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].

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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).
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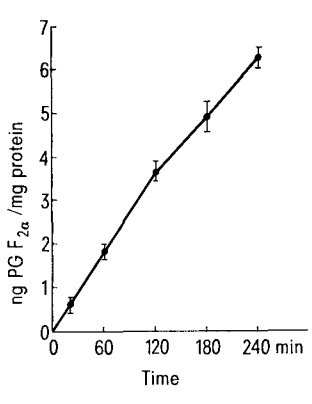
at 37 °C. 100 ng PGE_2 is a saturating substrate concentration and 1 m*M* NADPH is a maximally effective coenzyme concentration. Without the addition of NADPH, enzyme activity is less than 10% as compared to that in the presence of 1 m*M* NADPH. NADH cannot replace NADPH as an effective coenzyme. While PGE_1 and 15(S)-15-methyl-PGE_2 are also substrates for the enzyme, 15-keto-PGE_2 and 13, 14-dihydro-15-keto-PGE_2 are not converted to a measurable degree to the corresponding metabolites of $PGF_{2\alpha}$. Using only a radio-immuno assay for $PGF_{2\alpha}$, we cannot exclude the generation of $PGF_{2\beta}$ from PGE_2 by PGE-9-keto-reductase. However, the product of this enzyme reaction in other organs has been identified as $PGF_{2\alpha}$ by HENSBY[6] using combined gas chromatography-mass spectrometry and by LEVINE et al.[11] using immunological methods.

High concentrations of indometacin were found to inhibit rat brain PGE-9-keto-reductase (ID_{50} 4×10^{-5} *M*). Similarly, it has been shown that high concentrations of indometacin inhibit other PG-metabolizing enzymes[12, 13].

The regional distribution of the activity of PGE-9-keto-reductase in rat brain and spinal cord is shown in the Table. Enzyme activity can be measured in all regions of the central nervous system examined. It is highest in striatum and midbrain and lowest in cortex, spinal cord and cerebellum. The exact localization of the enzyme within the various compartments of the brain tissue, as well as the possible presence of endogenous modulators of enzyme activity, remain unknown. LEE and LEVINE[14] found an endogenous stimulator for PGE-9-keto-reductase in chicken heart and monkey brain. The same authors[7] postulated the presence of an endogenous inhibitor for the type II 15-hydroxy-PG-dehydrogenase of swine kidney medulla and monkey brain. We cannot, therefore, exclude the possibility that the uneven distribution of PGE-9-keto-reductase activity in rat brain is caused by an uneven distribution of endogenous enzyme inhibitors or stimulators and not of the enzyme itself. Nevertheless, the results indicate that enzyme activity is highest in regions consisting mainly of gray matter like striatum and midbrain, with the exception of medulla-pons, and lowest in regions containing much white matter. Contrary to the rat brain, PGE-9-keto-reductase was equally distributed in swine kidney cortex and medulla[15].



Formation of prostaglandin $F_{2\alpha}$ on incubation of 100,000 $\times g$ supernatant of rat brain with prostaglandin E_2 as substrate and 1 m*M* NADPH as coenzyme.

Specific activity of prostaglandin E-9-keto-reductase in the 100,000 $\times g$ supernatant of various regions of rat brain (mean$\pm$SEM, $n = 10$)

Brain region	pg $PGF_{2\alpha}$ formed/mg wet weight/120 min
Striatum	257.2$\pm$43.7
Midbrain	194.3$\pm$29.8
Medulla-pons	187.3$\pm$38.0
Hippocampus	133.4$\pm$24.1
Hypothalamus	118.1$\pm$18.0
Cortex	116.8$\pm$19.4
Spinal cord	86.1$\pm$19.6
Cerebellum	82.6$\pm$13.4

Under the conditions used, the PGE-9-keto-reductase reaction was not reversible. Incubation of 100,000 $\times g$ supernatant of rat brain as enzyme source with 1 m*M* NADP and 100 ng $PGF_{2\alpha}$ as substrate did not result in the formation of measurable amounts of PGE_2. Instead, relatively large amounts of 13, 14-dihydro-15-keto-$PGF_{2\alpha}$ are formed under such incubation conditions (6.2 $\pm$1.4 ng/mg protein/60 min, mean$\pm$SEM, $n = 4$). The enzymes responsible for this reaction are a type II 15-hydroxy-PG-dehydrogenase and Δ^{13}-reductase. The 15-hydroxy-PG-dehydrogenase was identified as a type II enzyme by its coenzyme requirement and substrate specificity. NAD is less effective than NADP as a coenzyme in this reaction. $PGF_{2\alpha}$ is a better substrate than PGE_2 for the type II 15-hydroxy-PG-dehydrogenase[7]. The activity of Δ^{13}-reductase was specifically measured by incubation of 100,000 $\times g$ supernatant of rat brain with 1 m*M* NADH or NADPH and 100 ng of 15-keto-$PGF_{2\alpha}$ as substrate. Under such incubation conditions 13, 14-dihydro-15-keto-$PGF_{2\alpha}$ is formed. Specific activity of rat brain Δ^{13}-reductase in the 100,000 $\times g$ supernatant was found to be 81.2 $\pm$ 14.6 ng 13, 14-dihydro-15-keto-$PGF_{2\alpha}$ formed /mg protein/60 min (mean $\pm$ SEM, $n = 6$). As in our results, HENSBY[6], using enzyme from sheep blood, did not observe reversibility of the PGE-9-keto-reductase reaction. On the other hand, the reaction was found to be reversible with cytoplasmic fractions of rat heart, monkey liver and pigeon heart and purified chicken heart PGE-9-keto-reductase[4, 16]. The reason for the discrepancy of the results is not clear, but it is not the simultaneous presence of a type II 15-hydroxy-PG-dehydrogenase in the 100,000 $\times g$ supernatant of rat brain, which decreases substrate concentration for the backward reaction. Using 15(S)-15-methyl-derivatives of the natural PGs, which are not substrate for 15-hydroxy-PG-dehydrogenase[17], we found conversion of 15(S)-15-methyl-PGE_2 to 15(S)-15-methyl-$PGF_{2\alpha}$, but no measurable backward reaction (to be published).

The function of the type II 15-hydroxy-PG-dehydrogenase and Δ^{13}-reductase of rat brain is probably biological inactivation of PGs, since 15-keto- and 13, 14-dihydro-15-keto-metabolites have almost no biological activity[18]. The function of rat brain PGE-9-keto-reductase, however, which converts one biologically active PG into another active PG, remains to be elucidated. In view of the different and often antagonistic actions of PGE and PGF in the central nervous system[19], the enzyme might play an important regulatory role in rat brain function.

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