Biochemical Pharmacology, Vol. 34, No. 12, pp. 2151–2156, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

MUTAGENIC ACTIVITY OF VARIOUS CHEMICALS IN *SALMONELLA* STRAIN TA100 AND GLUTATHIONE-DEFICIENT DERIVATIVES

ON THE ROLE OF GLUTATHIONE IN THE DETOXIFICATION OR ACTIVATION OF MUTAGENS INSIDE BACTERIAL CELLS

PETER R. M. KERKLAAN,* CLEMA E. M. ZOETEMELK† and GEORGES R. MOHN*

* Department of Radiation Genetics and Chemical Mutagenesis and † Department of Pharmacology, Subfaculty of Pharmacy of the State University of Leiden, Wassenaarseweg 72, 2333 AL Leiden, The Netherlands

(*Received* 4 *October* 1984; *accepted* 14 *January* 1985)

Abstract—Several mutants with decreased levels of reduced glutathione (GSH) were isolated from the sensitive mutagen tester strain *Salmonella typhimurium* TA100 after treatment with u.v. and selection for resistance to *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine (ENNG) and its methyl analogue MNNG. Estimation of the GSH concentration and GSH *S*-transferase activity in extracts of these strains and of TA100 indicates that the GSH^- derivatives contain 10–30% of the GSH level found in TA100, and that they exhibit normal GSH *S*-transferase activity.

The mutagenic activities of 7 chemicals, namely, MNNG, ENNG, 1,2-dibromoethane (DBE), 1-chloro-2,4-dinitrobenzene (CDNB), styrene-7,8-oxide (STOX), *N*-ethyl-*N*-nitrosourea (ENU) and methyl methane sulphonate (MMS) were compared in TA100 and in one representative GSH^- strain, denominated NG-57. MNNG, ENNG, DBE and CDNB are potent to extremely potent mutagens in TA100, but induce very low levels of His^+ mutants in NG-57. Pretreatment of NG-57 with 1 mM GSH (partially) restores the mutant yields to the levels usually found in TA100. The mutagenic activities of STOX, ENU and MMS are similar in both strains.

These results support some previous findings, namely that ENNG, MNNG and DBE, but not ENU are activated to mutagens inside the test bacteria, and also suggest that CDNB is activated by bacterial GSH. The latter finding is in contrast with the current view that CDNB is detoxified by GSH, as is also presently evidenced by a strong reduction of the compound's mutagenicity in the presence of extracts of rat liver, which contains GSH and GSH *S*-transferase activity. The results with STOX indicate that GSH plays in bacteria a much less important role in the detoxification of xenobiotics than in mammalian tissue, presumably due to a much lower GSH *S*-transferase activity in the first organism.

Glutathione is commonly present in cells of living organisms. Its presence, mainly in the reduced state (GSH)‡, has also been shown in a number of prokaryotic species, including *Salmonella typhimurium* and *Escherichia coli* [1–3]. In analogy to its function in mammalian cells, GSH is assumed to have mainly a protective function in bacteria, e.g. detoxification of reactive molecules of endogenous or exogenous origin that may cause damage to the cell. On the other hand, reports have also been made on the potentiating effect of GSH on certain xenobiotics. Alkylnitrosoguanidines such as MNNG and ENNG, for example, can be activated by GSH to products of enhanced alkylating potential [4–6]. Some vicinal haloalkanes are activated after enzymatic conjugation with GSH to bacterial mutagens [7, 8] and DNA alkylating species [9, 10]. The use of GSH-deficient mutants of *E. coli* strains [11, 12] and *Salmonella* TA1535 [13] has permitted the conclusion that MNNG, ENNG and DBE are also transformed inside the bacterial cell to products of higher mutagenicity. To investigate the effects of GSH on a broader spectrum of mutagenic chemicals, attempts were made in the present work at isolating GSH^- mutants of the sensitive and standardized *Salmonella* tester strain TA100 [14], which is a substrain of TA1535 containing the mutator plasmid pKM 101 [15]. The strain does contain GSH and has GSH *S*-transferase activity at levels comparable to those observed in TA1535 [2, 3]. The purpose of the present study was furthermore to compare the mutagenic activity of various chemicals in a TA100/GSH^- strain and in the GSH^+ wildtype. Consequently, 7 mutagens were selected: ENNG, MNNG and DBE in view of their known ability to react with GSH and producing more reactive species; STOX and CDNB because their conjugation to GSH presumably leads to inactivation of these compounds as shown in mammalian tissues and *in vitro* assays [3, 16, 17]; ENU, the mutagenic activity of which is not expected to be affected by GSH conjugation [12, 13]; and MMS was selected because its reactivity towards GSH is

‡ Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; DBE, 1,2-dibromoethane; DCNB, 1,2-dichloro-4-nitrobenzene; DMSO, dimethylsulphoxide; ENNG, *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine; ENU, *N*-ethyl-*N*-nitrosourea; GSH, reduced glutathione; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; MMS, methyl methanesulphonate; STOX, styrene-7,8-oxide.

scarcely documented, except that significant conjugation to GSH has been reported during *in vivo* metabolism of the compound in rats [18].

MATERIALS AND METHODS

Chemicals. ENU and ENNG were purchased from Pfaltz and Bauer, Stamford, CN; MNNG from Koch–Light Laboratories, Colnbrook; MMS from Merck-Schuchart, München, CDNB from Fluka, Buchs; DBE and STOX from Baker, Deventer. GSH and L-cysteine were obtained from Sigma Chemical Co., St. Louis, MO.

Bacterial strain. Salmonella typhimurium strain TA100 was kindly provided by Prof. B. N. Ames, Berkeley, CA. The isolation of GSH^- derivatives is described below.

Growth and suspension media. Phosphate buffered saline (pH 7.0), nutritent broth, and complete agar medium was prepared as described previously [19]. Soft agar contained 6 g of Bacto-agar (Difco) and 5 g of NaCl per litre and was kept at 50° before use. His^+ agar medium contained inorganic salts [20], 1.5% agar, 0.5% D-glucose, trace elements, 0.5 mg L-histidine and 1 mg D-biotine per litre, and was supplemented in the mutagenicity experiments with 50 mg cysteine per litre.

GSH^- mutant isolation. The method was similar to that described for the isolation of GSH^- mutants of *E. coli* [12] and *Salmonella* TA1535 [13], i.e. on the basis of resistance to MNNG and ENNG. About 10^8 stationary TA100 cells were treated with u.v. light (4 J/m^2), suspended in 1 ml of nutrient broth and grown overnight for expression of GSH^- phenotype. Aliquots (0.1 ml) were then spread over complete agar medium plates, and 10, 20 and 50 μl of solutions of ENNG and MNNG (10 mg per ml DMSO) were dropped on the middle of the plates. The plates were incubated for 48 hr at 37°. Single colonies that grew at the border of the growth inhibition zone of the chemicals were isolated and passaged twice on complete agar medium devoid of MNNG and ENNG, but supplemented with ampicilline (2 μg/ml), to ensure the presence of plasmid pKM 101. A total of 80 presumptive alkylnitroguanidine-resistant mutant colonies were isolated in this way. Resistance to ENNG and MNNG was checked in spot tests: seven substrains gave smaller inhibition zones compared to TA100. These seven strains were further tested for u.v. sensitivity, histidine dependence, crystal violet sensitivity and ampicilline resistance, as recommended by Maron and Ames [14]. Four strains were finally chosen for further characterization, including determination of intracellular levels of GSH and GSH S-transferase activity. One representative strain, denominated NG-57, was then retained for the comparative mutagenicity tests.

Determination of GSH content. The fluorimetric assay described by Hissin and Hilf [21], with minor modifications (see ref. 13) was used to determine the GSH concentration in bacterial extracts.

Determination of GSH S-transferase activity. GSH *S*-transferase activity was assayed with two substrates, CDNB and DCNB [22] with the method described by Baars *et al.* [23]. The bacterial extracts

Table 1. Some characteristics of *Salmonella* TA100 and its GSH-deficient derivatives

Strain	GSH content (nmoles/mg protein)*	% Survival after treatment with:†		Dependence on cysteine or GSH for optimal growth and phenotypic expression	Spontaneous His^- mutation frequency His^+ mutants/plate (± S.E.M.)
		0.2 mM MNNG	0.2 mM ENNG		
TA100	10.6 ± 2.2	0.01	0.01	–	49 ± 2
NG-11	1.4 ± 0.5	40	60	+	60 ± 10
NG-41	3.3 ± 1.7	55	50	+	40 ± 7
NG-54	1.3 ± 0.5	55	55	+	55 ± 7
NG-57	1.1 ± 0.4	55	65	+	56 ± 5

* Average values ± S.E.M. of 3 separate determinations.

† 10^9 stationary cells of each strain were treated with 0.2 mM MNNG and 0.2 mM ENNG or incubated without mutagen during 1 hr in phosphate saline buffer. After the treatment the incubation mixtures were diluted and aliquots of appropriate dilutions were spread in triplicate on complete agar medium. The colonies were counted after 24 hr.

were prepared by sonication of bacterial suspensions (1 g of wet cell pellet in 4 ml phosphate-saline buffer) during 5 × 1 min at 0°. The final assay mixtures contained 0.1 to 0.3 ml suspension per 1.5 ml incubation mixture. The protein content of the extracts was measured with the method of Lowry *et al.* [24].

Mutagenicity tests. Stock solutions of the mutagens were prepared in phosphate-saline buffer (ENU and MMS), 1% DMSO (MNNG and ENNG), 10% DMSO (CDNB) or 50% ethanol (STOX and DBE). The concentration of DMSO and ethanol in the final incubation mixtures never exceeded 2.5%. 0.5–1 × 10^8 stationary bacterial cells were incubated with solutions of the compounds in phosphate–saline buffer (total volume 1 ml) during 1 hr at 37° in the dark and under rotary shaking. After incubation 2.5 ml of molten top agar was added to the incubation mixtures, the total was mixed and spread over His^+ agar medium containing cysteine; His^+ revertant colonies were counted after incubation of the plates in the dark for 48 hr.

RESULTS

The ENNG- and MNNG-resistant strains designated NG-11, NG-41, NG-54 and NG-57 showed the same u.v. sensitivity, histidine dependence, crystalviolet sensitivity and ampicilline resistance as did strain TA100 (data not shown). Table 1 gives some further characteristics of the four mutant strains: firstly, the GSH content in stationary cultures of the strains as determined with the fluorimetric assays is roughly 10% of the levels found in TA100, with the exception of NG-41 (30%); secondly, the strains are very resistant to both ENNG and MNNG compared to TA100 in liquid incubation assays; thirdly, for optimal growth and expression of His^+ revertants addition of cysteine or GSH to the medium is required. The strains are, however, not fully auxotrophic for cysteine or GSH since they can still grow, but much slower, in the absence of either thiol compound. A similar dependence on cysteine or GSH was observed with the GSH^- derivative of TA1535 [13] and indicates possibly a more general phenomenon for GSH-deficient strains of *Salmonella*. Fourthly, the level of spontaneous His^+ revertants is comparable to that seen with TA100.

The values for GSH *S*-transferase activity assayed in extracts of TA100 and of the 4 GSH^- derivatives are given in Table 2. For comparison, activity found in the cytosolic fraction of rat liver extracts (S100) is also shown in Table 2. Some differences in transferase activity for CDNB and DCNB conjugation between the various strains can be observed, with NG-41 having the highest and NG-57 having the lowest average values. The activities found in the GSH^- strains are in the same range as those observed in TA100. In general, the specific activity of the bacterial GSH *S*-transferase(s) is several orders of magnitude lower than of its mammalian counterpart. The significance of the differences between the strains may therefore be questioned. Conjugation of CDNB to GSH in the presence of bacterial extracts is 10 × faster as conjugation of DCNB, as is also observed in the presence of the rat liver extract. This may be explained by a higher overall specificity of the bacterial and mammalian enzyme for CDNB, as was found earlier with purified extracts of rat liver [22].

The mutagenic activities of the 7 compounds were compared in TA100 and one representative GSH^- strain, NG-57, with and without pretreatment of the bacteria with GSH. The results are shown in Fig. 1. NG-57 is not, or hardly, mutagenized by the alkylnitroguanidines ENNG and MNNG, by DBE and CDNB. Pretreatment of the GSH^- cells with GSH, which results in an initially increased concentration of GSH inside the bacteria during incubation [13], (partially) restores mutant yields induced by all four compounds to levels observed in TA100. Pretreatment of TA100 with GSH also increases the mutant yield slightly, with the exception of DBE. At the concentration ranges tested no toxicity of the compounds for both strains was observed. The results with MNNG, ENNG, DBE and CDNB therefore are a strong indication that activation of these compounds is mediated by GSH *inside* the bacterial cell. The mutagenic activities of MMS, STOX and ENU seem not to be affected by the presence of GSH in the bacterial cell, since no or only small differences in number of His^+ revertants are observed between the strains at equimolar concentrations of the compounds.

The results with CDNB were unexpected since this compound is known to react strongly with bacterial GSH, indicated by its ability to deplete GSH inside *Salmonella* cells [3]. Conjugation of CDNB to GSH in the presence of mammalian liver extracts outside

Table 2. Specific activities of GSH *S*-transferase(s) for CDNB and DCNB in extracts of various bacterial strains and of rat liver

Extract from	CDNB conjugated (nmoles/mg protein/ml)	DCNB conjugated (nmoles/mg protein/ml)*
TA100	4.99 ± 0.70	0.39 ± 0.08
NG-11	2.28 ± 0.56	0.27 ± 0.11
NG-41	5.51 ± 1.04	0.74 ± 0.11
NG-54	3.45 ± 0.33	0.42 ± 0.06
NG-57	2.17 ± 0.19	0.21 ± 0.08
Rat liver (S100)†	1300	110

* Average values ± S.E.M. of 3–5 determinations.

† Rat liver S100 was prepared and assayed for GSH S-transferase activity as described by Baars *et al.* [23].

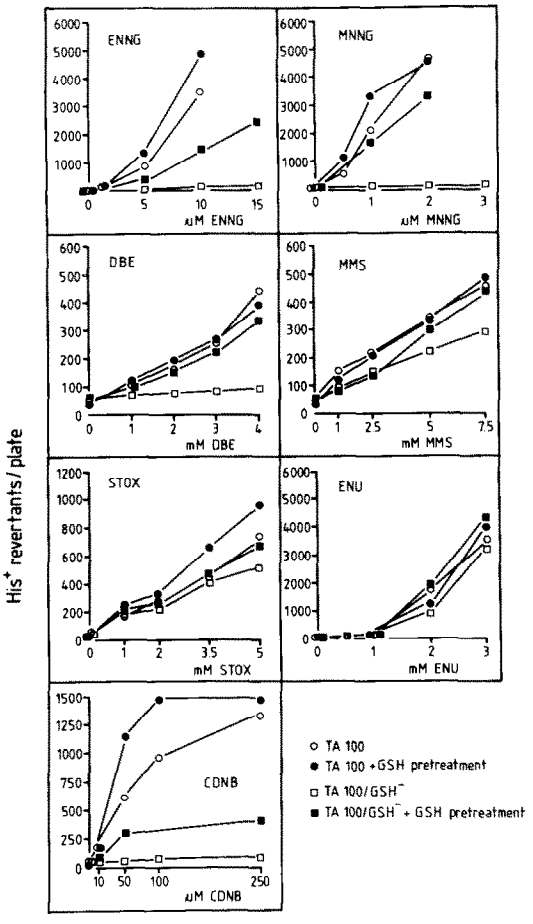

Fig. 1. Mutagenicity of ENNG, MNNG, DBE, MMS, STOX, ENU and CDNB in TA100 (○, ●) and NG-57 (□, ■). The bacteria were pretreated with 1 mM GSH (●, ■) or not (○, □); to attain this, overnight cultures of the strains were centrifuged and resuspended in 1 mM GSH in phosphate–saline buffer, or in buffer without GSH, and incubated for 15 min at 37°; after the incubation the bacteria were centrifuged, washed with buffer and resuspended in phosphate–saline buffer. This suspension was then distributed in the mutagenicity incubation mixtures. The experimental points represent average values obtained from at least 3 separate experiments.

the indicator bacteria is, furthermore, known to reduce its mutagenic activity strongly [3]. This led us to expect that a high concentration of GSH inside the cells would lead to deactivation of the compound. However, the opposite effect was observed. This result cannot be explained by toxic effects (killing, impairment of growth on the plates) of CDNB specifically in NG-57, since (i) no reduced survival of the bacterial cells during incubation in liquid medium was observed, as mentioned above, and (ii) removal of the compound from the medium before addition of the soft ager (by centrifugation of the bacterial suspensions) did not essentially alter the mutant yields observed in the experiments with CDNB still present on the plates (results not shown). To confirm that under the present conditions conjugation of CDNB to GSH outside the bacteria would indeed lead to a strong decrease in mutagenic activity of the compound, the compound was incubated with TA100 or NG-57 in the presence of rat liver extract and additional GSH (Fig. 2). The presence of S9 alone considerably lowers the mutagenic activity of CDNB in TA100, and when GSH was added its activity is completely abolished. Addition of GSH alone to the incubation mixtures increases the mutant yield in both TA100 and NG-57, in analogy to the results obtained with GSH pretreatment of the strains (see Fig. 1). This is probably due to the fact that the presence of 1 mM GSH in the incubation mixtures will increase the intracellular concentration of GSH to the same extent as during pretreatment. In any case, these results indicate that conjugation of CDNB to GSH outside the cell *decreases* its mutagenic activity, while inside the bacterial cell GSH strongly *potentiates* the compound's activity.

DISCUSSION

Selection of bacterial strains for resistance to ENNG and MNNG has proven a fast and simple method to obtain mutants with decreased levels of GSH. This paper describes the isolation of five of such mutants from *Salmonella* TA100, a sensitive mutagen tester strain. Primary characterization indicates that these derivatives are identical to the parent strain in all but one respect: they contain only 10–30% of the normal endogenous level of GSH. It is this deficiency that probably impairs their growth and, therefore, the optimal expression of mutations in medium devoid of sulfhydryl compounds. In contrast to what we suggested in a previous report [13], namely that the GSH⁻ derivative of *Salmonella* TA1535 could be a *cys* mutant, we now believe that the GSH deficient mutants of *Salmonella* isolated and characterized in this paper may rather be allelic to *gsh*A or *gsh*B mutants of *E. coli* described by Apontoweil and Berends [26, 27] and Fuchs and Warner [28]. The biochemical confirmation of the possible deficiencies in γ-glutamylcysteine synthetase (*gsh*A) or glutathione synthetase (*gsh*B) activity are presently underway.

The results on the activity of mutagens presented here, which may be considered as an extension of data gathered with GSH⁻ derivatives of *E. coli* and *Salmonella* TA1535 published earlier [12, 13], strongly suggest the importance of interaction of certain xenobiotics with GSH in the bacterial cell. Results with 4 of the 7 compounds tested in one representive GSH⁻ derivative of TA100, suggest dependence on GSH for mutagenic activity of these chemicals. The alkylnitroguanidines MNNG and ENNG, for instance, are much less mutagenic in the GSH⁻ derivative than in the GSH⁺ wild-type, while exogenous supply of GSH into the bacterial cells increases the mutant yields considerably. This result, in addition to earlier and similar findings with these compounds in GSH⁻ derivatives of bacteria [12, 13] provides strong evidence for activation of these extremely potent mutagens by GSH inside the various strains of bacteria. This corroborates results from studies on *in vitro* activation by mammalian GSH or other thiols and in mammalian cells [4, 6]. The results obtained with exposure of TA100 and its GSH⁻ derivative to DBE supports the indications that this compound is also activated by GSH inside the *Sal-*

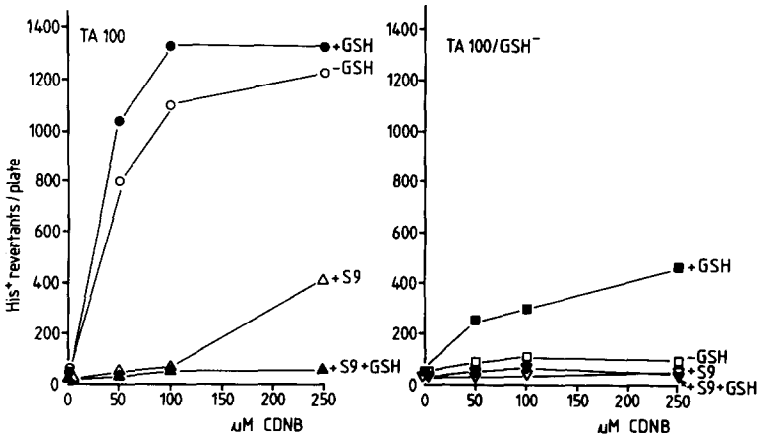

Fig. 2. Mutagenic activity of CDNB in TA100 (left) and NG-57 (right) in the presence of 1 mM GSH (●, ■), 10% (v/v) rat liver S9 (△, ▽), 10% S9 and 1 mM GSH (▲, ▼) or without additional factors (○, □). The rat liver S9 was prepared as described previously [25]. It contained 29 mg protein/ml, as determined with the method of Lowry *et al.* [24]. The experimental points represent average values from 2 separate experiments.

monella bacteria [13]. Direct evidence that DBE is indeed transformed by GSH into a DNA alkylating product through mediation by mammalian GSH *S*-transferases has recently been presented [10]. Earlier work of van Bladeren *et al.* [8] on the mutagenic activity of DBE in TA100 in the presence of crude rat liver extracts containing GSH and *S*-transferases, had already indicated that extracellular conjugation of DBE to GSH can give rise to products of higher mutagenic potency. It may therefore be assumed that the direct mutagenic activity of the compound in *Salmonella* strains (that is, in the absence of mammalian enzymatic activity) is a results of endogenous formation of a DNA alkylating product. This reactive species is formed after reaction of the parent compound with bacterial GSH.

The data obtained with CDNB also suggest a strong influence of GSH interaction on the mutagenic activity of the compound, but the implications are less clear-cut. CDNB conjugation to GSH in the medium, in the presence of mammalian *S*-transferases and GSH, leads to a less mutagenic product, while inside the bacteria GSH seems to potentiate the compound's mutagenicity. The following explanation may help to resolve this apparent paradox: the product formed from the reaction of GSH with CDNB (*S*-(2′-4′-dinitrobenzyl)glutathione) may be mutagenic for TA100 because this aromatic product contains nitro-substituents that should be considered as substrates for bacterial nitroreductases. This enzymatic activity, which is also present in *Salmonella* strains, catalyzes the formation of more reactive hydroxylamino-, nitroso- and hydroxamic ester derivatives from nitroaromatics [29–31]. It has been suggested that the strong mutagenic activity of 2,4-dinitroanisole, a compound structurally related to the 2,4-dinitrobenzene-GSH-adduct mentioned above, is due to nitroreduction inside the *Salmonella* test bacteria [32]. Formation of such a CDNB–GSH reaction product outside the bacterial cells may lead to inactivation of CDNB because of difficult penetration of the product into the bacteria, or to inactivation by factors present in the rat liver extract (S9).

STOX, the major oxidative metabolite of styrene in mammals [33, 34] and a potent mutagen in TA100 [35, 36] was expected to exert a stronger mutagenic effect in the GSH-deficient derivative of TA100 than in TA100 itself, since the compound is easily enzymatically conjugated in mammalian organs to GSH, which leads to a less reactive product [37]. In the present experiments, however, no increase in the mutagenic activity in the GSH⁻ strain was observed. This suggests that conjugation to bacterial GSH does not play a significant role in the inactivation of styrene oxide. An explanation may be that the activity of GSH *S*-transferase in the test bacteria is too low to effectively detoxify the compound. Its has been shown in this paper and by others [2, 3] that mammalian (liver) tissue contains levels *S*-transferase activity that are several orders of magnitude higher than in bacterial cells. With ENU, a compound that is not expected to interact strongly with GSH *in vitro* [38] or in bacteria [12, 13], no large differences in mutagenic activity in TA100 and NG-57 could be observed. This presents a further confirmation of the finding that the presently isolated GSH-mutants are isogenic to the parent strain TA100 except for their content of endogenous GSH. The results with MMS, finally, do not provide a clear indication for a strong reaction of the compound with bacterial GSH. These and earlier results [12, 13] indicate that the isolation and use of GSH⁻ derivatives of *Salmonella* and *E. coli* strains represent a useful and simple method to study GSH-interacting properties of (mutagenic) xenobiotics in intact bacterial cells. It is plausible that similar reactions can take place in mammalian cells, as has been shown for some of the compounds, although there they may be more important than in bacteria, because of much higher levels GSH S-transferase activity.

Acknowledgements—We thank Mr. P. de Knijff for his help with the confirmation of the various genotypes of

the strains. This research was sponsored by the Koningin Wilhelminafonds of The Netherlands, Grant No. SG-81.92.

REFERENCES

1. R. C. Fahey, W. C. Brown, W. B. Adams and M. B. Worsham, *J. Bacteriol.* **133**, 1126 (1978).
2. J. Meyer, J. W. DePierre and U. Rannug, *Chem.-Biol. Interact.* **31**, 247 (1980).
3. K.-H. Summer, W. Göggelmann and H. Greim, *Mutation Res.* **70**, 269 (1980).
4. U. Schulz and D. R. McCalla, *Can. J. Chem.* **47**, 2021 (1969).
5. R. Süssmuth and F. Wingens, *Z. Naturforsch.* **246**, 903 (1969).
6. P. D. Lawley and C. J. Thatcher, *Biochem. J.* **116**, 693 (1970).
7. U. Rannug, A Sundvall and C. Ramel, *Chem.-Biol. Interact.* **20**, 1 (1978).
8. P. J. van Bladeren, D. D. Breimer, G. M. T. Rotteveel-Smijs, R. A. W. de Jong, W. Buys, A. van der Gen and G. R. Mohn, *Biochem. Pharmac.* **29**, 2975 (1980).
9. N. Ozawa and F. P. Guengerich, *Proc. natn. Acad. Sci. U.S.A.* **80**, 5266 (1983).
10. P. E. Inskeep and F. P Guengerich, *Carcinogenesis* **5**, 805 (1984).
11. B. Sedgewick and P. Robins, *Molec. Gen. Genet.* **180**, 85 (1980).
12. G. Mohn, P. de Knijff and A. Baars, *Mutation Res.* **111**, 25 (1983).
13. P. Kerklaan, S. Bouter and G. Mohn, *Mutation Res.* **122**, 257 (1983).
14. D. M. Maron and B. N. Ames, in *Handbook of Mutagenicity Test Procedures*, 2nd Edn (Eds. B. J. Kilbey, M. Legator, W. Nichols and C. Ramel), p. 93. Elsevier, Amsterdam (1984).
15. B. N. Ames, J. McCann and E. Yamasaki, *Mutation Res.* **31**, 347 (1975).
16. J. van Anda, B. R. Smith, J. R. Fouts and J. R. Bend, *J. Pharmac. exp. Ther.* **211**, 207 (1979).
17. K. Yoshikawa, M. Isobe, T. Watabe and E. Takabatake, *Mutation Res.* **78**, 219 (1980).
18. E. A. Barnsley, *Biochem. J.* **106**, 18P (1968).
19. M. H. Adams, *Bacteriophages*. Wiley, New York (1959).
20. H. J. Vogel and D. M. Bonner, *J. biol. Chem.* **218**, 97 (1956).
21. P. J. Hissin and R. Hilf, *Analyt. Biochem.* **74**, 214 (1976).
22. W. H. Habig, M. J. Pabst and W. B. Jakoby, *J. biol. Chem.* **249**, 7130 (1974).
23. A. J. Baars, M. Jansen and D. D. Breimer, *Biochem. Pharmac.* **27**, 2487 (1978).
24. O. H. Lowry, H. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
25. P. Kerklaan, G. Mohn and S. Bouter, *Carcinogenesis* **2**, 415 (1981).
26. P. Apontoweil and W. Berends, *Biochim. biophys. Acta* **399**, 10 (1975).
27. P. Apontoweil and W. Berends, *Molec. Gen. Genet.* **141**, 91 (1975).
28. J. A. Fuchs and H. R. Warner, *J. Bacteriol.* **124**, 140 (1975).
29. E. C. McCoy, H. S. Rosenkranz and R. Mermelstein, *Environm. Mutagen.* **3**, 421 (1981).
30. D. F. Callen, in *Chemical Mutagens: Principles and Methods for their Detection*, Vol. 7 (Eds. F. J. de Serres and A. Hollaender), p. 163. Plenum Press, New York (1982).
31. H. S Rosenkranz and R. Mermelstein, *Mutation Res.* **114**, 217 (1983).
32. C. W. Chiu, L. H. Lee, C. Y. Wang and G. T. Bryan, *Mutation Res.* **58**, 11 (1978).
33. T. Watabe, M. Isobe, T. Sawahata, K. Yoshikawa, S. Yamada and E. Takabatake, *Scand. J. Work Environ. Health* **4** (suppl. 2), 142 (1978).
34. H. Vainio, *Chem.-Biol. Interact.* **22**, 117 (1978).
35. C. de Meester, F. Poncelet, M. Roberfriod, J. Rondelet and M. Mercier, *Mutation Res.* **56**, 147 (1977).
36. L. Busk, *Mutation Res.* **67**, 201 (1979).
37. J. W. Steele, B. Yagen, O. Hernandez, R. H. Cox, B. R. Smith and J. R. Bend, *J. Pharmac. exp. Ther.* **219**, 35 (1981).
38. G. P. Wheeler and B. J. Bowdon, *Biochem. Pharmac.* **21**, 265 (1972).