respective chemical properties of 2-mercaptoethanol and acrolein, we suggested that acrolein inhibited the DNA polymerase by oxidizing the active thiol groups of the enzyme. This hypothesis was consistent with the fact that *E. coli* DNA polymerase I, devoid of SH groups in its active centre, was not inhibited but activated by acrolein. In agreement with these results, our present work demonstrates that (^{3}H) acrolein binds 10 to 20 times more to regenerating rat liver DNA polymerase than to *E. coli* enzyme. The antagonistic action of 2-mercaptoethanol and (^{3}H) acrolein with respect to the regenerating rat liver DNA polymerase and *E. coli* enzyme confirms that the unsaturated aldehyde attaches to the thiol groups of the enzymes and that the fixation is irreversible. Besides, for an (^{3}H) acrolein molarity similar to that measured in vivo in regenerating rat liver[3], the binding of (^{3}H) acrolein to the enzyme is at least 100 times higher than that measured in vivo for the total regenerating rat liver protein.

This result suggests a great affinity of acrolein for the regeneryting rat liver DNA polymerase. ALACRON[4] has demonstrated that acrolein was produced by enzymatically oxidized spermine and spermidine and also during the oxydative degradation of 2 antitumor agents[5,6]. He emphasized that the unsaturated aldehyde might be an effective cell growth inhibitor. Recently, CONNORS et al.[7] pointed out that phosphoramide mustard and acrolein, resulting from the in vitro metabolism of cyclophosphamide, had the highest cytotoxicity for Walker tumor cells. The inhibition of DNA and RNA synthesis that we observed in vivo and in vitro, the affinity of acrolein for regenerating rat liver DNA polymerase and also the ability of acrolein to bind DNA account for the cell growth inhibitory properties of acrolein[8].

Résumé. La fixation de l'acroléine ^{3}H à l'ADN polymérase de foie de rat en régénération et à l'ADN polymerase I de *E. coli* a été étudiée. L'acroléine ^{3}H inhibe l'activité de l'ADN polymérase de foie de rat en régénération en se fixant irréversiblement sur les groupements SH essentiels à son activité. Par ailleurs, la fixation de l'acroléine ^{3}H aux modèles, ADN ou polymères synthétiques, est de 1 molécule pour 10^3 nucléotides et correspond aux fixations à l'ADN de foie de rat mesurées in vivo.

NICOLE MUNSCH, ANNE-MARIE DE RECONDO and C. FRAYSSINET

Institut de Recherches Scientifique sur le cancer, Boîte postale No. 8, F-94800 Villejuif (France), 29 April 1974.

[3] N. MUNSCH and C. FRAYSSINET, manuscript in preparation.
[4] R. A. ALACRON, Arch. Biochem. Biophys. *137*, 365 (1970).
[5] R. A. ALACRON and J. MEIENHOFER, Nature New Biol. *233*, 250 (1971).
[6] R. A. ALACRON, J. MEIENHOFER and E. ATHERTON, Cancer Res. *32*, 2519 (1972).
[7] T. A. CONNORS, P. J. COX, P. B. FARMER, A. B. FOSTER and M. JARMAN, Biochem. Pharmac. *23*, 115 (1974).
[8] Acknowledgments. We are greatly indebted to Miss O. FICHOT for the rat liver enzyme purification. This work was supported by a grant from the C.E.A. and a subvention from Ministère de l'Environnement.

Multiple DNA-Dependent RNA Polymerases of *Neurospora*

Multiple DNA-dependent RNA polymerases have been demonstrated and characterized in fungi[1-4] as well as in other eucaryotes[5,6]. In a recent report[7], RNA polymerase activities were characterized from isolated nuclei of the *Neurospora crassa* mutant 'slime'. In that study, 4 peaks of enzyme activity were observed upon elution from DEAE-cellulose. All of these activities were insensitive to rifampicin, while one was completely sensitive to α-amanitin and one was partially sensitive.

In this study, DNA-dependent RNA polymerases were isolated from a crude preparation of nuclei from wild type *N. crassa* using different techniques, and when chromatographed on DEAE-sephadex, only 2 major peaks of RNA polymerase activity were resolved. These activities are identified as RNA polymerases I and II. The reason for the difference between these results and those obtained using *N. crassa* 'slime' are unclear at this time. *Neurospora crassa*, strain 853A, was grown in Vogel's medium N containing 2% (w/v) sucrose for 23 h at 25 °C with vigorous aeration. Conidia for obtaining large amounts of mycelia were produced as described by DAVIS and DESERRES[8]. Each 8 l culture was inoculated with conidia obtained from one 2.5 l Fernbach flask. Buffer H: 0.05 *M Tris*-HCl, pH 7.3 at 4 °C, 0.01 *M* $MgCl_2$, 1.0 m*M* $CaCl_2$, 0.5 m*M* dithiothreitol (DTT), 0.1 m*M* EDTA, 0.5 *M* sucrose, 10% (v/v) glycerol. Buffer A: 0.05 *M Tris*-HCl, pH 7.5 at 4 °C, 0.01 *M* $MgCl_2$, 0.50 *M* $(NH_4)_2SO_4$, 0.5 m*M* DTT, 1.0 m*M* EDTA, 0.1 m*M* phenylmethylsulfonylfluoride (PMSF), 10% (v/v) glycerol. Buffer B: 0.05 *M Tris*-HCl, pH 8.2 at 4 °C, 0.01 *M* $MgCl_2$, 0.01 *M* $(NH_4)_2SO_4$, 0.5 m*M* DTT, 1.0 m*M* EDTA, 0.1 m*M* PMSF, 20% (v/v) glycerol.

The reaction mixture (0.25 ml) for the assay for RNA polymerase consisted of 0.04 *M Tris*-HCl, pH 7.9 at 30 °C, 0.1 m*M* EDTA, 2.0 m*M* $MgCl_2$, 0.5 m*M* DTT, 0.5 mg/ml bovine serum albumin, 0.15 m*M* ATP, CTP, GTP, 0.015 m*M* 5-^{3}H UTP, specific activity 300 Ci/mole, 0.20 mg/ml calf thymus DNA, 0.4 m*M* potassium phosphate, pH 7.5, and 30% (v/v) glycerol. In addition, for assays for RNA polymerase I: 0.03 *M* $(NH_4)_2SO_4$, 1.0 m*M* $MnCl_2$, and for RNA polymerase II: 0.08 *M* $(NH_4)_2SO_4$ and 2.0 m*M* $MnCl_2$.

Reactions were started by the addition of enzyme, incubated 30 min at 30 °C, and stopped by the addition of 2 ml of cold 5% (w/v) trichloroacetic acid (TCA). After 30 min, precipitates were collected on Whatman GF/C filters and washed 10× with 5 ml of cold 5% TCA. Filters were dried at 80 °C and counted in a toluene based liquid

[1] T. M. BROGT and R. J. PLANTA, FEBS Lett. *20*, 47 (1972).
[2] H. PONTA, U. PONTA and E. WINTERBERGER, FEBS Lett. *18*, 204 (1971).
[3] P. A. HORGEN and D. H. GRIFFIN, Proc. natn. Acad. Sci., USA *68*, 338 (1971).
[4] H. PONTA, U. PONTA and E. WINTERBERGER, Eur. J. Biochem. *29*, *110* (1972).
[5] S. P. BLATTI, C. J. INGLES, T. J. LINDELL, P. W. MORRIS, R. F. WEAVER, F. WEINBERG and W. J. RUTTER, Cold Spring Harb. Symp. quant. Biol. *35*, 649 (1970).
[6] P. A. HORGEN and J. L. KEY, Biochim. biophys. Acta *294*, *227* (1973).
[7] M. T. TELLY DE INON, P. D. LEONI and H. N. TORRES, FEBS Lett. *39*, 91 (1974).
[8] R. H. DAVIS and F. J. DESERRES, Meth. Enzym. *27*, 79 (1970).

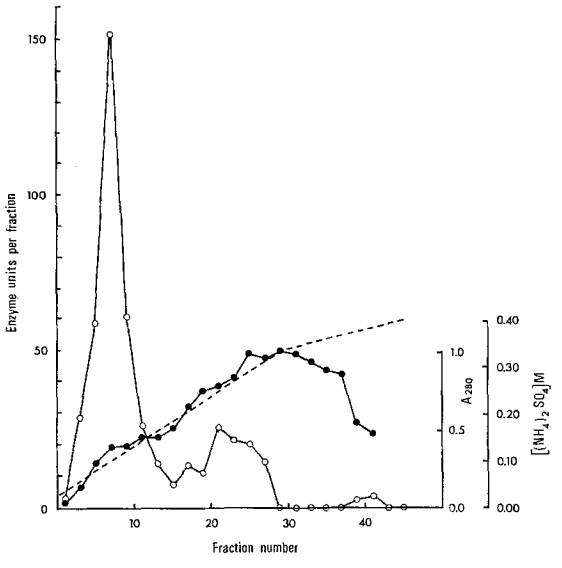

Fig. 1. Elution of RNA polymerase from DEAE-sephadex. The diluted enzyme preparation was adsorbed to a 2.5×15 cm DEAE-sephadex column, washed with buffer B, and eluted with an $(NH_4)_2SO_4$ gradient. Fractions, 2 ml, were collected and 50 µl aliquots assayed for RNA polymerase activity. The reaction mixture contained 2 m*M* $MnCl_2$ and no added $(NH_4)_2SO_4$. The concentration of $(NH_4)_2SO_4$ (----) was monitored by conductivity measurements. ●–●–●, A_{280}; ○–○–○, enzyme activity.

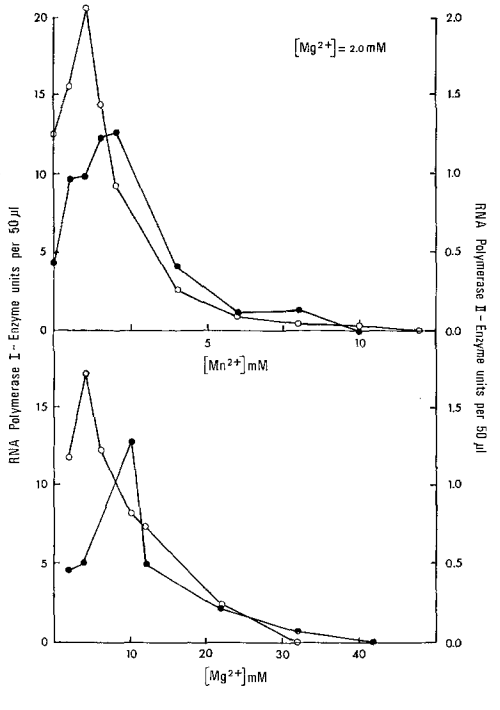

Fig. 2. The effect of Mn^{2+} and Mg^{2+} on RNA polymerase I and II activity. The concentrations of $MnCl_2$ or $MgCl_2$ were varied in the standard reaction mixture. The concentration of $(NH_4)_2SO_4$ was 0.03 *M* for polymerase I (○–○–○) and 0.08 *M* for polymerase II (●–●–●).

scintillation solution. One enzyme unit is the incorporation of 1 picomole of UMP into TCA-precipitable polynucleotides per 10 min.

For RNA polymerases isolation, two 8 l cultures were harvested by filtration through nylon net. The unwashed mycelium was suspended in 250 ml of buffer H and homogenized with glass beads (0.2 mm diameter) in an Omnimixer (Sorvall, Norwalk, Conn., USA) for 5 min at half full speed. The homogenate was filtered through 2 layers of flannel and the filtrate centrifuged at 10,000 ×*g* for 30 min. The pellet, containing nuclei and mitochondria, was suspended in 30 ml of buffer A and 300 µg of DNase I was added. The suspension was stirred for 15 min and centrifuged at 15,000 ×*g* for 15 min. The pellet was re-extracted with 10 ml of buffer A and centrifuged as above. The supernatants were combined, solid $(NH_4)_2SO_4$ was added to 29% of saturation, the solution was stirred for 30 min, and centrifuged at 40,000 ×*g* for 15 min. Solid $(NH_4)_2SO_4$ was added to the supernatant to 55% of saturation, the solution was stirred for 30 min and centrifuged at 40,000 ×*g* for 30 min. The pH was maintained at 7.5 during the $(NH_4)_2SO_4$ fractionation by the addition of 1 *M* NH_4OH. The pellet was dissolved in buffer B and diluted with the same buffer until the specific conductivity (0 °C) was 4.5 n Ω. The diluted preparation was then applied at a rate of 100 ml/h to a 2.5×15 cm DEAE-sephadex A 50 column pre-equilibrated with buffer B. The column was washed at 100 ml/h with buffer B until the A_{280} of the effluent was 0.05, and the enzyme eluted with a 100 ml, 0.05–0.40 *M* $(NH_4)_2SO_4$ gradient in buffer B at 60 ml/h. The peak fractions of each activity were pooled and stored in liquid N_2 where they were stable for at least 6 weeks. All operations were carried out at 0–4 °C as rapidly as possible.

Reagents were obtained as follows: nucleoside triphosphates, Boehringer; PMSF, calf thymus DNA (Type 1), DNase I (1 × crystallized), Sigma; bovine serum albumin (A grade), rifampicin, DTT, cycloheximide, Calbiochem; double-distilled glycerol, *tris* base, Merck; uridine [5-^{3}H]-5′-triphosphate (ammonium salt, 15 Ci/mmole), Amersham Radiochemical Centre. α-Amanitin was a gift from Dr. T. Wieland, Max Planck Inst., Heidelberg, Germany. All other chemicals were reagent grade.

Figure 1 shows the profile of enzyme activity eluted from DEAE-sephadex. 2 major peaks eluted at 0.10 *M* and 0.25 *M* $(NH_4)_2SO_4$ while a third, small peak eluted at 0.37 *M*. Due to the fact that very little of this last activity was present in any preparation and often was absent, its properties were not studied.

The nucleoside triphosphate and DNA dependency and drug sensitivity of RNA polymerases I and II

Treatment	Control (%) Polymerase I	Polymerase II
Complete	100	100
– ATP	3	0
– CTP	11	0
– GTP	16	11
– DNA	0	0
+ cycloheximide (200 µg/ml)	99	93
+ α-amanitin (40 µg/ml)	94	6
+ rifampicin (10 µg/ml)	99	85

Each activity was assayed under optimal conditions and the specified compound added or removed.

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.

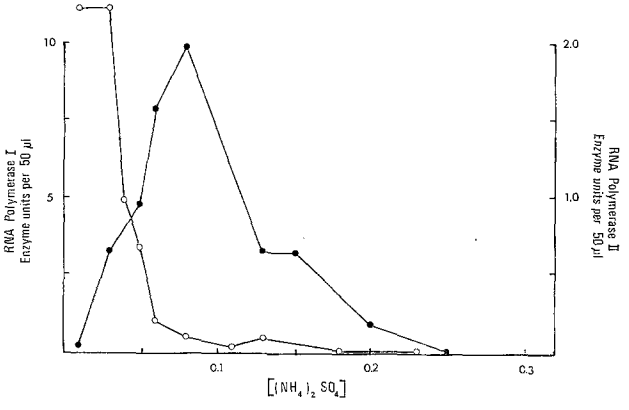

Fig. 3. The effect of $(NH_4)_2SO_4$ on RNA polymerase I and II activity. The concentration of $(NH_4)_2SO_4$ was varied in the standard reaction mixture. The concentration of $MgCl_2$ was 2.0 m*M* while that of $MnCl_2$ was 1.0 m*M* for polymerase I (○–○–○) and 2.0 m*M* for polymerase II (●–●–●).

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.

W. E. TIMBERLAKE and G. TURIAN[12]

Laboratoire de Microbiologie, Département de Biologie Végétale, Université de Genève, CH-1211 Genève 4 (Switzerland), 5 September 1974.

[9] S. T. JACOB, E. M. SAJDEL and H. N. MUNRO, Biochem. Biophys. Res. Commun. *38*, 765 (1970).
[10] F. STIRPE and L. FIUME, Biochem. J. *105*, 779 (1967).
[11] H. KUNTZEL and K. P. SCHAFER, Nature New Biol. *231*, 265 (1971).
[12] This research was supported by a grant from the Fonds National Suisse de la Recherche scientifique. We thank Mme CHRISTIANA GALASSI for her technical assistance and Dr. K. PUROHIT for initiation of this study.

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.

[1] D. JERINA, J. DALY, B. WITKOP, P. ZALTZMAN-NIRENBERG and S. UDENFRIEND, Archs Biochem. Biophys. *128*, 176 (1968).
[2] E. W. MAYNERT, R. L. FOREMAN and T. WATABE, J. biol. Chem. *245*, 5234 (1970).
[3] E. BOYLAND, G. S. RAMSAY and P. SIMS, Biochem. J. *78*, 736 (1961).
[4] A. R. JONES, Drug Metab. Rev. *2*, 71 (1973).
[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)).
[7] E. IVASHKIV and J. M. DUNHAM, J. pharm. Sci. *63*, 285, (1973).
[8] H. JACKSON, I. S. C. CAMPBELL and A. R. JONES, Nature, Lond. *226*, 86, (1970).