ger). When no further decrease in absorbance at 340 nm was observed, the (4-^{3}H) $NADP^+$ was purified in a manner analogous to that used for 4S-(4-^{3}H) NADPH with the exception that 10 mM Na phosphate, pH 6.0, was used as buffer.

4R-(4-^{3}H) NADPH (A-NADPH) was obtained from (4-^{3}H) $NADP^+$ and unlabeled D-glucose using conditions otherwise identical with those used for the preparation of 4S-(4-^{3}H) NADPH.

The following procedure was used to determine which of the 2 hydrogen atoms, pro-4S or pro-4R, was transferred to the aldehyde substrate. Aldehyde reductase, purified by the procedure of Wermuth et al.[1], was incubated in a mixture containing 10 mM Na phosphate pH 7.0, 1 mM 4S-(4-^{3}H) NADPH or 4R-(4-^{3}H) NADPH and 1 mM 4-nitrobenzaldehyde. When no further decrease in absorbance at 340 nm was observed the reaction mixture was subjected to ion exchange chromatography under the same conditions used for the purification of (4-^{3}H) $NADP^+$.

(1-$^3H_1$) n-Butanol was prepared enzymatically with aldehyde reductase from n-butanal and 4R-(4-^{3}H) NADPH. An excess of butanal was reduced by the addition of $NaBH_4$ and the (1-$^3H_1$) butanol was extracted with ether. H_2O was added and the ether evaporated. The concentration of butanol was determined enzymatically with alcohol dehydrogenase in the presence of an excess of NAD^+ and semicarbazide according to the method of Bücher and Redetzki[3]. When no further increase in absorbance at 340 nm occurred the reaction mixture was diluted with water to a conductivity of 5 mS and applied to DEAE-Sepharose equilibrated against 10 mM NH_4HCO_3, pH 8.0. Elution of the NADH was accomplished by a linear gradient 0-400 mM NaCl in 10 mM NH_4HCO_3.

Results and discussion. The results of our studies on the stereospecificity of hydrogen transfer catalyzed by human liver aldehyde reductase are summarized in the table. Most of the tritium label (92%) remained on the coenzyme when 4S-(4-^{3}H) NADPH was used for the reduction of the aldehyde substrate.

In contrast, in the presence of 4R-(4-^{3}H) NADPH less than 1 percent of the initial radioactivity was recovered in $NADP^+$ and essentially all of the label (102%) was transferred to 4-nitrobenzaldehyde. These results indicate that aldehyde reductase catalyzes the transfer of the pro-4R hydrogen atom of the dihydronicotinamide ring to the aldehyde substrate without exchange with protons of the medium and thus belongs to the A-side specific dehydrogenases.

In order to determine the stereospecificity of aldehyde reductase with respect to the addition of the hydrogen atom to the carbonyl carbon atom of the substrate, (1-^{3}H) n-butanol, synthesized from n-butanal and 4R-(4-^{3}H) NADPH in the presence of aldehyde reductase, was reoxidized with alcohol dehydrogenase. The results given in the table show that most of the label was transferred from (1-^{3}H) butanol to the coenzyme. This indicates the same stereospecificity of aldehyde reductase and alcohol dehydrogenase, which is known to add the hydrogen atom of the coenzyme to the *re* face of the carbonyl carbon atom of the substrate[4]. A similar stereospecificity has been reported for the recution of glyceraldehyde by aldehyde reductase from pig kidney cortex[5]. Aldose reductase, a monomeric dehydrogenase showing striking similarities to aldehyde reductase is also an A-side specific enzyme[6]. On the other hand, rabbit tissues contain an aldehyde reductase with a stereospecificity for the pro-4S hydrogen of NADPH[7,8]. Thus the determination of the stereospecificity distinguishes between aldehyde reductases and may reveal structural and mechanistic differences in a class of enzymes which are otherwise very similar in physical and kinetic properties.

1 B. Wermuth, J.D.B. Münch and J.P. von Wartburg, J. biol. Chem. *252*, 3821 (1977).
2 B. Wermuth, J.D.B. Münch, J. Hajdu and J.P. von Wartburg, Biochim. biophys. Acta *566*, 237 (1979).
3 T. Bücher and H. Redetzki, Klin. Wschr. *29*, 615 (1951).
4 G. Popják, in: The Enzymes, vol. 2, 3rd edn p. 115. 1970.
5 T.G. Flynn, J. Shires and D.J. Walton, J. biol. Chem. *250*, 2933 (1975).
6 H.B. Feldman, P.A. Szczepanik, P. Havre, R.J.M. Corrall, L.C. Yu, H.M. Rodman, B.A. Rosner, P.D. Klein and B.R. Landau, Biochim. biophys. Acta *480*, 14 (1977).
7 H.W. Culp and R.E. McMahon, J. biol. Chem. *243*, 848 (1968).
8 R.L. Felsted, D.R. Richter and N.R. Bachur, Fedn Proc. *37*, 1692 (1978).

Occurrence of mitochondrial monoamine oxidase in human semen

O. Suzuki*, M. Oya, Y. Katsumata, T. Matsumoto and S. Yada

Department of Legal Medicine and Division of Oncology, First Department of Surgery, Nagoya University School of Medicine, Nagoya 466 (Japan), 30 January 1979

Summary. Mitochondrial monoamine oxidase (MAO) was found in human semen, showing its K_m and V_{max} values of 91.7 μM and 290 pmoles/mg of protein/60 min, respectively, with kynuramine as substrate. A major part of the activity was due to type A MAO.

Amine oxidases in mammalian tissues can be classified into 2 groups[1]. One is a flavin-containing amine oxidase (EC 1.4.3.4), which is known to be located in mitochondrial membranes. The other is a copper- and pyridoxal-containing group (EC 1.4.3.6) including diamine oxidase, serum monoamine oxidase (MAO) and an amine oxidase in connective tissues. Although diamine oxidase was found in human semen[2,3], to our knowledge, the presence of mitochondrial MAO in human semen has never been reported. In the present study, we found mitochondrial MAO in human semen and characterized it briefly.

Materials and methods. Human semen was collected at the Department of Urology, Nagoya University Hospital. It was centrifuged at 1500 × g for 5 min. The resulting supernatant fraction was centrifuged at 18,000 × g for 20 min and the pellet was suspended in 0.25 M sucrose solution. The suspension was recentrifuged at 18,000 × g for 20 min and the pellet was resuspended in 0.1 M sodium phosphate buffer (pH 7.4). This was used as an enzyme source.

The determination of MAO activity with kynuramine as substrate was carried out fluorometrically by the method of Kraml[4] with a slight modification[5]. The concentration of

the substrate was 82 μM. For each assay, 0.09–0.38 mg of protein was used. The assays were carried out at 37 °C and pH 7.4 for 60 min. Under these conditions, the assays were linear during incubation for at least 60 min.

For inhibition experiments, clorgyline, a selective inhibitor of type A MAO[6], was generously supplied by May & Baker Ltd, Dagenham, England. Deprenyl, a selective inhibitor of type B MAO[7], was kindly donated by Prof. J. Knoll, Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary. It was confirmed that all inhibitors used did not quench the fluorescence of 4-hydroxyquinoline, the reaction product by MAO from kynuramine. The assay mixture was preincubated at 37 °C with each inhibitor for 10 min to ensure maximal enzyme inhibition. For a kinetic study, MAO activities were measured over the range of 5.1-1636 μM kynuramine. Protein was determined by a slight modification of the conventional biuret method[8].

Results and discussion. The kynuramine-deaminating activity was discovered in the 18,000× g-precipitate of human semen, showing the K_m and V_{max} values of 91.7 μM and 290 pmoles/mg of protein/60 min, respectively. The activity was also observed in the 1500× g-precipitate, but the specific activity was much lower than that in the 18,000× g-precipitate; no activity was found in the 18,000× g-supernatant fraction.

To determine the specific amine oxidase involved in the present kynuramine-oxidation, the effects of various inhibitors at the concentration of 1.0 mM were tested. As can be seen in the table, the inhibition by carbonyl reagents, such as semicarbazide and aminoguanidine, was low, while pargyline, an inhibitor of flavin-containing MAO, revealed almost complete inhibition. These results show that the kynuramine-oxidizing activity is due to flavin-containing (mitochondrial) MAO. Thus, this enzyme may be located in mitochondria of spermatozoa.

Sulhydryl reagents, such as p-chloromercuribenzoic acid and iodoacetic acid, showed potent MAO inhibition. These results are compatible with the observation that sulhydryl groups are involved in the reaction of mitochondrial MAO[9]. Potassium cyanide, a chelator of metals, hardly affected the activity.

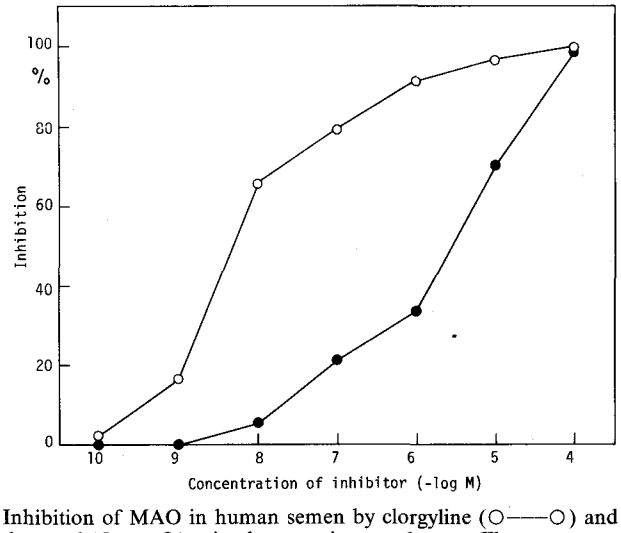
In recent years, mitochondrial MAO is believed to exist in 2 functional forms called type A and type B[6,10,11]. Type A MAO has been shown to be active with 5-hydroxytryptamine, norepinephrine and epinephrine[12] as substrates, and sensitive to inhibition by a low concentration of clorgyline. Type B MAO has been shown to be active with β-phenylethylamine and benzylamine, and sensitive to inhibition by a low concentration of deprenyl. Some substrates, such as kynuramine, tyramine, tryptamine and dopamine, are oxidized by either type of MAO. It should be noted that the concept of the 2 types of MAO is primarily based on the sensitivity of MAO to clorgyline. Therefore, to identify type A and type B MAO in human semen, we investigated the inhibition by clorgyline and deprenyl using kynuramine as substrate according to the method of Squires[13]. As illustrated in the figure, clorgyline, when present in the incubation medium at a concentration of 10^{-7} M, inhibited the kynuramine oxidation by about 80%. Much higher concentrations of deprenyl were required to inhibit the activity by the same extent. These results indicate that a major part of MAO activity in human semen is of type A enzyme. If we regard spermatozoa as an undifferentiated cell, our results are consistent with the idea that in undifferentiated or immature tissues type A MAO predominates[14].

Epinephrine, a substrate for type A MAO[12], is known to exist as a normal constituent of human semen in a relatively high concentration and may be related to the contractions of seminal vesicles[15]. Therefore, MAO in human semen may be responsible for the degradation of the amine.

Inhibition of human seminal MAO by various inhibitors*

Inhibitor	Percent inhibition
Pargyline	98.3
Semicarbazide	3.3
Aminoguanidine	10.8
Iodoacetic acid	35.0
p-Chloromercuribenzoic acid	96.7
Potassium cyanide	4.2

* Concentration of the inhibitors was 1.0 mM. Each value is the mean obtained form duplicate determinations.



Inhibition of MAO in human semen by clorgyline (○——○) and deprenyl (●——●) using kynuramine as substrate. The concentration of the substrate was 82 μM. Each point represents the mean obtained from duplicate determinations.

* Present address: Department of Legal Medicine, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431-31, Japan.

1 H. Blaschko, Rev. Physiol. Biochem. Pharmac. *70*, 83 (1974).
2 E.A. Zeller and C.A. Jöel, Helv. chim. Acta *24*, 968 (1941).
3 O. Suzuki, M. Oya, Y. Katsumata, S. Yada and K. Higashide, Jap. J. legal Med. *33*, 1 (1979).
4 M. Kraml, Biochem. Pharmac. *14*, 1684 (1965).
5 B. Century and K.L. Rupp, Biochem. Pharmac. *17*, 2012 (1968).
6 J.P. Johnston, Biochem. Pharmac. *17*, 1285 (1968).
7 J. Knoll and K. Magyar, in: Advances in Biochemical Psychopharmacology, vol. 5, p. 393. Ed. E. Costa and M. Sandler. Raven Press, New York 1972.
8 O. Suzuki, E. Noguchi and K. Yagi, Brain Res. *135*, 305 (1977).
9 A.L. Symes and T.L. Sourkes, Can. J. Biochem. *53*, 910 (1975).
10 H.-Y.T. Yang and N.H. Neff, J. Pharmac. exp. Ther. *187*, 365 (1973).
11 H.-Y. T. Yang and N.H. Neff, J. Pharmac. exp. Ther. *189*, 733 (1974).
12 M.D. Houslay and K.F. Tipton, Biochem. J. *139*, 645 (1974).
13 R.F. Squires, in: Advances in Biochemical Psychopharmacology, vol. 5, p. 355. Ed. E. Costa and M. Sandler. Raven Press, New York 1972.
14 T.J. Mantle, N.J. Garrett and K.F. Tipton, FEBS Lett. *64*, 227 (1976).
15 T. Mann, in: The Biochemistry of Semen and of the Male Reproductive Tract, p. 216. Methuen & Co. Ltd, London 1964.