The effects of Mn^{2+} and Mg^{2+} concentration on the RNA polymerases are shown in Figure 2. RNA polymerase I (eluted at 0.10 *M* $(NH_4)_2SO_4$) had maximum activity at 1 m*M* Mn^{2+} and at 4 m*M* Mg^{2+}, while RNA polymerase II (eluted at 0.25 *M* $(NH_4)_2SO_4$) had maximum activity at 2 m*M* Mn^{2+} and 10 m*M* Mg^{2+}. The ratio of Mn^{2+} activity to Mg^{2+} activity was 1.21 for form I and 0.97 for form II.

Figure 3 shows the effect of $(NH_4)_2SO_4$ concentration on enzyme activity for both polymerases I and II. The optimum for form I was 0.01–0.03 *M* and for form II 0.08 *M*.

The Table shows that both RNA polymerase activities were dependent on the addition of ATP, CTP, GTP, and DNA. Polymerase I was insensitive to cycloheximide, α-amanitin, and rifampicin, while polymerase II is significantly inhibited only by α-amanitin.

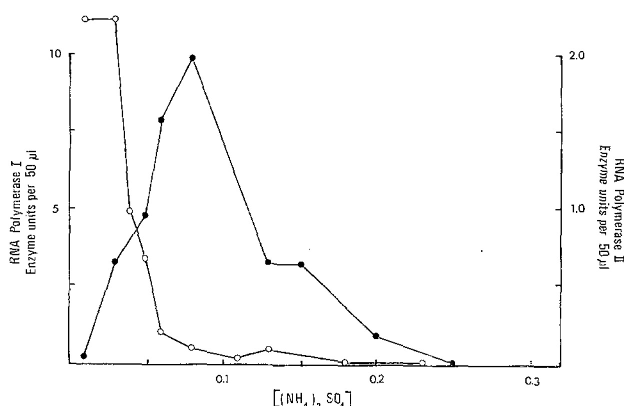

Fig. 3. The effect of $(NH_4)_2SO_4$ on RNA polymerase I and II activity. The concentration of $(NH_4)_2SO_4$ was varied in the standard reaction mixture. The concentration of $MgCl_2$ was 2.0 m*M* while that of $MnCl_2$ was 1.0 m*M* for polymerase I (○–○–○) and 2.0 m*M* for polymerase II (●–●–●).

The data presented here show that *N. crassa* has 3 RNA polymerases which can be resolved by DEAE-sephadex chromatography. The activities eluting at 0.10 *M* and 0.25 *M* $(NH_4)_2SO_4$ are RNA polymerases I and II, respectively, as is shown by their salt requirements and sensitivity to rifampicin and α-amanitin[3,5,9–11]. The third form, eluting from DEAE-sephadex at 0.37 *M* $(NH_4)_2SO_4$, was not studied, but might be mitochondrial RNA polymerase.

In a recent report[7], four DNA-dependent RNA polymerases were isolated from nuclei of the *N. crassa* mutant 'slime'. While the reasons for the differences between that report and this one are not presently clear, they may be 1. the strain of *N. crassa* used, or 2. differences in the method of enzyme preparation. Our preliminary results indicate that perhaps the use of sonication during extraction of polymerases from *N. crassa* results in modification of native enzymes.

Résumé. Des ARN polymérases, dépendantes de l'ADN, ont été isolées de *Neurospora crassa*. Deux pics majeurs d'activité enzymatique ont été séparés par chromatographie sur DEAE-sephadex, et, sur la base de leurs exigences salines et leur sensibilité à l'α-amanitine, identifiés comme ARN polymérases I et II.

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The Comparative Metabolism of 3-Bromo-propane-1,2-diol and 3-Bromopropanol in the Rat

The involvement of epoxides as intermediates in the metabolic conversion of olefins to vicinal diols[1,2] and premercapturic acids[3,4] is well documented as epoxide formation in vivo is typified by the production of hydroxylated metabolites. If the original molecule is already in the hydroxylated form, the metabolites are not indicative of epoxide formation. In these cases evidence may be derived from an examination of the comparative metabolism of the hydroxylated and un-hydroxylated compound.

From i.p. administration of 3-bromopropane-1, 2-diol (I) to rats (50 mg/kg), the urinary metabolites were identified as S-(2, 3-dihydroxypropyl)cysteine (V) and the corresponding mercapturic acid, N-acetyl-S-(2, 3-dihydroxypropyl)cysteine (VI). As no unchanged compound was excreted[5], the relative efficiency of the detoxification process (the metabolites represented over 25% of the administered compound[6]) raised the possibility of a more active alkylating intermediate since most alkyl halides produce minor amounts of cysteineconjugated metabolites as well as being excreted unchanged[4]. In assessing the alkylating ability of (I) towards glutathione, it was found that no reaction occurs in the range pH 7–8.5 though at pH 9 alkylation produces the conjugate S-(2, 3-dihydroxypropyl)glutathione. However at this pH, (I) is quantitatively converted to 2, 3-epoxypropane-1-ol (glycidol, III)[7]. As glycidol itself conjugates with glutathione at pH 7–8.5, it represents in vitro alkylating species and as it gives rise to the same metabolites as (I) in the rat[8], it can be inferred that it is the in vivo intermediate.

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[5] On a 3 ft × 2 mm column of Porapak Q at 180°, authentic 3-bromopropane-1,2-diol had a retention time of 7.10 min. On a 3 ft × 2.5 mm column of 20% diethylene glycol succinate on HMDS chromosorb W at 180°, authentic 3-bromopropanol had a retention time of 2.15 min. Neither compound could be detected in continuous ether extracts of urine from treated animals.
[6] Assessed by administration of the ^{14}C-labelled compound (A. R. JONES, J. Labelled Cpds. *9*, 697 (1973)).
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The elimination of bromide by the rat following administration of (I) is given in Figure 2. The excretion rate approximates to that of an equivalent dose of KBr indicating rapid debromination in vivo. From the related compound, 3-bromopropanol (II), which cannot undergo direct epoxidation and is not excreted unchanged, bromine elimination is more rapid suggesting the excretion of a bromine-containing metabolite[9]. Consequently the metabolism of (II) was investigated and the major metabolite indentified as the oxidation product 3-bromopropionic acid (IV), GLC showing the presence of trace amounts (less than 2%) of the mercapturic acid, N-acetyl-S-(3-hydroxypropyl)cysteine.

The different metabolic pathways of the 2 compounds (Figure 1) can be explained by participation of the epoxide (III) as the reactive intermediate in the detoxification of 3-bromopropane-1,2-diol (I). Whether the conversion of (I) to (III) is enzyme catalyzed is not known but an enzyme (halohydrin epoxidase) has been isolated from a *Flavobacterium Sp.* which converts a number of α-bromohydrins, including I, to glycerol via glycidol[11]. In mammalian systems however, glycidol would undergo rapid conjugation with glutathione either directly or by a glutathione-S-epoxide transferase mediated reaction[12] as well as being hydrolyzed to glycerol. The conversion of 3-bromopropanol to 3-bromopropionic acid (and further oxidation products in some micro-organisms[13,14]) probably represents the major metabolic pathway of alkyl halides in general, since the majority of those that have been investigated are extensively oxidised and produce mercapturic acids only in low yields[4].

The bio-transformation of other α-bromohydrins to epoxides has been suggested[15,16] and the scope of this conversion with a number of α-halohydrins is being further investigated.

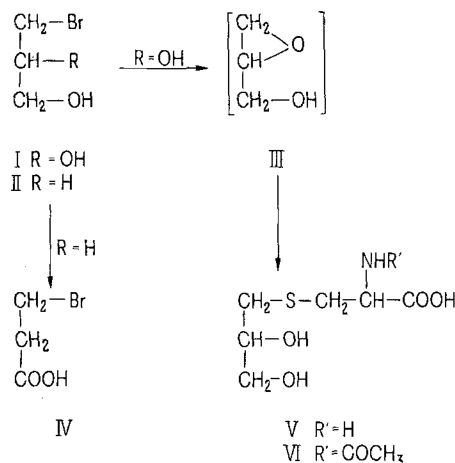

Fig. 1. Metabolic pathways of 3-bromopropane-1,2-diol (I) and 3-bromopropanol (II) in the rat. The cysteine conjugates were isolated from urine as previously described[17] and their chromatographic mobilities compared with those of authentic compounds for which satisfactory analyses were obtained. On Merck pre-coated silica gel G plates (0.25 mm) in butanol: glacial acetic acid: water (4:2:1), the Rf values were 0.18 and 0.63 for (V) and (VI), respectively. The metabolites were interconverted either by acetylation (acetic anhydride) or hydrolysis (5 *N* HCl at 95° for 1 h, or acylase at pH 7.4 and 37° for 16 h). Treatment of (VI) and acetylated (V) with diazomethane gave N-acetyl-S-(2,3-dihydroxypropyl)cysteine methyl ester which had identical GLC retention times to authentic material (retention times 3.81 m on 15% Apiezon L on chromosorb P at 180°). 3-bromopropionic acid (IV) was isolated from the ether extract of acidified urine, purified by preparative TLC (Rf 0.42 in ethanol: 880 ammonia (100:1) on 1 mm precoated silica gel G plates) and identified by reference to authentic material by mass spectroscopy[18] and GLC of the methylesters (retention times 2.80 m on 3% OV-225 on chromosorb W at 190°).

Zusammenfassung. Nachweis, dass das 3-Bromopropan-1,2-diol (I) von Ratten schnell zu den Cysteinkonjugaten (V) und (VI) metabolisiert wird, während das 3-Bromopropanol (II) hauptsächlich zu 3-Bromopropionsäure (IV) oxydiert. Das beträchtliche Ausmass der Konjugation von (I) ist ein Hinweis auf die Beteiligung des Epoxids (III) als reaktives Zwischenprodukt in vivo.

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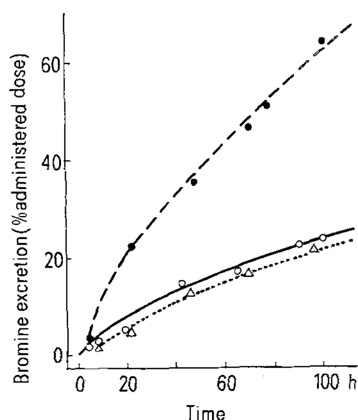

Fig. 2. Excretion of total bromine in the urine of rats receiving 3-bromopropan-1,2-diol (△---△); 3-bromopropanol (●--●); and KBr (○—○). Compounds were administered as aqueous solutions by i.p. injection at a dose level of 100 mg/kg. Bromine assays were determined in triplicate by the method of Hunter[19].

[9] Renal excretion of bromide is slow due to equilibration with chloride and selective resorption[10]. High values in the bromine assay indicate excretion of organically bound bromine.

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