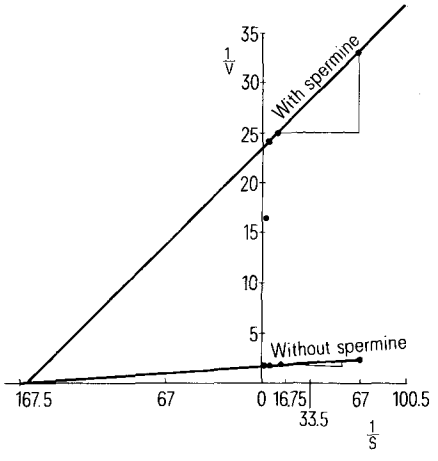
Results and discussion. Results of our study demonstrate that spermine, like theophylline[3], inhibited the phosphodiesterase activity of human spermatozoa (table). Inhibition of the enzyme activity by spermine was dose-related. On studying the kinetics, this inhibition was found to be non-competitive (figure). The apparent K_m-value of the enzyme was 6.18 µM.

In our earlier studies, the effect of spermine on sperm adenylyl cyclase was determined in the presence of theophylline (a potent inhibitor of sperm phosphodiesterase[3]). Increased levels of cyclic AMP observed by us in these experiments were therefore due to the increased formation of cyclic AMP as a result of activation of adenyl cyclase by spermine. The results of the present studies suggest that spermine also decreased the degradation of cyclic AMP by virtue of its action on phosphodiesterase. The observed effects of spermine on accumulation of cyclic AMP by spermatozoa are therefore the net result of its effect on formation as well as on degradation of cyclic AMP. These effects of spermine on cyclic AMP levels may be of physiological importance as the cyclic AMP is known to be involved in all processes starting from the motility to the capacitation of the human spermatozoa prior to fertilization[1,3].

It has been reported that cyclic nucleotide phosphodiesterase inhibitors such as caffeine, theophylline, and papaverine markedly increased motility of bovine epididymal spermatozoa incubated with pyruvate, acetate, oxal-acetate or β-hydroxybutyrate[3]. Spermine, by virtue of its phosphodiesterase-inhibitory action may have an important role in the regulation of sperm motility and metabolism, especially when it is present in the unusually high concentrations found in human semen[9-11].



The Lineweaver-Burk plot showing the change in the phosphodiesterase activity of human spermatozoa sonicate (supernate) with variation in the substrate concentration (mM^{-1}) in presence or absence of 5.6 mM spermine in the reaction mixture.
The K_m-value was found to be 6.18 µM and the inhibition seems to be non-competitive.

1 J.J. Hicks, N. Pedron, Martinez-Manatou and A. Rosado, Fert. Steril. *23*, 886 (1972).
2 J. Tash and T. Mann, J. Reprod. Fert. *35*, 591 (1973).
3 D.L. Garbers, N.L. First and H.A. Lardy, J. biol. Chem. *248*, 875 (1972).
4 G.V. Shah, A.R. Sheth, P.P. Mugatwala and Shanta S. Rao, Experientia *31*, 631 (1975).
5 A.R. Sheth, G.V. Shah and Shanta S. Rao, Indian J. Biochem. Biophys. *13*, 129 (1976).
6 R.W. Butcher and E.W. Sutherland, J. biol. Chem. *237*, 1244 (1962).
7 C.H. Fiske and Y. Subba Rao, J. biol. Chem. *66*, 375 (1925).
8 O.H. Lowry, N.J. Rosebrough, A. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
9 A. Leeu Wenhoek as quoted by T. Mann, in: Biochemistry of semen and of male accessory reproductive tract. Methuen & Co., London 1964.
10 T. Tabor and C.W. Tabor, Pharmac. Rev. *16*, 245 (1964).
11 A.N. Thakur, A.R. Sheth, S.S. Rao and D.S. Pardhanani, Indian J. Biochem. Biophys. *10*, 134 (1973).

Cysteine oxidase and cysteine sulfinic acid decarboxylase in developing rat liver

C. Loriette and Fernande Chatagner[1]

Laboratoire de Biochimie, 96, Boulevard Raspail, F-75006 Paris (France), 18 January 1978

Summary. The patterns of development of cysteine oxidase (CO) and cysteine sulfinic acid decarboxylase (CSD) in rat liver are not similar. It was observed that CO is not under sex control as CSD is. The results obtained agree with the idea that, in liver, as well as in brain, CSD is the limiting factor for the regulation of taurine biosynthesis.

The physiological importance of taurine has been recognized since it was known that, in mammals, lipids are absorbed by the intestine as complexes with taurine and glycocolle bile salts (principally taurine conjugates in the rat). In addition, during recent years, taurine was shown to have other functions specially in the central nervous system[2]: in vision[3], in the heart[4], in skeletal muscles[5] and taurine is probably involved in endocrine[6] and reproductive[7] processes.

It is well accepted that the most significant biosynthetic pathway of taurine in the rat liver begins with the oxidation of cysteine to cysteine sulfinic acid. This acid is then decarboxylated into hypotaurine which is oxidized into taurine. Cysteine oxidase(CO) (EC 1.13.11.20) catalyses the 1st step, cysteine sulfinic acid decarboxylase (CSD) (EC 4.1.1.29) and hypotaurine oxidase catalyse respectively the 2nd and the 3rd steps. In rat liver, CO and CSD are well defined enzymes whereas little is known about hypotaurine oxidase.

The purpose of this study was to determine the developmental patterns of CO and CSD in liver of male and female rats.

Materials and methods. Albino rats of local breeding (from 2 days a.p. to 150 days p.p.) were used. From weaning they were maintained on a commercial stock diet (UAR 103). DL 3 ^{14}C cysteine and L 1 ^{14}C cysteine sulfinic acid (CSA) were purchased from CEA, Saclay, France. Rats were killed by decapitation and the livers quickly removed were homogeneized at 4 °C in water to a 20% (w/v) suspension. In experiments with fetuses, livers from several animals were pooled. All enzyme assays were performed immediately after the preparation of the tissue extracts. CO

activity was assayed according to Yamaguchi et al.[8]. CSD activity was measured by the method of Bergeret et al.[9] with minor modifications: the assay mixture contained L $1^{14}C$ CSA (1 µCi) instead of only unlabelled CSA and the final volume was 1.1 ml. $^{14}CO_2$ was trapped in hyamine hydroxide and counted in a Intertechnique liquid scintillation spectrometer after addition of 10 ml of scintillation solution (toluene, PPO,POPOP).

The results are respectively expressed as µmoles of CSA produced and µmoles of cysteine sulfinic acid decarboxylated per g wet tissue per h. They are presented the mean ± SEM; the statistical analysis was performed according to Student's t-test.

Results. Cysteine oxidase activity (figure 1): It increased rather sharply from 12 µmoles of CSA produced/g of liver/h in a 19-day fetal liver to 47 µmoles/g of liver in 21-day young rat liver. Until the 21th day there was no difference between male and female CO activities. Between approximately the 30th and 50th day, female CO activity was constant and higher than male CO activity but this difference was not statistically significant. After the 50th day male and female activities were similar. When rats became older (100-day), CO activity slightly decreased. We observed that the activity in the 100-day liver was almost the same than that in the 21-day liver.

Cysteine sulfinic acid decarboxylase activity (figure 2): It was very low in fetal liver and until about the 10th day p.p. In male liver CSD activity regularly increased and reached a plateau on about the 35th day (28 µmoles CSA decarboxylated/g of liver/h). After the 50th day CSD activity slightly decreased (20 µmoles/g of liver/h in the 150-day male rat). In female liver, the highest level of CSD activity occured between the 35th and 40th day. From the 40th day CSD activity rapidly fell and its value is significantly lower ($p < 0.01$) than that in male rats of the same age. When females were 150 days old, CSD activities were similar to that in the 10-day rat. We did not observe any difference between liver activities in pregnant and non-pregnant rats at the same age. Weaning did not lead to a change in the increasing of CO and CSD activities.

Conclusions. The present observations show that the developmental patterns of rat liver CO and CSD activities differ markedly. The most stricking differences are:

1. A high level is more quickly reached for CO activity than for CSD activity: therefore it appears that rat liver is able to synthesize CSA rapidly after birth whereas CSA decarboxylation is slowly developing.
2. CO activity is higher than CSD activity: this observation suggests that, in liver, CSD decarboxylation might be the limiting factor for the production of taurine as already reported for the brain[10]. In both cases, however, the contribution of the 3rd step, namely the oxidation of hypotaurine to taurine, is still unknown. In addition, in liver, the lack of correlation between the CSD activity and the taurine concentration was reported years ago[11,12] and was also observed in our laboratory.
3. Adult male and female liver CO activities are very similar. On the contrary when puberty is reached male liver CSD activity is much more higher than female liver CSD activity. This significant difference was already reported[13].

As male rat brain is the only tissue on which studies on the development of CO activity[14] and CSD activity[11,15] were performed, it is worthwhile to compare liver and brain. The development of CO activity is different in liver and in brain, and in adult rat CO activity is much more higher in brain than in liver. The development of CSD activity is similar in brain and in liver and CSD activity is lower in brain than in liver. Furthermore no difference was observed between male and female brain CSD activities[13].

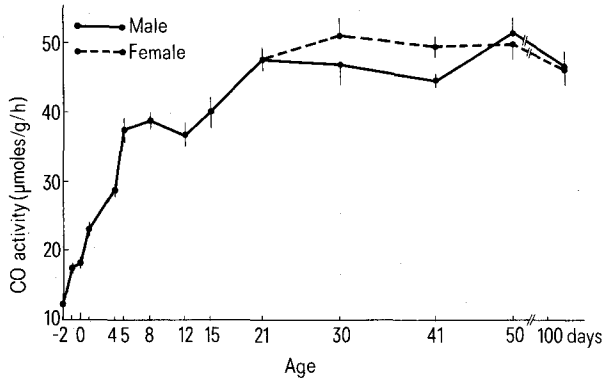


Fig. 1. Cysteine oxidase activity in fetal, newborn and adult rat liver (µmoles CSA produced/g of liver/h). Each point represents the mean ± SEM of 4–14 determinations.

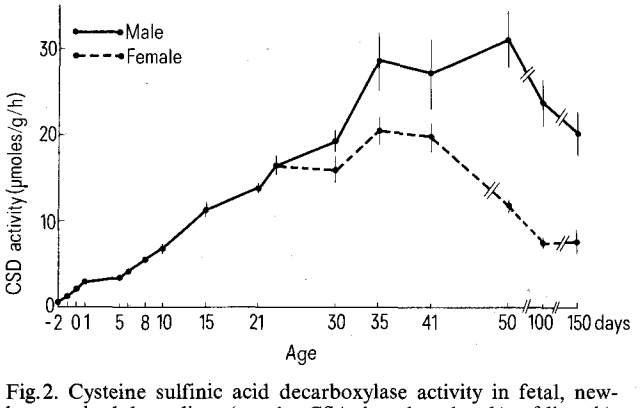


Fig. 2. Cysteine sulfinic acid decarboxylase activity in fetal, newborn and adult rat liver (µmoles CSA decarboxylated/g of liver/h). Each point represents the mean ± SEM of 5–15 determinations.

1 Acknowledgments: The authors thank CEA for financial support for the purchasing of labelled substrates.

2 A. Barbeau, N. Inoue, Y. Tsukada and R.F. Butterworth, Life Sci. *17*, 669 (1975).
3 H. Posantes-Morales, R. Salceda and A.M. Lopez-Colome, in: Taurine, p. 191, Ed. R. Huxtable and A. Barbeau. Raven Press, New York 1976.
4 D.S. Grosso and R. Bressler, Biochem. Pharmac. *25*, 2227 (1976).
5 R. Huxtable, J. Chubb and R. Bressler, Proc. West. pharmac. Soc. *18*, 101 (1975).
6 P.D. Thut, R.E. Hruska, R. Huxtable and R. Bressler, in: Taurine, p. 357. Ed. R. Huxtable and A. Barbeau, Raven Press, New York 1976.
7 C.D. Kochakian, in: Taurine, p. 375. Ed. R. Huxtable and A. Barbeau. Raven Press, New York 1976.
8 K. Yamaguchi, S. Sakakibara, K. Koga and I. Ueda, Biochim. biophys. Acta *237*, 502 (1971).
9 B. Bergeret, F. Chatagner and C. Fromageot, Biochim. biophys. Acta *17*, 128 (1955).
10 H. Pasantes-Morales, C. Loriette and F. Chatagner, Neurochem. Res. *2*, 671 (1977).
11 H.C. Agrawal, A.N. Davison and L.K. Kaczmarek, Biochem. J. *122*, 759 (1971).
12 D.G. Spaeth and D.L. Schneider, Proc. Soc. exp. Biol. Med. *147*, 855 (1974).
13 F. Chatagner and B. Bergeret, Bull. Soc. Chim. Biol. *38*, 1159 (1956).
14 C.H. Misra and J.W. Olney, Brain Res. *97*, 117 (1975).
15 H. Pasantes-Morales, C. Mapes, R. Tapia and P. Mandel, Brain Res. *107*, 575 (1976).