Effect of partial hepatectomy on removal of *O*⁶-methylguanine from alkylated DNA by rat liver extracts

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1. The activity of an enzyme catalysing the loss of *O*⁶-methylguanine from methylated DNA was increasing during liver regeneration after partial hepatectomy. Activity was increased 3-fold by 24 h and was maximal (6-fold increase) over the period 48–72 h after operation. 2. This activity could also be induced by chronic treatment with dimethylnitrosamine, but the maximal response amounted to a 2–3-fold change (with the greater effect in male rats) after 4–6 weeks of exposure to daily doses of 2 mg of dimethylnitrosamine/kg. 3. Neither partial hepatectomy nor treatment with dimethylnitrosamine increased the activities of two other enzymes repairing alkylated DNA, DNA (7-methylguanine)-glycosylase and DNA (3-methyladenine)-glycosylase. 4. These results therefore indicate that there is a selective induction of the *O*⁶-methylguanine removal system during hepatocyte proliferation. Since this product is known to lead to mutations and its persistence in DNA throughout cell replication has been implicated in tumour initiation, this induction may play a role in resistance to carcinogenesis by alkylating agents.

The mutagenic and carcinogenic actions of certain alkylating agents have been linked to the formation of *O*⁶-alkylguanine in DNA (Lawley, 1974; Pegg, 1977; Margison & O'Connor, 1979; Rajewsky, 1980). This product is chemically stable in DNA at physiological pH, and its disappearance from DNA *in vivo* with time after treatment with *N*-nitroso-carcinogens therefore indicates the presence of an enzymic mechanism for its removal. There is evidence that the ability to remove *O*⁶-methylguanine from DNA differs from one organ to another and correlates with resistance to carcinogenesis. However, there is at present little information on the physiological factors regulating the activity of this DNA repair system. A similar system in *Escherichia coli* is known to be inducible after exposure to alkylating agents (Samson & Cairns, 1977; Jeggo *et al.*, 1977; Schendel & Robins, 1978), and there is preliminary evidence that the system in rodent liver cells may be inducible by chronic exposure to chemical carcinogens (Cooper *et al.*, 1978; Montesano *et al.*, 1979, 1980; Margison *et al.*, 1979; Buckley *et al.*, 1979; Pegg, 1980; Swann & Mace, 1980). These experiments were performed by analysing the labelled methylated DNA isolated at various times after treatment with labelled dimethyl-

nitrosamine. This method is laborious, expensive and lacking in sensitivity. Furthermore, it is unsuitable for comparisons between conditions where the rate of activation of dimethylnitrosamine to the alkylating species may be different, and the presence of unlabelled carcinogen adducts remaining from the pretreatment may lead to underestimation of the extent of increase in activity.

In the present work we have used an assay method *in vitro* to measure the ability of liver cell extracts to catalyse the removal of *O*⁶-methylguanine from alkylated DNA. Previous studies have shown that activities measured in this system *in vitro* parallel those measured *in vivo* with respect to differences between tissues (Pegg, 1978*a*, 1980), saturation and loss of activity after treatment with high doses of alkylating agents (Pegg, 1978*b*; Stumpf *et al.*, 1979; Pegg & Balog, 1979) and effects of hormones (Pegg *et al.*, 1978; Pegg, 1980). The present studies confirm that chronic treatment with dimethylnitrosamine brings about a 2–3-fold increase in the activity removing *O*⁶-methylguanine from DNA, but show that a substantially greater effect (6-fold) is brought about by partial hepatectomy and consequent liver regeneration. Neither liver regeneration nor chronic treatment with dimethylnitrosamine produced any increase in the activities of DNA (3-methyladenine)-glycosylase or DNA (7-methyl-

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guanine)-glycosylase, which are other enzymes repairing bases altered by alkylation (Lindahl, 1979; Margison & Pegg, 1981; Singer & Brent, 1981).

Methods and materials

Treatment of animals

Male Sprague-Dawley rats weighing 200–250 g were subjected to two-thirds partial hepatectomy or to a sham operation in which no liver was removed under ether anaesthesia (Higgins & Anderson, 1931). Male and female Sprague-Dawley rats weighing 150 g at the start of the treatment were given doses of 2 mg of dimethylnitrosamine/kg daily by intraperitoneal injections of a solution of 1 mg/ml in 0.9% NaCl. Control rats received daily injections of saline.

Preparation of tissue extracts

For the assay of *O*⁶-methylguanine removal activity, livers were homogenized in 3 vol. of ice-cold buffer solution (50 mM-Tris/HCl buffer, pH 7.5, containing 1 mM-dithiothreitol and 0.1 mM-EDTA) in a Polytron homogenizer (Brinkman Instruments, New York, NY, U.S.A.). The homogenate was centrifuged at 12000 *g* for 5 min, and the supernatant was removed and saved. A further volume of the buffer solution equal to the original weight of the tissue was then added to the precipitate, and, after homogenization, the homogenate was sonicated for three periods of 30 s each as described (Pegg & Hui, 1978). The sonicated extract was then combined with the original supernatant fraction and centrifuged at 22000 *g* for 30 min. The supernatant was removed, and (NH₄)₂SO₄ was added to 80% saturation. The resulting precipitate was collected by centrifugation at 22000 *g* for 10 min, dissolved in 50 mM-Tris/HCl buffer, pH 7.5, containing 1 mM-dithiothreitol and 0.1 mM-EDTA and dialysed overnight against 100 vol. of this solution. The dialysed sample was centrifuged at 22000 *g* for 15 min and the supernatant was used for the assays. Approx. 55% of the total activity was present in the original supernatant solution, and the remaining 45% was released from the crude nuclear pellet by the sonication treatment. The two fractions were combined in order to assess the total activity.

For the assay of DNA (3-methyladenine)-glycosylase and DNA (7-methylguanine)-glycosylase activities extracts were prepared by homogenization of the livers in 2 vol. of ice-cold 50 mM-Tris/HCl buffer, pH 8.0, containing 1 mM-2-mercaptoethanol and 1 mM-EDTA in the Polytron homogenizer. The extract was centrifuged at 3000 *g* for 10 min and the supernatant was saved. The pellet was rehomogenized with a further 1 vol. of the extraction buffer, and the mixture was combined with super-

natant and centrifuged at 105 000 *g* for 60 min. The supernatant was removed and used for the assays.

Assay of O⁶-methylguanine removal

[³H]Methylated DNA was prepared by treating 84 mg of calf thymus DNA (Sigma Chemical Co., St. Louis, MO, U.S.A.) with 1 mCi of *N*-[³H]methyl-*N*-nitrosourea (1.01 Ci/mmol; New England Nuclear, Boston, MA, U.S.A.) in 16 ml of 80 mM-sodium phosphate buffer, pH 8, for 30 min at 37°C. The DNA was then precipitated by the addition of 2 vol. of ethoxyethanol, washed four times with ethanol and once with ether, dissolved in assay buffer and dialysed overnight before use. The assay mixtures contained 0.8 mg of [³H]methylated DNA (containing 2.6 pmol of *O*⁶-methylguanine), 200 μmol of Tris/HCl buffer (final pH 8.3), 4 μmol of dithiothreitol, 1 μmol of EDTA and 0.5–12 mg of protein in a total volume of 3 ml. After incubation of the mixture at 37°C for 30 min (in some experiments linearity of reaction for up to 90 min was tested), the reaction was stopped by the addition of 1.0 ml of cold 1M-HClO₄. The precipitate was collected by centrifugation (all centrifugations were at 10000 *g* for 10 min) at 2°C, and the pellet was washed twice at 2°C with cold 0.25 M-HClO₄. The precipitated DNA was then hydrolysed by suspension in 0.75 ml of 0.1 M-HCl and heating at 70°C for 30 min. The mixture was centrifuged and the supernatant was removed. The pellet was again suspended in 0.75 ml of 0.1 M-HCl and heated at 70°C for 30 min. After centrifugation, the supernatant was combined with the earlier supernatant and a sample was subjected to high-pressure liquid chromatography for separation of the methylated bases. A 0.75 ml portion was applied with appropriate marker bases to a column (0.46 cm × 25 cm) packed with Partisil 10SCX cation-exchange medium (Whatman, Clifton, NJ, U.S.A.). The column was eluted at 50°C with 20 mM-ammonium formate buffer, pH 4, at a flow rate of 2 ml/min with the use of a Micromeritics (Norcross, GA, U.S.A.) model 750 solvent-delivery system, model 730 universal injector, model 731 column oven and a model 740 micro-processor. The fractions were monitored for absorbance at 254 nm with a Waters (Milford, MA, U.S.A.) model 440 absorbance monitor, and fractions corresponding to adenine, guanine, 7-methylguanine and *O*₆-methylguanine were collected. The recoveries of adenine and guanine were determined by measurement of absorbance, and the amounts of the methylated purines were determined by addition of 2 vol. of Formula 947 liquid-scintillation 'cocktail' (New England Nuclear) and counting of radioactivity in a Beckman Instruments liquid-scintillation spectrometer at an efficiency of 28%. The results of incubation with the protein extracts were compared with the content of methylated bases in DNA from

tubes in which the reaction was stopped at zero time or incubated without protein. Only *O*⁶-methylguanine was lost from DNA under these incubation conditions. Less than 5% of the 7-methylguanine or purine bases was removed from the acid-precipitable DNA during a 1 h incubation, showing that the reaction observed was specific for *O*⁶-methylguanine.

Assay of DNA (3-methyladenine)-glycosylase

The substrate for this reaction was prepared by treating 32 mg of calf thymus DNA with 5 mCi of di[³H]methyl sulphate (2.4 Ci/mmol; New England Nuclear) in 40 ml of 0.2 M-sodium cacodylate buffer, pH 7.2, for 1.5 h at 37°C. The DNA was precipitated by the addition of 2 vol. of cold ethanol and washed with 80% (v/v) ethanol. The DNA was then dissolved in 6 ml of 10 mM-NaCl and dialysed overnight against 100 vol. of this solution. Samples of the DNA solution were then used for the enzyme assays. The assays were performed by incubating a total volume of 0.35 ml containing 0.3 ml of the tissue extract, prepared as described above and diluted with the extraction buffer so that up to 5 mg of protein was added, 35 μmol of KCl, 25 μmol of Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]/KOH buffer, pH 7.8, 0.35 μmol of EDTA, 0.35 μmol of dithiothreitol and 15 μg of [³H]methylated DNA (55 nCi; containing about 4.6 pmol of 3-methyladenine). After incubation at 37°C for 1 h, the reaction was halted by the addition of 0.035 ml of 2 M-NaCl and 1.05 ml of ethanol at 2°C. The tubes were left in ice for 10 min, and the precipitate was removed by centrifugation at maximum speed in an Eppendorf 3400 microcentrifuge at 4°C. Samples (0.5 ml) of the supernatant were removed and the radioactivity of each was determined after addition of 5 ml of Formula 947 liquid-scintillation 'cocktail'. A further sample was evaporated to dryness, dissolved in 0.5 ml of 0.1 M-HCl and analysed by high-pressure liquid chromatography after addition of unlabelled markers of 7-methylguanine and 3-methyladenine. More than 75% (all samples were in the range 79–88%) of the enzymically released radioactivity was present as 3-methyladenine. Results are expressed as fmol of 3-methyladenine released/h per mg of protein. The 3-methyladenine released into the supernatant fraction by spontaneous hydrolysis was determined in parallel incubation mixtures containing 5 mg of bovine serum albumin instead of the liver protein and was subtracted from that obtained in the enzyme assays. This blank value represented about 5–15% of that obtained in the assays.

Assay of DNA (7-methylguanine)-glycosylase

The substrate for this reaction was prepared by incubating the [³H]methylated DNA prepared by

reaction with dimethyl sulphate as described above with DNA (3-methyladenine)-glycosylase from *E. coli* to remove all 3-methyladenine. The mixture contained 300 μg of the labelled DNA, 1 mM-EDTA, 1 mM-2-mercaptoethanol, 70 mM-Hepes/KOH buffer, pH 7.6, 5% (v/v) glycerol and about 5 mg of protein (fraction III) isolated from *E. coli* B (Riazuddin & Lindahl, 1978). After incubation for 4 h at 37°C, the DNA was extracted by shaking with an equal volume of chloroform/3-methylbutan-1-ol (9:1, v/v). The mixture was centrifuged at 10000g for 5 min, and the aqueous phase was removed and dialysed twice against 1 litre of 20 mM-Tris/HCl buffer, pH 7.5, containing 0.1 mM-EDTA for 18 h. The resulting DNA solution contained 9000 d.p.m./μg in 7-methylguanine and less than 10 d.p.m./μg in 3-methyladenine.

The assays of DNA (7-methylguanine)-glycosylase were performed by incubation of 1 μg of the [³H]methylated DNA (containing about 1.7 pmol of 7-methylguanine), 35 μmol of KCl, 25 μmol of Hepes/KOH buffer, pH 7.8, 0.35 μmol of EDTA, 0.35 μmol of dithiothreitol and 0.3 ml of the diluted tissue extract containing up to 4 mg of protein. After incubation at 37°C for 1 h, the reaction was stopped and the radioactivity released into the ethanol supernatant fraction was determined as described for the assay of DNA (3-methyladenine)-glycosylase. High-pressure liquid-chromatographic analysis of this radioactivity revealed that more than 95% corresponded to 7-methylguanine. The results were corrected by subtraction of the 7-methylguanine released into the supernatant fraction by spontaneous hydrolysis. This was measured by substitution of 4 mg of bovine serum albumin instead of the liver protein and amounted to 30–45% of that obtained in the enzymic reaction. The results were expressed as fmol of 7-methylguanine released/h per mg of protein.

Other methods

Protein was determined by the method of Bradford (1976) with the use of reagents from Bio-Rad Laboratories (Richmond, CA, U.S.A.) and bovine serum albumin as standard. Unlabelled methylated purine bases, dimethylnitrosamine and other reagents were obtained or synthesized as described previously (Pegg, 1978a,b; Pegg & Hui, 1978).

Results

As previously reported (Pegg, 1978a; Pegg & Hui, 1978), liver extracts were able to catalyse the loss of *O*⁶-methylguanine from alkylated DNA (Fig. 1). Under the conditions used for the assay in the present experiments the reaction was proportional to the time of incubation for up to 1 h and there was no significant degradation of the DNA substrate to

Fig. 1. Effect of protein concentration on removal of O^6 -methylguanine from alkylated DNA

Liver extracts were prepared from control rat livers (▼), livers of rats treated for 44 days with 2 mg of dimethylnitrosamine/kg per day (■) and livers of rats subjected to two-thirds partial hepatectomy 3 days before they were killed (●). The amount of protein shown was then incubated at 37°C with 0.8 mg of [3 H]methylated DNA (containing 2.6 pmol of O^6 -methylguanine), 200 μ mol of Tris/HCl buffer (final pH 8.3) at 37°C, 1 μ mol of EDTA and 4 μ mol of dithiothreitol in a total volume of 3.0 ml. At the end of this period the amounts of alkylated bases remaining in the acid-precipitable DNA were determined as described in the Methods and materials section. The amount of O^6 -methylguanine lost from the DNA during the incubation is shown as the mean of three determinations for each point which agreed within $\pm 10\%$. There was no significant loss ($< 5\%$) of 7-methylguanine from the DNA during the incubation with any of the extracts, indicating the absence of significant nuclease activity under these assay conditions.

acid-soluble material due to nuclease action. It can be seen from Fig. 1 that the removal of O^6 -methylguanine from DNA catalysed by control rat liver extracts was proportional to the amount of protein added over the entire range tested up to

Fig. 2. Time course in change of O^6 -methylguanine-removal activity in liver extracts after partial hepatectomy

Liver extracts were prepared from rats subjected to two-thirds partial hepatectomy (■) or sham operation (●), and liver extracts were prepared and assayed for the ability to catalyse loss of O^6 -methylguanine from DNA as indicated in Fig. 1. Results were taken from the range of protein concentration over which activity was proportional to the amount of protein added. Results are shown $\pm$ S.D. for at least four separate animals for each point.

12 mg of protein. When extracts were prepared from rats subjected to partial hepatectomy or to chronic treatment with dimethylnitrosamine, the activity was greater and was proportional to protein concentration only when less than 65% of the O^6 -methylguanine present in the DNA substrate was removed. All comparisons were made by expressing results as pmol of O^6 -methylguanine removed/mg of protein as determined by measurements over a range in which activity was proportional to protein concentration.

The effect of partial hepatectomy on the ability to remove O^6 -methylguanine from DNA is shown in Fig. 2. Activity was increased significantly within 24 h after the operation and reached a peak at 48–72 h. These increases parallel the induction of DNA synthesis and cell replication in the liver. Sham operation did not lead to any significant change in O^6 -methylguanine removal. Chronic treatment with dimethylnitrosamine also enhanced the ability of liver extracts to remove O^6 -methylguanine from DNA (Table 1). Sprague–Dawley rats of both sexes were tested for this activity after treatment for various times. In male rats a maximal response was produced by 3–4 weeks of treatment with daily doses of 2 mg/kg, and the activity remained at this

Table 1. *Effect of partial hepatectomy and chronic treatment with dimethylnitrosamine on the ability of liver extracts to catalyse the removal of O⁶-methylguanine, 3-methyladenine and 7-methylguanine from methylated DNA*

Treatment with dimethylnitrosamine (2 mg/kg per day for 44 days) and partial hepatectomy (72 h before measurement) were performed as described in the Methods and materials section. All results are given as the means $\pm$ S.D. for at least five determinations.

Treatment of rats	O ⁶ -Methylguanine- removal activity (fmol/h per mg)	DNA (3-methyladenine)- glycosylase activity (fmol/h per mg)	DNA (7-methylguanine)- glycosylase activity (fmol/h per mg)
Control male	152 $\pm$ 16	94 $\pm$ 8	8.0 $\pm$ 1.3
Control female	192 $\pm$ 19	102 $\pm$ 11	8.8 $\pm$ 1.5
Dimethylnitrosamine-treated male	448 $\pm$ 46	85 $\pm$ 18	7.1 $\pm$ 1.3
Dimethylnitrosamine-treated female	382 $\pm$ 98	98 $\pm$ 10	8.5 $\pm$ 1.6
Partially hepatectomized male	916 $\pm$ 140	71 $\pm$ 14	7.8 $\pm$ 0.5
Sham-operated male	158 $\pm$ 28	76 $\pm$ 21	6.2 $\pm$ 1.3

elevated value, so that by 6 weeks the activity was highly significantly increased to about 3 times that of the controls. The result agrees well with previous results in which induction of O⁶-methylguanine removal activity was observed in male BD IV rats given oral doses of 2 mg of dimethylnitrosamine/kg, although in this case the enhancement was only 2.2-fold and insufficient animals were tested for the individual variability in response to be determined (Montesano *et al.*, 1980). In our present experiments activity of the O⁶-methylguanine removal system was also induced by dimethylnitrosamine treatment in female rats, although there was greater variability in the response and at least 3–4 weeks of exposure was required for a significant effect. However, as shown in Table 1, by 6 weeks of treatment activity was increased by 2-fold, and this difference is highly significant ($P < 0.01$).

The increased activity removing O⁶-methylguanine from DNA brought about by partial hepatectomy or chronic exposure to dimethylnitrosamine was not due to changes in the amount of inhibitory or stimulatory substances in the extracts. When extracts from control and treated animals were mixed the observed effects were as predicted from the sum of the activities observed when the two extracts were tested separately.

Other methylated purines produced by the action of carcinogenic methylating agents on DNA include 3-methyladenine and 7-methylguanine (Lawley, 1974). These products are lost from mammalian DNA partly by spontaneous depurination and partly by the action of specific glycosylases (Lawley, 1974; Margison & Pegg, 1981). The activity of these enzymes was measured in extracts from livers of rats after partial hepatectomy and chronic treatment with dimethylnitrosamine (Table 1). The activity of these enzymes was not increased by either treatment, showing that the enhancement of O⁶-methylguanine removal is a specific effect and not part of a general stimulation of DNA repair enzymes dealing with alkylated products. (It should be noted that the assay conditions used in the experiments of Table 1

were designed to yield results in which the activity measured was proportional to the amount of protein added, but do not indicate the maximal activity for any of the enzymes measured. In particular, the concentration of methylated DNA was not saturating in either of the DNA glycosylase assays.)

Discussion

These results provide direct evidence that the activity of an enzyme involved in the repair of a specific lesion in DNA damaged by a chemical carcinogen is induced by a stimulus producing enhanced cell replication. This may be a more widespread phenomenon, since a substantial increase in DNA (uracil)-glycosylase activity occurs in lectin-stimulated lymphocytes (Sirover, 1979) and during proliferation of human fibroblasts (Gupta & Sirover, 1980). There is also evidence that the nucleotide-excision repair activity of human lymphocytes exposed to chemical carcinogens is increased after stimulation with concanavalin A (Scudiero *et al.*, 1976). It should be noted that, contrary to previous reports based on comparisons of single XP-derived cell lines with controls (Goth-Goldstein, 1977; Bodell *et al.*, 1979; Altamirano-Dimas *et al.*, 1979), inability to remove O⁶-methylguanine from DNA did not correlate with xeroderma pigmentosum when more comprehensive studies were performed (Sklar & Strauss, 1981). Since this disease is associated with a defect in nucleotide-excision repair (Cleaver & Bootsma, 1975), it is clear that, as suggested by assays performed *in vitro* (Pegg, 1978a,b; Pegg & Balog, 1979) and by analogy with the bacterial system (Jeggo *et al.*, 1977; Karran *et al.*, 1979), O⁶-methylguanine removal is not mediated via the nucleotide-excision pathway. Increased activity of all DNA repair systems is not seen during proliferation, since DNA (3-methyladenine)-glycosylase and DNA (7-methylguanine)-glycosylase activities were not increased at 72 h after partial hepatectomy or by treatment with dimethylnitrosamine.

The present results provide experimental evidence for the possibility, suggested by Cleaver & Kaufman (1980), that the capacity of the liver to remove *O*⁶-alkylguanine may be enhanced during a period of cell proliferation. Such an increase is also in agreement with the results obtained by Rabes *et al.* (1979). They used infusion of hydroxyurea to synchronize hepatocytes in regenerating liver and found a higher *O*⁶-methylguanine/7-methylguanine ratio in DNA when dimethylnitrosamine was administered during S and G₂M phases compared with G₁ and G₀ phases. Our results differ from the finding that excision of *O*⁶-methylguanine and 7-methylguanine is decreased during S phase in mouse fibroblasts (Smith *et al.*, 1980), and suggest that the repair of alkylated DNA is controlled differently in liver cells.

It is of particular interest that proliferating cells increase their capacity to remove the potentially mis-coding lesion, *O*⁶-methylguanine, from DNA since there is strong correlative evidence to implicate this product in tumour initiation and its removal in protection against carcinogenesis (Lawley, 1974; Pegg, 1977; Margison & O'Connor, 1979). Craddock (1975*a,b*) has demonstrated convincingly that partial hepatectomy increases the carcinogenic potential of dimethylnitrosamine, but even with the 6-fold increase in excision capacity the vast increase in DNA synthesis occurring during liver regeneration greatly increases the chances of replication taking place at sites containing *O*⁶-methylguanine. Therefore the present findings are not contradictory to the hypothesis that the presence of *O*⁶-methylguanine during DNA replication can be an initiating event in carcinogenesis.

It appears quite probable that the apparent induction of *O*⁶-methylguanine-removal capacity in the liver by administration of acetamidofluorene is due to the cellular proliferation produced by the prolonged exposure to diets containing this compound (Buckley *et al.*, 1979). It is less clear whether the increased activity due to chronic treatment with dimethylnitrosamine could be produced in this manner. The maximal increase produced by such treatment occurred after only a few weeks' exposure and amounts to about half of that produced by partial hepatectomy. Detailed investigation of the kinetics and cellular distribution of the restorative hyperplasia and increased *O*⁶-methylguanine-removal activity will be needed to evaluate the mechanism of induction in this case. The comparable process for loss of *O*⁶-methylguanine from DNA of *E. coli* is clearly induced by exposure to alkylating agents (Samson & Cairns, 1977; Schendel & Robins, 1978; Jeggo *et al.*, 1977). The liver and bacterial systems resemble one another in a number of respects. Both processes are saturable, excising only a certain number of lesions after exposure to

high doses of alkylating agents (Schendel & Robins, 1978; Pegg, 1978*b*; Robins & Cairns, 1979), and appear to involve the transfer of the alkyl group from *O*⁶-alkylguanine (Pegg, 1978*a*; Pegg & Balog, 1979; Karran *et al.*, 1979; Pegg, 1980).

Note added in proof (received 8 April 1981)

After this work was completed we became aware that others had observed an increase in DNA (3-methyladenine)-glycosylase activity after partial hepatectomy in rats (Gombar, 1981). This group found that the activity rose to a peak between 18 and 24 h after operation and then declined to basal values by 48 h. This finding is therefore in agreement with our results that DNA (3-methyladenine)-glycosylase was not enhanced 72 h after partial hepatectomy.

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