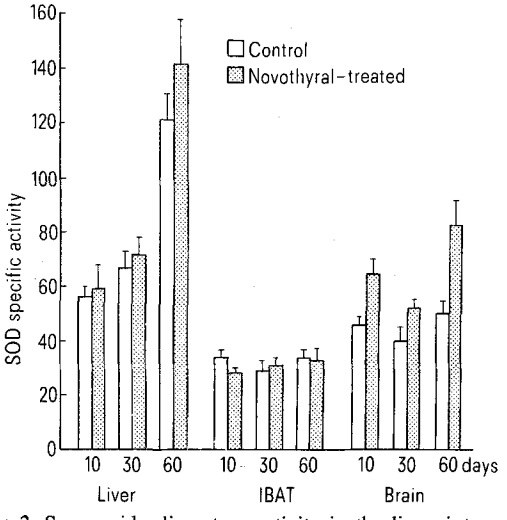

Figure 2. Superoxide dismutase activity in the liver, interscapular brown adipose tissue and brain of 10-, 30- and 60-day-old rats treated with thyroid hormones. Mean ± SEM of 8 or 10 animals. Difference between treated and control 10-, 30-, 60-day-old animals for the brain: $p < 0.01$, $p < 0.05$ and $p < 0.01$, respectively.

1 Weisiger, R.A., and Fridovich, I., J. biol. Chem. *248* (1973) 3582.
2 Petkau, A., and Chelack, W.S., Biochim. biophys. Acta *433* (1976) 445.
3 Hassan, H.M., and Fridovich, I., Archs Biochem. Biophys. *196* (1979) 385.
4 Petrović, V.M., Gudz, Tatjana, and Saičić, Zorica, Experientia *37* (1981) 14.
5 Petrović, V.M., Saičić, Zorica, Spasić, M., Radojičić, R., and Milić, Biljana, Bull. Acad. Serbe Sci. *72* (1980) 11.
6 Misra, H.P., and Fridovich, I., J. biol. Chem. *247* (1972) 3170.
7 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
8 Van Den Berg, C.J., Biochemistry of the developing brain, vol. 2, 149. Ed. W. Himwich. Dekker, New York 1974.
9 Bohnekamp, W., and Weser, U., Hoppe-Seyler's Z. physiol. Chem. *356* (1975) 747.
10 Van Balgooy, J.N.A., and Roberts, E., Comp. Biochem. Physiol. *62* (1978) 263.
11 Mavelli, I., Mondovi, B., Federico, R., and Rotilio, G., J. Neurochem. *31* (1977) 363.
12 Yoshioka, T., Utsumi, K., and Sekiba, K., Biol. Neonate *32* (1977) 147.
13 Sterling, K., Milch, P.O., Brenner, M.A., and Lazarus, J.H., Science *197* (1977) 996.
14 Sterling, K., Lazarus, J.H., Milch, P.O., Sakurada, T., and Brenner, M.A., Science *201* (1978) 1120.