sponge utilizes preferably cholesterol during the biosynthesis of the A-nor-steranes and cholestanol, although it can act as precursor of these latter, does not seem an intermediate in the conversion of cholesterol into 3β-hydroxymethyl-A-nor-5 α-cholestane.

[1] L. MINALE and G. SODANO, J. chem. Soc. Perkin I, *1972*, 2380.
[2] M. DE ROSA, L. MINALE and G. SODANO, Experientia *31*, 408 (1975).
[3] The incubation conditions are given in reference [2]; the conditions of extraction and isolation of stanols are given in references [1] and [2]; the labelled stanols, after dilution with carrier A-nor-stanols, were hydrogenated on palladium-charcoal and converted to the nor-ketones (experimental details in reference [2]).
[4] S. P. COLOWICK and N. O. KAPLAN, Meth. Enzymol. *4*, 804 (1957).
[5] A. G. SMITH and L. J. GOAD, Biochem. J. *146*, 35 (1975).
[6] The 3β-hydroxymethyl-A-nor-5α-steranes are less polar than cholesterol (Rf on silica gel TLC in chloroform 0.45 as against Rf 0.4).

Ontogenesis of Monoamine Oxidase in the Thyroid Gland of Rats

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Summary. During postnatal development, MAO activity in the thyroid gland of male rats increases until day 16, when adult values are reached.

Monoamine oxidase (MAO, E.C.1.4.3.4) is a degradating enzyme catalyzing oxidation of primary and secondary amines. MAO has been found in various tissues of different animal species[1]. It is present also in the thyroid gland[2]. The final products of MAO activity are aldehyde and hydrogen peroxide. Hydrogen peroxide enzymatically generated in the thyroid under the catalytic influence of peroxidases is needed for oxidation of iodide to a more reactive state (I^+)[3]. The H_2O_2-peroxidase system participates also in the synthesis of thyroxine and triiodothyronine[4]. BAUDHUIN[5] has found that the activity of MAO in the thyroid gland is substantially higher in comparison with other tissues and localized in both mitochondria and microsomes. It is not known whether this enzyme has a direct effect on the biogenesis of thyroid hormones. However the significance of MAO in the thyroid is supported by the presence of catecholamines in thyroid tissue.

The aim of the present work was to study the activity of MAO in the thyroid gland of rats during postnatal development.

Materials and methods. Wistar albino male rats were used. The animals were killed by decapitation. Thyroids were removed, placed into ice-cold 0.25 *M* sucrose and homogenized in a glass-Contes homogenizer. Details of the assay for MAO have been described by WURTMAN and AXELROD[6]. The reaction mixture contained 25 μl (6.25 nmol) of tryptamine C^{14} (specific activity 2.7 mCi/mmol, Amersham, England), 250 μl of 0.5 *M* potassium phosphate buffer (pH 7.4) and various amount of tissue homogenate in a final volume 0.3 ml. The reaction, carried out at 37 °C, was stopped after 20 min with 2 *N* HCl and the radioactive products were extracted into 10 ml of toluene; 4 ml of toluene extracts were measured for radioactivity in 10 ml of Bray's solution by a Packard scintillation counter. The activity of MAO during ontogenesis was assayed in 0.5 mg samples in triplicates and two series of experiments were performed[7].

Results. Figure 1 shows the effect of varying concentrations of thyroid tissue upon the rate of C^{14}-indolacetic acid formation. The amount of acid was proportional to concentration in the range between 0.1 and 1 mg. The

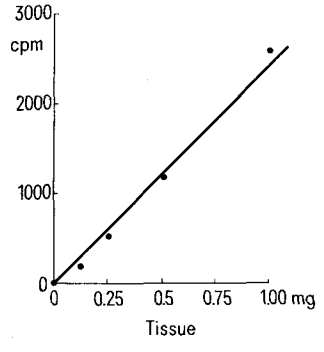

Fig. 1. C^{14}-indolacetic acid production during 20 min incubation vs. amount of tissue.

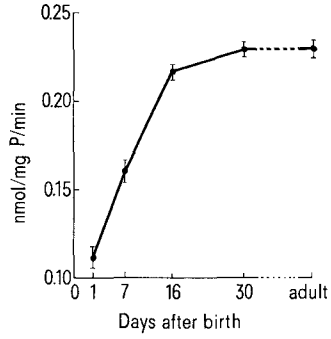

Fig. 2. MAO activity during development in 0.5 mg thyroid incubated for 20 min. Means of 10 values ± SE (results of experiments 1 and 2 were pooled).

[1] H. C. STANTON, R. A. CORNEJO, H. J. MERSMANN, L. J. BROWN and R. L. MUELLER, Arch. intern. Pharm. Therapie *213*, 128 (1975).
[2] K. BHAGVAT, H. BLASCHKO and D. RICHTER, Biochem. J. *33*, 1338 (1939).
[3] L. J. DE GROOT, New Engl. J. Med. *272*, 297 (1965).
[4] L. LAMAS, M. L. DORRIS and A. TAUROG, Endocrinology *90*, 1417 (1972).
[5] P. BAUDHUIN, Y. BRAUFAY, O. RAHMAN-LI, R. SELLINGER, P. WATTIAUX and C. DE DUVE, Biochem. J. *92*, 179 (1964).
[6] R. J. WURTMAN and J. AXELROD, Biochem. Pharmac. *12*, 1439 (1963).
[7] C. V. ATACK, L. E. ERICSON and A. MELANDER, J. Ultrastruct. Res. *41*, 484 (1972).

change of MAO activity during ontogenesis is illustrated in Figure 2. MAO activity increases from birth through about 2 weeks of age. After day 16, however, no significant change was found and on day 30 and 90 (adult animals), enzyme activity was almost the same.

Discussion. In this study we used tryptamine as a substrate for measurement of MAO. Most of studies were performed with other substrates, but our results show that in thyroid tissue tryptamine is suitable (Figure 1). Although MAO has been localized in mitochondria and microsomes[8], we used whole homogenates because they more probably reflect total activity of the organ. MAO activity during ontogenesis has been studied in various tissues of several species of experimental animals; however, no data have been published concerning the thyroid gland. In the rat GRIPOIS and ROFFI[9] have measured MAO activities in liver, kidney and heart homogenates and found that adult values in liver and kidney are reached at the 18th day after birth, whereas in the heart they occurred later. In our studies MAO increased during the early postnatal period. Its maturation occurred during the first 2 weeks after birth and adult levels were reached on the 16th day. The changes of enzymatic activity of MAO during ontogenesis of rat thyroid suggest the importance of the role of this enzyme in thyroid function. MAO could be significant in providing thyroid hydrogen peroxide from catecholamines which have been found in the thyroid of mammals[7].

[8] I. K. MUSHAHWAR, L. OLINER and A. R. SCHULZ, Can. J. Biochem. *50*, 1035 (1972).
[9] D. GRIPOIS and J. ROFFI, Ann. Biol. Anim. Bioch. Biophys. *12*, 631 (1972).

On the Metabolism of Prostaglandins by Rat Brain Homogenate[1]

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Summary. Using radio-immuno assays for prostaglandins and prostaglandin metabolites, three prostaglandin metabolizing enzymes were found in the 100,000 × *g* supernatant of rat brain, 15-hydroxy-prostaglandin-dehydrogenase, Δ^{13}-reductase and prostaglandin E-9-keto-reductase. Specific activity of the latter enzyme was highest in striatum and midbrain and lowest in cortex, cerebellum and spinal cord.

Reports on the activity of prostaglandin (PG) metabolizing enzymes in rat brain are controversial. SIGGINS et al.[2], using a histochemical technique, found 15-hyrdoxy-PG-dehydrogenase activity to be low or absent in all regions of rat brain examined, except the cerebellar cortex. NAKANO et al.[3] reported that 10,000 × *g* supernatant of homogenates of cerebral cortex and cerebellum of both rat and dog metabolized very little PGE_1. Recently, however, LESLIE and LEVINE[4] described the occurrence of PGE-9-keto-reductase in various rat tissues including brain. NADH was the only cofactor studied. Later, NADPH dependent PGE-9-keto-reductase activity was demonstrated to occur in the cytoplasmic fraction of several tissues including brain of pigeon and monkey[5]. A similar enzyme activity has been described for sheep blood[6]. Furthermore, LEE and LEVINE[7] demonstrated the presence in monkey and human brain of a type II 15-hydroxy-PG-dehydrogenase, which, contrary to the well-known type I enzyme, uses NADP as a coenzyme more effectively than NAD. The present work was undertaken to measure the activity of PGE-9-keto-reductase in rat brain and to study some of the properties of the enzyme including its regional distribution. Furthermore, a type II 15-hydroxy-PG-dehydrogenase and Δ^{13}-reductase activity were found in the 100,000 × *g* supernatant of rat brain.

Material and methods. Male Wistar rats (250–400 g) were killed by dislocation of the neck. The brains were rapidly removed and homogenized in 30 ml of ice-cold Bucher medium (0.02 *M* KH_2PO_4, 0.072 *M* K_2HPO_4, 0.0276 *M* nicotinamide, 0.0035 *M* $MgCl_2$ pH 7.4). After centrifugation at 10,000 × *g* at 4 °C for 15 min the supernatant was recentrifuged at 100,000 × *g* for 1 h. 0.15 ml of the clear supernatant as enzyme source was incubated with 1 m*M* coenzyme (NAD or NADP for 15-hydroxy-PG-dehydrogenase, NADH or NADPH for PGE-9-keto-reductase and Δ^{13}-reductase) and 100 ng substrate (PGE_2 or $PGF_{2\alpha}$ for 15-hydroxy-PG-dehydrogenase and PGE-9-keto-reductase, 15-keto-$PGF_{2\alpha}$ for Δ^{13}-reductase) at 37 °C. Enzyme reactions were stopped by incubation in a boiling water bath for 1 min. The incubates were diluted to 4.0 ml with ice-cold distilled water. Products and remaining substrates in aliquots of the samples were determined using radioimmunoassays for PG and PG metabolites as described previously[8]. For studies on the regional distribution of PGE-9-keto-reductase activity, rat brains were dissected as described by GLOWINSKI and IVERSEN[9]. Protein was determined by the method of LOWRY et al.[10]. All unlabelled PGs and PG metabolites used were a generous gift of Dr. J. PIKE, Upjohn Co., Kalamazoo, USA.

Results and discussion. As shown in the Figure, incubation of the 100,000 × *g* supernatant of rat brain as enzyme source with 1 m*M* NADPH and 100 ng PGE_2 results in the formation of $PGF_{2\alpha}$. The enzyme reaction is linear with time for at least 2 h, and has a broad pH optimum between 7.0 and 9.5 and a temperature optimum

[1] This work was supported by the Deutsche Forschungsgemeinschaft (SFB No. 70).
[2] G. SIGGINS, B. HOFFER and F. BLOOM, Ann. N. Y. Acad. Sci. *180*, 302 (1971).
[3] J. NAKANO, A. V. PRANCAN and S. E. MOORE, Brain Res. *39*, 545 (1973).
[4] C. A. LESLIE and L. LEVINE, Biochem. biophys. Res. Commun. *52*, 717 (1973).
[5] S. C. LEE and L. LEVINE, J. biol. Chem. *249*, 1369 (1974).
[6] C. N. HENSBY, Biochim. biophys. Acta *348*, 145 (1974).
[7] S. C. LEE and L. LEVINE, J. biol. Chem. *250*, 548 (1975).
[8] B. M. PESKAR and B. A. PESKAR, Biochim. biophys. Acta *424*, 430 (1976).
[9] J. GLOWINSKI and L. L. IVERSEN, J. Neurochem. *13*, 655 (1966).
[10] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).