chlorohydrin and an acidic metabolite, β-chlorolactic acid (BCLA, III). During the 24 h after administration of α-chlorohydrin, 16% of the radioactive label is eliminated as $^{36}Cl^-$ indicating extensive dehalogenation by epoxidation (I→II), since $^{36}Cl^-$ is excreted slowly due to dilution with the normal Cl^- pool[7]. BCLA appears as a urinary metabolite 3–5 h after administration of α-chlorohydrin and continues to be excreted, together with traces of $^{36}Cl^-$, 7 days later. The amount of BCLA excreted indicates that the oxidative metabolic pathway (I→III) occurs with approximately 25% of the α-chlorohydrin given at this dose level. Administration of BCLA (100 mg/kg, oral or i.p.) to male rats, however, did not induce visible epididymal lesions though histological sectioning of the testis-epididymis complex showed the ductuli efferentes to be either dilated or choked with sperm, similar to the early symptoms of spermatocoele formation by α-chlorohydrin[5].

During these studies it was observed that α-chlorohydrin and BCLA (100 mg/kg) have a diuretic action on male rats. Higher doses of either compound (120–150 mg/kg) cause anuresis and death by renal failure, histology revealing glomerular nephritis due to a crystalline renal deposition. Furthermore, the urines of the diuretic animals were examined microscopically and found to contain envelope-shaped crystals, characterized as calcium oxalate. Consequently the metabolism of ^{36}Cl- and ^{14}C-BCLA (100 mg/kg, i.p.) was investigated; whereas ^{36}Cl-BCLA appeared unchanged together with $^{36}Cl^-$ in the urine, radioactivity from ^{14}C-BCLA was localized in the kidneys and oxalic acid (IV), as calcium oxalate, identified as a urinary metabolite. Administration of oxalic acid (50 mg/kg, i.p.) to male rats caused a brief phase of diuresis and at autopsy approximately 50% of the treated animals exhibited visible mono-lateral spermatocoeles. Higher doses of oxalic acid were toxic due to renal deposition of calcium oxalate[8] similar to the effects seen with high doses of both α-chlorohydrin and BCLA.

The mechanism by which oxalic acid induces the formation of spermatocoeles is not known. It may involve interference with the reabsorption of testicular fluid from the ductuli efferentes and caput epididymis to the blood stream[9] since in primary hyperoxaluria, extrarenal deposits of calcium oxalate are frequently found in the walls of veins, arteries and arterioles associated with the male urinogenital tract[10]. Previous studies have concluded that spermatocoele formation by a single high dose of α-chlorohydrin involves interference with an absorptive role of the epididymis[11] or conus epididymis[5] possibly by causing chemical changes in vascular permeability in this region[12,13]. If this action of α-chlorohydrin is due to the sustained in vivo release of oxalic acid, other compounds metabolized to oxalic acid may also be expected to induce similar lesions. Long-term administration of ethylene glycol mono-ethyl ether, which produces oxalic acid via ethylene glycol[14], is reported as causing bilateral testicular lesions in over 60% of treated male rats[15]. Three other male antifertility agents producing spermatocoeles are α-bromohydrin[5]. 1-amino-3-chloropropan-2-ol[16] and ethane-1,2-dimethanesulphonate[17]. It is possible that these three compounds could be metabolized to oxalic acid via β-bromolactic acid, BCLA[18] and ethylene glycol, respectively. Preliminary results show that these three antifertility agents induce diuresis in male rats and that the urine contains calcium oxalate. Their metabolism together with the effect of oxalic acid on the male rat reproductive tract, is at present being investigated.

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Developmental Characteristics of Histamine Methyltransferase and Phenylethanolamine -N-Methyltransferase of Rat Brain

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Summary. The specific activity of histamine methyltransferase of rat brain increases rapidly from the 16th until the 25th day of gestation (7 days after birth). The specific activity of phenylethanolamine-N-methyl-transferase shows a rapid increase during the 1st and the 2nd week after birth, the adult values being obtained by the end of the 2nd week.

Histamine might be a central neurotransmitter[2] and a regulator of tissue growth as well[3]. The major pathway for histamine metabolism in the brain of cat, mouse and rat is methylation of the imidazole ring to yield 1-methyl (-aminoethyl)-imidazole (methyl-histamine)[4]. Histamine methyltransferase (HMT) is distributed all over the brain of the rat, the highest levels being found in the hypothalamus[5,6].

Phenylethanolamine-N-methyltransferase (PNMT) in mammals is highly localized within the adrenal medulla[7].

Although only small amounts of epinephrine have been detected in the mammalian brain[8], POHORECKY, et al.[9] demonstrated the existence of PNMT in various brain regions of the rat, cat, hen and turtle. CIARANELLO et al.[10] studied the in vitro synthesis of epinephrine in different areas of the rat brain and the subcellular localization of PNMT as well. SAAVEDRA and AXELROD[11] and INWANG et al.[12] identified phenylethylamine and phenylethanolamine in the mammalian brain. SABELLI et al.[13] postulated that phenylethanolamine may be a possible neurotransmitter.

This paper describes the developmental characteristics of histamine and phenylethanolamine methylating enzymes of the rat brain.

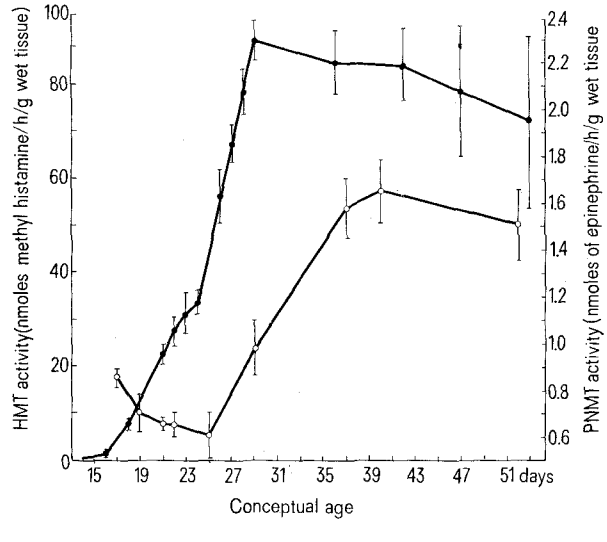
Materials and methods. Pregnant Winstar rats, which were established as sperm-positive on a definite day, were maintained in separate cages. Parturition occurred on the 22nd day of gestation. Rats were killed by decapitation, the brains were immediately removed and homogenized in a glass homogenizer with a teflon pestle in 10 vol. (W/V) of ice cold KCl 0.15 *M*. The homogenates were centrifuged at 13,000 *g* for 1 h and the supernatant fluid was used for the assay. In the case of fetal brains, 2–4 were pooled and treated as described above. In some experiments, the brains were dissected in cerebral cortex, hypothalamus, brain stem, diencephalon and cerebellum or in hemispheres, and brain stem. Pools of up to 4 of the separate parts of the brain were homogenized in 0.32 *M* sucrose and centrifuged at 13,000 *g* for 1 h. The precipitate was resuspended in 0.32 *M* sucrose containing 0.15% Triton-X 100. For the determination of HMT activity, the method described by BROWN et al.[14] was used.

The determination of PNMT activity was done as described by POHORECKY et al.[9] with the following modification: The organic phase (butanol), which contained the extracted (^{14}C)-epinephrine, was transferred in the scintillation vials and evaporated to dryness. The residue was dissolved in ethanol and counted. All enzyme assays were run in duplicate.

Results. Our results (Figure and Table) indicate that fetal rat brain at 14 days of gestation shows a slight HMT activity. The enzyme activity increases rapidly from the 16th day of gestation until the 29th day of gestation (7 days after birth). The possibility that the increase of HMT activity was caused by either the appearance of an activator or the disappearance of an inhibitor, rather than by an increase in enzyme protein, was considered. Mixtures of extracts from the 19th fetal and 10th postnatal days manifested HMT activities equalling the sum of the activities of the two; this fact is compatible with the idea of increased enzyme protein content during development. The results presented in the Table indicate that by the 10th postnatal day the specific activity of the enzyme was lowest in the cerebral cortex and highest in the hypothalamus, as was also found for adult animals[6]. Hypothalamus, diencephalon and cerebellum show a significant increase of HMT activity between the 6th and 10th day after birth, while cerebral cortex and brain stem do not show any change during this period.

PNMT activity is found in the supernatant fraction at 17 days of gestation and, after a slight decrease, a rapid increase follows during the 1st and 2nd week after birth, the adult values being obtained by the end of the 2nd week (Figure). Mixtures of supernatants from 21st fetal and 16th postnatal day manifested PNMT activities equalling the sums of the activities of the two.

Discussion. Rat brain histamine content undergoes a biphasic change during postnatal development. It shows a sharp rise shortly after birth and then, after the end of the 1st week, it declines to reach the adult levels[15–17]. Histidine decarboxylase activity shows the opposite changes during this period, while histidine concentration initially rises and afterwards falls with the peak value on the 1st day after birth[15]. The above observations were interpreted by SCHWARTZ et al.[15], as indicating that the changes of histamine during the first days are due to the changes of histidine concentration, and he assumed that histidine decarboxylase is, like in the rat stomach, an adaptive enzyme whose activity is regulated by the local concentration of histamine.



Developmental changes of HMT and PNMT in rat brain. Each point represents the arithmetic mean of the assays from 10 different rats ± SEM. ●, HMT; ○, PNMT.

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A) HMT activity in the supernatant and particulate fractions of various regions of the rat brain during development, expressed in nmoles/g tissue/60 min

	2 Days		6 Days		10 Days		Adult	
Brain regions	S	P	S	P	S	P	S	P
Cortex	33.7±27.9	13.7±10.1	68.5±18.1 [c]	19.7±11.0 [b]	71.1±16.8	22.7±12.5	62.1±18.5	19.7±12.2
Hypothalamus	86.8±15.0	62.3±15.3	109.1±19.4 [c]	86.6±19.2 [c]	182.9±19.2 [c]	131.0±17.4 [c]	163.2±17.7 [b]	99.0±18.4
Diencephalon	75.0±18.0	22.9±18.1	84.6±14.0	27.1±15.1	107.1±17.1 [c]	32.0±15.0	89.1±20.4	35.9±14.4
Brain stem	105.3±17.0	22.1±15.3	122.3±15.9 [a]	38.7±19.2 [c]	125.6±19.1	36.6±12.9	110.2±18.7	33.2±12.8
Cerebellum	62.8±14.9	28.9±18.9	63.1±27.6	30.1±13.4	118.2±18.3 [c]	42.6±15.3 [b]	98.5±20.4	39.4±15.4

Each number is the mean value of the 10 animals ± SD.

B) PNMT activity in the supernatant and particulate fractions of the hemispheres and brain stem of the rat brain during development expressed in nmoles/g tissue/60 min

	9 Days		15 Days		21 Days	
Brain regions	S	P	S	P	S	P
Hemispheres	1.31±0.72	2.53±0.96	1.63±0.81	3.88±1.23	1.78±0.75	4.33±1.17
Brain stem	2.10±0.90	4.83±1.08	1.59±0.63	5.82±1.14	1.82±0.66	5.32±0.99

[a] $p < 0.02$; [b] $p < 0.01$; [c] $p < 0.001$
Each number is the mean value of 10 animals ± SD.

Our results suggest that the decline of brain histamine content and the apparent increase of histamine turnover after the 1st week of life, might be the result of enhanced methylation by the increased activity of HMT during this period. This is compatible with the observation of SCHWARTZ et al.[18] that endogenous histamine of rat brain rises sharply after inhibition of histamine methylation. Factors other than metabolism rate, such as modifications in compartmentation[17] and release of histamine, may also contribute to the above developmental changes.

PNMT shows almost a 2-fold increase in specific activity between 17 days of gestation and 14 days after birth. No significant change was observed after the end of the 2nd week, while, as shown by other investigators, tyrosine hydroxylase and dopamine-β-hydroxylase, two other enzymes of the same pathway, show a further increase during the following 2 weeks of life[19, 20].

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Alteration of Cholesterol Synthesis in Rat Liver as Induced by 4-Methyl-5-Hydroxy Valeric Acid

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Summary. The rate of cholesterogenesis in rat liver, measured by the incorporation of labelled acetate or mevalonate into cholesterol, was significantly supressed by the use of 4-methyl-5-hydroxy valeric acid sodium salt. This effect cannot be explained by changes in HMG CoA reductase activity.

It is generally agreed that the rate-limitting step in hepatic cholesterogenesis is the formation of mevalonic acid from HMG CoA (3-hydroxy-3-methylglutaryl CoA)[1]. This reaction, which is catalyzed by the enzyme HMG CoA reductase (EC 1.1.1.34), is altered by dietary cholesterol content[2], hormonal conditions[3] and drugs like cholestyramine[4]. Several compounds, including certain valeric acid derivatives[5], have been tested as hypolipemic agents, some of them having important inhibitory effect on hepatic cholesterogenesis by mechanisms not always involving direct action of the drug upon the regulatory step[6].

This report deals with the effect of 4-methyl-5-hydroxy valeric acid sodium salt (MHVA) on hepatic cholesterol biosynthesis in the rat. MHVA is obtained as a side product in the chemical synthesis of pregnenolone from diosgenin[7].

Materials and methods. Male Wistar rats, 120 to 150 g, maintained on standard laboratory chow supplemented as indicated, were used in all experiments. 4-methyl-5-hydroxy valeric acid was kindly supplied by Dr F. GIRAL (Nat. Univ. Mexico, Sch. Chem.). 1-^{14}C-sodium acetate (62 mCi/ mmole) and DL-2-^{3}H-mevalonic acid lactone (82 mCi/mmole) were obtained from The Radiochemical