The metabolism of the soil fumigant 1,2-dibromo-3-chloropropane in the rat[1]

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Summary. The soil fumigant 1,2-dibromo-3-chloropropane (**I**) undergoes hydrolysis in the rat to a series of epoxide metabolites. Alkylation of glutathione by these epoxides produces 2 urinary metabolites identified as the mercapturic acids **VI** (R=$COCH_3$) and **VII** (R=$COCH_3$). Hydrolysis of the epoxides produces the male antifertility agents α-chlorohydrin (**IX**, X=Cl) and α-bromohydrin (**IX**, X=Br) which are oxidatively metabolized to oxalic acid (**XII**), thus causing renal damage. These metabolic pathways can explain the toxic nature of the fumigant as a carcinogen, a male chemosterilant and as an agent causing kidney damage.

1,2-Dibromo-3-chloropropane (DBCP[2], **I**) was introduced as a nematocide in 1953 and gained such widespread acceptance that over 4 million kg was produced in the USA in 1969[3]. Whereas previous soil fumigants were toxic to plants, DBCP had the advantage that it was not phytotoxic and could be applied directly to soils in which crops were growing[4]. In mid-1977, press reports claimed that male employees involved in the manufacture and formulation of DBCP were becoming infertile. A subsequent investigation[5], conducted by the Environmental Protection Agency in the USA, confirmed these reports, acknowledging the known carcinogenicity of the compound[3,6], and that it could be detected in residues of root crops grown in treated soil[7]. The investigation found 'that human dietary consumption of DBCP constitutes a serious risk to the population of this country, since it appears that not only is DBCP a powerful carcinogen, but that it may also damage human reproductive functions, and may cause sterility in males'. On this basis, the Agency announced the 'intention to conditionally suspend all registrations of products containing DBCP[3].'

Apart from a study[8] showing that 'the substance is cleanly converted by soil water cultures to n-propanol', it is surprising for a compound classified[9] as 'extremely toxic', which has been readily available to the general public for nearly 25 years, that its metabolism has never been examined. For this reason, and in view of its male antifertility actions, we have determined the fate of the compound in the rat.

Experimental. After i.p. administration of DBCP (50 mg/kg in propylene glycol), 9% of the dose appears in the expired air within 2 h, a further 5% appears in the following 2 h and trace amounts after this. As expected for a highly lipophilic compound, none is excreted in the urine so that 85% undergoes bio-transformation. Bromide assays[10] of rat urine showed the rate of excretion of inorganic bromide to be delayed, approximating to that of an equivalent dose of KBr and indicating that DBCP is rapidly debrominated in vivo with the bromide being selectively retained by the kidney[11].

Continuous ether extraction of acidified urine from DBCP-treated rats enabled the isolation of 2 metabolites, hydrolysed by acylase and identified as S-(2,3-dihydroxypropyl)-

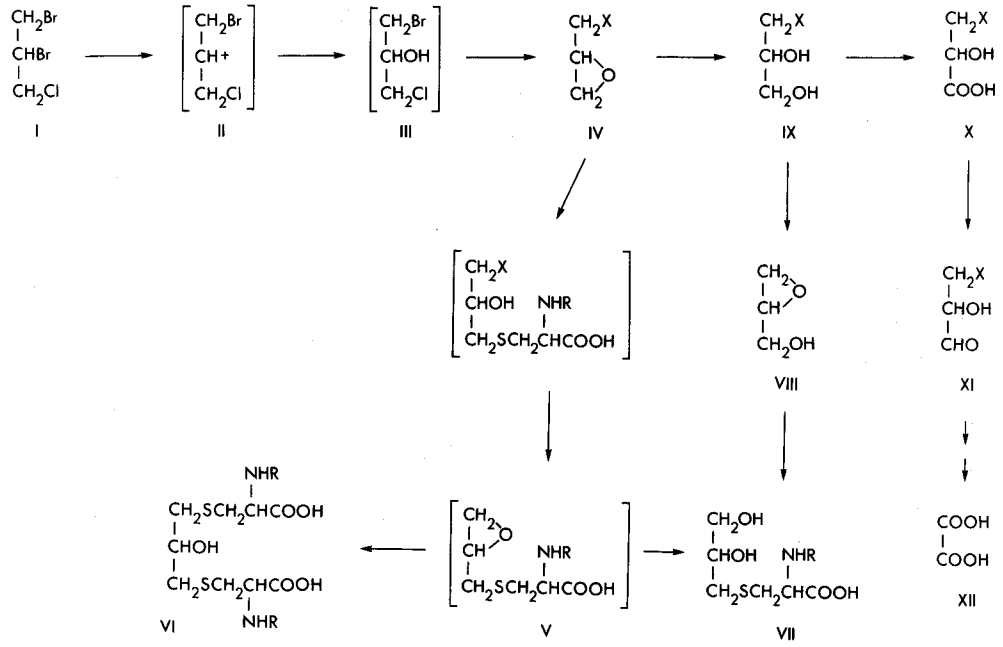

Fig. 1. The metabolism of 1,2-dibromo-3-chloropropane (DBCP, **I**) in the rat. Structures in parentheses represent proposed intermediates. DBCP was a gift from Shell Chemical (Australia) and was purified by distillation at reduced pressure to remove approximately 25% epichlorohydrin (GLC analysis). Epi-chlorohydrin (Hopkin & Williams, Chadwell Heath, Essex UK), epi-bromohydrin (Eastman Kodak, Rochester, New York) and α-chlorohydrin (Aldrich Chemicals, Milwaukee, Wisconsin) were purchased; 1-bromo-3-chloropropan-2-ol was synthesized from HCl and epi-bromohydrin; 1,3-(bis-cysteinyl)propan-2-ol was prepared from cysteine and epi-bromohydrin; α-bromohydrin[13], β-chlorolactate[13] and β-bromolactate[14] were synthesized according to the literature. For the isolation of metabolites, acidified urine was extracted continuously with ether for 72 h. Purification of this extract (TLC) followed by methylation (BF_3/methanol), enabled methyl β-chlorolactate and methyl β-bromolactate to be identified (GLC). Hydrolysis (5M HCl/90 °C/1 h) of the ether extract or treatment with acylase (pH 7.4/37 °C/16 h) liberated the S-alkylated cysteine conjugates which were purified by ion-exchange chromatography (Dowex 50W-X, H^+ form) and characterized, by reference to authentic samples[13], by amino-acid analysis and GLC-mass spectroscopy of their trimethylsilylated derivatives.

cysteine (**VII**, R=H) and 1,3-(bis-cysteinyl)propan-2-ol (**VI**, R=H) showing that the metabolites were the mercapturic acids **VII** (R=$COCH_3$) and **VI** (R=$COCH_3$). These are believed to arise from the nucleophilic attack by glutathione[12] on the intermediate epoxides **V** and **VIII**. Confirmation of this pathway was obtained by examining the metabolism of the proposed intermediates epi-chlorohydrin (**IV**, X=Cl) and epi-bromohydrin (**IV**, X=Br); each compound produced the same mercapturic acids **VI** (R=$COCH_3$) and **VII** (R=$COCH_3$) as urinary metabolites.

Opening of the epoxide ring of the epi-halohydrins (**IV**) by hydrolysis would produce α-chlorohydrin (**IX**, X=Cl) and α-bromohydrin (**IX**, X=Br) which are known[13] to undergo oxidation first to β-chlorolactate (**X**, X=Cl) and β-bromolactate (**X**, X=Br) and then, via their respective aldehydes (**XI**), to oxalic acid (**XII**). Consequently the ether extract of urine obtained from DBCP-treated rats was methylated and analysed by GLC. Methyl β-chlorolactate and methyl β-bromolactate were identified. As a result of their metabolism to oxalic acid, the halo-lactates (**X**) induce a phase of diuresis in rats by the deposition of calcium oxalate within the renal tubules[14]. Figure 1 shows that a single dose of DBCP (50 mg/kg) induces diuresis, resulting in the excretion of calcium oxalate and a 200-fold increase in the excretion of glucose.

Exposure of DBCP-saturated oxygen vapours to the Udenfriend oxidizing system[15] gave, after basification and addition of cysteine, S-(2,3-dihydroxypropyl)cysteine (**VII**, R=H), showing that dehalogenation is possible under these in vitro conditions. The initial in vivo process of dehalogenation is presumed to involve the loss of the $C_{(2)}$ bromine atom to give, ultimately, 1-bromo-3-chloropropan-2-ol (**III**) which, by epoxidation, could lead to both of the epi-halohydrins (**IV**). Evidence for this comes from the metabolism of **III** which produced the 2 mercapturic acids **VI** (R=$COCH_3$) and **VII** (R=$COCH_3$), both β-halolactates and a period of diuresis comparable in extent and duration to that induced by DBCP. In addition, studies on the effect of DBCP on growth rates of immature rats showed that the compound had an immediate rather than a delayed effect in reducing body weight which, according to Elson[16], classifies it as a compound acting by an S_Nl mechanism.

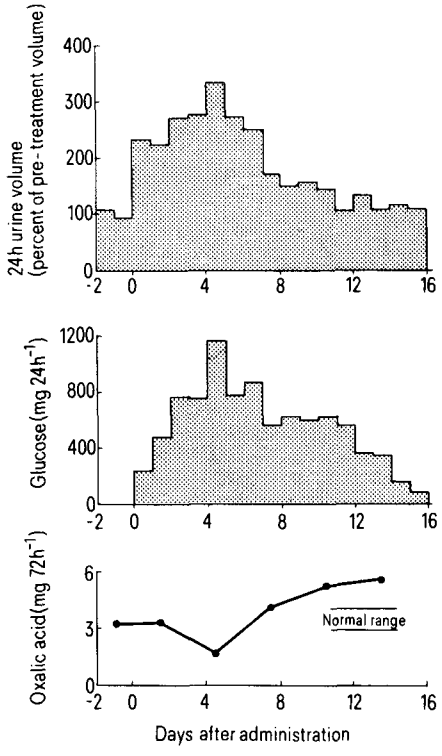

Fig. 2. The effect of a single i.p. dose of DBCP (50 mg/kg) on urine volume, urinary glucose and oxalic acid excretion by the rat. Six animals were dosed at day 0, the diuresis[14], glucose[26] and oxalic acid[27] being assayed according to the literature.

Results and discussion. The proposed metabolic pathway for DBCP in the rat is shown in figure 1. Formation of the cysteine conjugates **VI** (R=$COCH_3$) and **VII** (R=$COCH_3$) classifies DBCP as a biological alkylating agent[17], a class of compound not only carcinogenic, but which are known to affect spermatogenesis and cause testicular degeneration[18]. The production of α-chlorohydrin (**IX**, X=Cl) as a metabolite, which is a known male chemosterilant in the rat[19], could also account for the reported antifertility action in human males as this compound is known to have an inhibitory affect on glycolysis in mature human sperm[20]. Furthermore, by their oxidation to oxalic acid[14], α-chlorohydrin and α-bromohydrin are both capable of producing spermatocoeles or sperm retention cysts in the ductuli efferentes and caput epididymides of the rat[21]. By blocking the passage of testicular sperm to the proximal epididymal duct, these pathological lesions produce prolonged periods of infertility or even sterility, usually accompanied by testicular atrophy and a decrease in testicular weight[21].

Due to embryonic propinquity, the absorptive functions of the kidney and proximal epididymal duct appear to be related. Previous toxicological studies with DBCP in the rat indicated that the compound had deleterious effects on both of these organs[22]. This has been confirmed in the present study and the table shows the effects of a single dose of DBCP on the weights of the testis and the kidney. Although the increase in weight of the kidney is reversible and parallels the duration of the diuretic action, the weight of the testes decreases to nearly 50% by day 16 and apparently is non-reversible.

A number of chemicals with structures related to that of DBCP and its metabolites are used widely in the agriculture and foodstuff industries. The possibility arises, therefore, that these may undergo metabolism not only to compounds which could effect male fertility, but to oxalic acid which, as calcium oxalate, could cause renal damage. The metabolite of DBCP, epi-chlorohydrin (**IV**, X=Cl) is used as an industrial solvent to which 'chronic exposure can cause kidney injury'[23]. Our investigations with 1,2,3-tribromopropane, a nematocide[24] as effective as DBCP[25], have shown that it undergoes a similar series of biotransformations in the rat. We have identified β-bromolactate (**X**, R=Br), oxalic acid (**XII**) and the mercapturic acids **VI** and **VII** (where R=$COCH_3$) as urinary metabolites and determined its diuretic action (at 50 mg/kg) to be nearly twice as severe as that induced by DBCP.

The effect of a single i.p. dose of DBCP (50 mg/kg in propylene glycol) on testis and kidney weights, 8 and 16 days after administration. Control animals received an equivalent dose of propylene glycol. Results are significant ($p < 0.01$); 20 animals in each treatment group

Treatment	Body weight (g)	Right kidney (g/100 g b.wt)	Left kidney	Right testis	Left testis
Control	257	0.418	0.402	0.608	0.619
DBCP-treated (day 8)	253	0.640	0.599	0.463	0.460
DBCP-treated (day 16)	258	0.492	0.468	0.323	0.333

1 This work was supported in part by the National Health and Medical Research Council of Australia.
2 DBCP was the active ingredient in Fumazone and Nemagon, the trademarks of the Dow Chemical Company and the Shell Chemical Corporation, respectively.
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Karyology of the primitive salamanders, family Hynobiidae[1]

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Summary. Karyotypes have been studied in 3 species of *Hynobius* and in 1 species each of the remaining genera of Hynobiids *(Ranodon, Batrachuperus, Salamandrella* and *Onychodactylus).* All species have large diploid numbers, between 56 and 66, and asymmetrical and bimodal karyotypes. DNA contents (2C) were found to vary between 33 and 51 pg. Determination was not possible in *Onychodactylus* where higher values may be suspected. Some of the karyotypes investigated are similar to those of Cryptobranchids. Phylogenetic implications are discussed.

The members of the family of the Hynobiidae, found throughout palaearctic Asia, are the most primitive of the living Urodeles (Amphibia, Caudata). It is probable that all, or nearly all, the present-day families of the order were derived from ancient hynobiid forms[2, 3]. The relationships among the different genera of the family are not well known at present. Most of the species live in areas difficult to reach. According to Noble[2], *Hynobius* is the central genus from which the other Hynobiids were derived by independent specializations. Within the genus, the Japanese species are usually more evolved than the continental ones. However, other authors consider that *Ranodon* retains the greatest number of primitive characters[4], even though it is the only hynobiid able to fabricate a rudimentary spermatophore[5].

Salamandrella, which is monotypical, is often included in the genus *Hynobius.* It is the most widespread of the family and the only one which has reached Europe. Other genera, such as *Batrachuperus* and *Onychodactylus,* consist of a few species adapted to life in mountain waters. The species *boulengeri,* sometimes assigned to the genus *Pachypalaminus,* is more probably a species of *Hynobius*[6]. The karyology of various species of *Hynobius* and of *Salamandrella keyserlingii* was studied by Makino[7] and Sato[8], who showed the chromosomal affinities between the Hynobiids and the Cryptobranchids, and the differences between these 2 families (often combined in the suborder Cryptobranchoids) and the other families of the Urodeles. Other investigators have since confirmed these conclusions in general[9-11].

In this note we report on the chromosomes of *S. keyserlingii* and of 3 species of *Hynobius* (described also by the Japanese investigators) and describe those of 1 species each of the genera *Ranodon, Batrachuperus* and *Onychodactylus.* We have also evaluated the amounts of nuclear DNA (2 C) in nearly all of these species. The karyology of these primitive Urodeles is also compared to that of the other families of the order[11].

Materials and methods. The species investigated were *Ranodon sibiricus, Batrachuperus mustersi, Salamandrella keyserlingii, Hynobius dunni, H. tsuensis, H. nebulosus* and *Onychodactylus japonicus*[12]. In the case of *Ranodon,* we were able to find only 1 juvenile specimen of undifferentiated sex. In the other cases, except for *Onychodactylus,* we had available live animals of both sexes, from which we obtained fresh material suitable for investigation on the karyotypes and also histophotometrical assay of nuclear DNA. For *Onychodactylus* only fixed material was available, and therefore the data on the karyotype must be considered as approximate and provisional, while DNA measurements were not possible. The methods used for the investigation of the chromosomes and for the determination of the genome size were those described in a previous paper[13].

Results. Ranodon sibiricus (2n = 66) has 5 pairs of large biarmed chromosomes (metacentric, or M), 9 pairs of chromosomes with only 1 arm or with the 2nd arm extremely short (acrocentric, or A), and finally 19 pairs of very small, dot-like chromosomes (microchromosomes) (figure 1). The