fers with the reduction of 3,5-dinitrosalicylic acid, the chromogen by reducing sugars, product of amylolysis; and hence remains inconclusive. The size of the hydrated cation seems to be irrelevant, since $CsCl_2$ could activate the enzyme almost to the same degree as by sodium, potassium and ammonium chlorides. Sodium bromide activated the enzyme to some extent, while both $NaNO_3$ and CH_3COONa had no such action. The enzymic activity was inhibited by Na_2CO_3 as expected, since the pH of the reaction mixture was raised. These observations regarding bromide and acetate are also in agreement with the earlier findings. According to BERNFELD[4], NO_3^- exhibits some activation but we failed to get the same as MYRBÄCK and others.

In a recent report[5], perhaps the only one so far, sodium ion has been claimed to be the activator of pancreatic α-amylases of toad, reptile, pigeon and rat. Using NaCl, Na_2CO_3, KCl and $CuCl_2$ in phosphate buffer, they had shown that while NaCl and Na_2CO_3 activated the enzyme both $CuCl_2$ and KCl did not. It is already reported in the literature[8] that rat salivary and liver amylases are activated by KCl, as also confirmed by us. The failure to get any activation by $CuCl_2$ is rather to be expected and the reasons are discussed earlier. Unlike the findings of these authors, activation by Na_2CO_3 could not be confirmed in our laboratory since additions of Na_2CO_3, as low as 2.5 μmoles in the 2 ml of reaction mixture caused a change in pH. However, with *Tris*-carbonate buffer at pH 7.0 similar activity was obtained as with *Tris*-phosphate. It can be mentioned here that methodology adapted and concentrations of KCl, $CuCl_2$ and Na_2CO_3 used by us were the same as reported by them[5].

In view of the above, the following conclusions have been drawn. Chloride ion shows increased activation from pH 6.0 to 7.0 while most other anions including phosphate do the reverse. Activation by chloride ion is higher compared to other ions at both the pH values. The presence and size of the cation is irrelevant. Essentiality of Ca^{++} as anticipated through earlier investigation can evidently be ruled out as shown by the results of oxalate, acetate and aspartate in *Tris* buffer.

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The Oxidative Metabolism of α-Chlorohydrin and the Chemical Induction of Spermatocoeles

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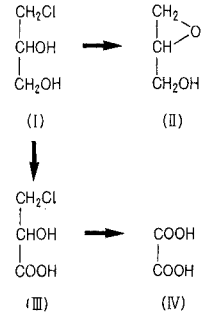
Summary. α-Chlorohydrin (I) is oxidatively metabolized to β-chlorolactic acid (III) and oxalic acid (IV). Deposition of calcium oxalate within the renal tubules is responsible for the toxic effects of α-chlorohydrin and a similar action on the epididymis or epididymal blood vessels could initiate the formation of spermatocoeles from this and other male antifertility agents.

The male antifertility agent[2] α-chlorohydrin (3-chloropropan-1,2-diol, I) has two effects on the male rat reproductive tract. Consecutive low daily doses (5 × 10 mg/kg) cause an immediate and reversible phase of infertility[3] by inhibiting sperm glycolysis[4], whereas a single high dose (100 mg/kg) produces epididymal lesions or spermatocoeles[5]. These lesions occlude the ductuli efferentes, block the passage of testicular sperm and produce prolonged or even permanent infertility. As the main metabolite[6] of α-chlorohydrin, the epoxide glycidol (2,3-epoxypropan-1-ol, II) has a similar antifertility action to the low dose regime of α-chlorohydrin[3], but does not induce spermatocoele formation, the possibility arose that α-chlorohydrin may be converted by another route to a metabolite responsible for the epididymal lesions.

Chromatograms of rat urine collected within 8 h of either oral or i.p. administration of ^{36}Cl-α-chlorohydrin (100 mg/kg) revealed the presence of 3 radioactive components. These were identified as Cl^-, unchanged α-



The metabolism of α-chlorohydrin in the Rat. β-chlorolactic acid (BCLA, III) was isolated from the acidified urine of male Wistar rats dosed with α-chlorohydrin (100 mg/kg), by continuous ether extraction and chromatography on a column of Merck silica gel G, elution with ethyl acetate: 40–60° petroleum ether (2:1) giving white plates, m.p. and mixed[20] m.p. 78–79°. Characterization was by reverse isotope dilution, mass spectroscopy {M^+ 125/127, base peak 79/81 $(M-H-CO_2)$} and GLC (retention time of a spiked sample 11.30 m on a 1 m × 3 mm i.d. 5% FFAP on 80–100 mesh Chromosorb G at 150° with N_2 inlet pressure of 14 psi). Urinary α-chlorohydrin (I) was identified as the *bis*-benzoyl derivative as previously described[6], chloride ion by the method of SEILER and KAFFENBERGER[21] and oxalic acid (IV) according to PARKE and WILLIAMS[22]. ^{36}Cl-BCLA and uniformy-labelled ^{14}C-BCLA were prepared from ^{36}Cl-α-chlorohydrin[6] and uniformly-labelled ^{14}C-α-chlorohydrin[23], respectively, by mild nitric acid oxidation[24]. Rf values (Merck pre-coated silica gel G TLC plates, 0.25 mm) were α-chlorohydrin (0.73), BCLA (0.30), calcium oxalate (0.10) and Cl^- (0.01–0.15) in chloroform:methanol (7:3).

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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].