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EFFECT OF STREPTOZOTOCIN ON THE GLUTATHIONE *S*-TRANSFERASES OF MOUSE LIVER CYTOSOL

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Abstract—Streptozotocin (STZ) increased the activity of mouse hepatic glutathione (GSH) *S*-transferases assayed with 1-chloro-2,4-dinitrobenzene. Nicotinamide administered prior to STZ prevented the hyperglycemia indicative of STZ-induced diabetes, but had no effect on the increase in GSH *S*-transferase activity caused by the drug. Another diabetogenic agent, alloxan, did not alter GSH *S*-transferase activity. Thus, streptozotocin may be increasing GSH *S*-transferase activity directly, and not as a result of the diabetic state the drug induces. Two transferases were characterized from mouse liver cytosol. One was a homodimer with a subunit molecular weight of about 28,000 and a pI of about 8.2. The other was also a homodimer with a subunit molecular weight of about 27,500 and a pI of about 9.2. The pI 8.2 GSH *S*-transferase was induced by STZ, while the pI 9.2 transferase was decreased by the drug. At least one other transferase appeared to be induced by STZ. Two other nitroso compounds, chlorozotocin and diethylnitrosamine, also increased GSH *S*-transferase activity, suggesting that this effect may be nitroso related.

Streptozotocin, a nitrosourea derived from *Streptomyces acromogenes*, causes diabetes in rats [1] and mice [2]. This effect may be due to the destruction of β-cells of the pancreas [3]. The drug also has antitumor activity [2].

The glutathione *S*-transferases are a group of enzymes that function in detoxication by conjugating GSH‡ with many xenobiotics. In addition, these enzymes may serve as binding and/or storage proteins [4, 5]. Streptozotocin has been reported to increase CDNB-conjugating GSH *S*-transferase activity in mouse hepatic cytosol [6]. The drug decreases enzyme activity in the rat liver; this effect is reversed by insulin [1], suggesting that the STZ-induced diabetic state is responsible for the decreased activity. Similarly, the STZ-induced increase in certain monooxygenase activities in the mouse liver may be related to decreased insulin levels [7].

In this report, we present evidence indicating that the STZ-mediated increase in GSH *S*-transferase activity in the mouse liver, as assessed with CDNB, was due to a direct action of the drug rather than a manifestation of its diabetogenic action. This effect was the result of the induction by STZ of at least two separate GSH *S*-transferases. In addition, a third transferase was decreased following STZ administration. Effects of other nitroso compounds on GSH *S*-transferase activity are also discussed.

MATERIALS AND METHODS

Materials

CD-1 [Crl:CD-1(ICR)BR] (Charles River Breeding Laboratories, Inc., Wilmington, MA) female mice maintained on Rat Chow 5012 (Ralston Purine Co., St. Louis, MO) and water, *ad lib.*, were used in all experiments. They ranged in ages from 9 to 16 weeks and weighed from 25 to 35 g.

Streptozotocin was provided by Dr. William E. Dulin of the Upjohn Co. (Kalamazoo, MI). Chlorozotocin (CTZ) was a gift from the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD). BCNU [1,3-bis(2-chloroethyl)-1-nitrosourea] and CCNU [1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea] were obtained from Bristol Laboratories (Syracuse, NY). Diethylnitrosamine (DEN), dimethylnitrosamine (DMN), alloxan, Glucose Assay Kit No. 510, glutathione, and nicotinamide were purchased from the Sigma Chemical Co. (St. Louis, MO). TES-tape (glucose enzymatic test strip) was obtained from Eli Lilly & Co. (Indianapolis, IN). 1-Chloro-2,4-dinitrobenzene (CDNB) was purchased from the Eastman Kodak Co. (Rochester, NY). All materials for polyacrylamide gel electrophoresis were obtained from Bio-Rad Laboratories (Richmond, CA). Electrophoresis and gel filtration standards, Sephadex G-75 and G-100, and CM-Sephadex C-50 were purchased from Pharmacia Fine Chemicals (Piscataway, NJ). Ampholine pH 3.5–10 ampholyte used in isoelectric focusing was obtained from LKB (Rockville, MD).

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‡ Abbreviations: BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; CDNB, 1-chloro-2,4-dinitrobenzene; CTZ, chlorozotocin; DEN, diethylnitrosamine; DMN; dimethylnitrosamine; GSH, glutathione; NICO, nicotinamide; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; and STZ, streptozotocin.

Methods

Drug preparations. Streptozotocin was injected i.p. within 5 min after dissolving in 0.05 M citrate buffer, pH 4.5. The dose was 200 mg/kg body weight in 0.2 ml buffer. All other chemicals were injected i.p. in the indicated solutions and doses. All controls were injected with the solutions in which the chemicals were dissolved.

Organ excision and cytosol preparation. Mice were killed by cervical dislocation, the livers were perfused with ice-cold 0.05 M Tris–HCl, pH 7.4, containing 0.25 M sucrose, and the livers were excised and placed in the same ice-cold buffer. The organs were blotted dry, weighed, and then homogenized in the ice-cold buffer with twelve strokes in a tight-fitting Potter–Elvehjem homogenizer. Cytosol was prepared by centrifuging aliquots of the homogenates at 10,000 *g* for 10 min at 4° in a Sorvall RC2-B centrifuge (DuPont Instruments-Sorvall, Newtown, CT). The supernatant fractions were centrifuged at 100,000 *g* for 1 hr at 4° in a Beckman L5-50 ultracentrifuge with a TY-40 rotor (Beckman Instruments, Inc., Fullerton, CA). Care was taken to leave the lipid layer behind when removing the supernatant fractions after the final centrifugation. The cytosols were assayed for GSH *S*-transferase activity no later than 48 hr after they were prepared. Freezing the cytosols at −20° for up to 9 weeks did not affect the activity of the enzyme. Freezing the homogenates at −20° for 24–48 hr prior to the 100,000 *g* centrifugation did not affect the activity of the cytosolic glutathione *S*-transferases.

Assays. Glutathione *S*-transferase activity was measured using the method of Habig *et al.* [8]. The total volume of the assay mixture was 3 ml, and the assay was performed at room temperature (20–23°). A unit of glutathione *S*-transferase activity is defined as the amount of enzyme catalyzing the formation of 1 μmole of GSH conjugate per min at room temperature.

Whole blood obtained from the pericardial cavity was assayed for glucose according to the protocol in the glucose assay kit (No. 510) from the Sigma Chemical Co. Urinary glucose levels were estimated with TES-tape.

Polyacrylamide gel electrophoresis (PAGE). Slab gel polyacrylamide electrophoresis with sodium dodecyl sulfate (SDS) was performed on 10% and 15% gels (16 cm wide, 12 cm long, 1.5 mm thick) with 4% stacking gels (2 cm high) as described by Maizel [9]. The standard marker proteins phosphorylase *b* (mol. wt. = 94,000), bovine serum albumin (mol. wt. = 67,000), ovalbumin (mol. wt. = 43,000), carbonic anhydrase (mol. wt. = 30,000), and soybean trypsin inhibitor (mol. wt. = 20,100) were used to estimate the molecular weights of the sample protein bands [10]. Cytosolic samples were diluted with a buffer containing 0.15 M Tris, 45% glycerol, 6 mM EDTA, 7.5% SDS, and 15% 2-mercaptoethanol. The samples were heated for 2 min in boiling water just prior to being applied to the gels. Protein (5–42 μg) in from 8 to 50 μl was put in the wells. Bromphenol blue was added to a few empty wells so that the course of the electrophoresis could be followed. The gels were run at 12 or 24 mA constant current until the marker dye reached the separator gel (about 2 hr); then the current was increased to 30 or 50 mA and maintained at that level until the dye migrated to the bottom of the gel (about 3.5 hr). The gels were then stained in Coomassie Brilliant Blue R for 2 hr at 37°, destained in 30% methanol (37°, 8–12 hr overnight and then for 2–3 hr with several changes of methanol), and some scanned (white light source) on a Kontes Fiber Optic Scanner (model 800) connected to a Hewlett–Packard 3390A Integrator.

Gel filtration. Sephadex G-75 (1.5 × 81 cm) and G-100 (1.5 × 70 cm) columns were used to partially purify cytosol and the first peak eluted from CM-Sephadex C-50 columns to which cytosol from control and STZ-treated mice was applied. The columns were equilibrated and eluted with 0.01 M phosphate buffer, pH 7.2, containing 0.1 M NaCl. The void volume (V_o) of the G-75 column was determined with Blue Dextran 2000, and the following standard protein markers were used to calibrate the column: ribonuclease A (mol. wt. =13,700), chymotrypsinogen A (mol. wt. = 25,000), ovalbumin (mol. wt. = 43,000), and albumin (mol. wt. = 67,000). The molecular weight of the proteins eluted in the GSH *S*-transferase activity peak from the G-75 column was estimated by noting the elution volume in relation to those plotted for the standard proteins. Aliquots of 2 ml were collected from both the Sephadex G-75 and G-100 columns at a flow rate of 30 ml/hr, and the fractions with GSH *S*-transferase activity were pooled and concentrated by ultrafiltration. Aliquots of this material from both columns were applied to SDS-polyacrylamide gels.

Ion-exchange chromatography. Mouse liver cytosol was dialyzed against 1 liter of 10 mM sodium phosphate buffer, pH 7.4, at 4° for 22 hr, with a change of buffer after 12 hr. Ten milliliters of cytosol containing 210 mg of protein was applied to a CM-Sephadex C-50 column (2 × 15 cm) in the cold (4°) [11]. The flow rate was 16 ml/hr, and 2 ml aliquots were collected. After 50 ml of the phosphate buffer eluted, the GSH *S*-transferases retained by the ion exchanger were eluted with a 10–80 mM NaCl gradient (300 ml of 10 mM sodium phosphate buffer, pH 7.4, in one chamber, and 300 ml of 100 mM NaCl in the phosphate buffer in the other chamber of a gradient mixer).

Isoelectric focusing. Isoelectric focusing in polyacrylamide tube gels under nondenaturing conditions was performed according to the method of Wrigley [12]. The gels were cut into 5 mm slices and soaked overnight at 4° in 0.25 to 0.5 ml of potassium phosphate buffer, pH 6.5. The slices were then assayed for GSH *S*-transferase activity.

Protein determination. Proteins were determined using the method of Lowry *et al.* [13], with bovine serum albumin as a standard.

Statistical analysis. All statistical methods are from Snedecor and Cochran [14]. Student's *t*-test was used to determine if there was a significant difference between two means. One-way analysis of variance tested for significance among several treatment groups. If significance was indicated, the Newman–Keuls test was employed to determine which pairs of means were significantly different.

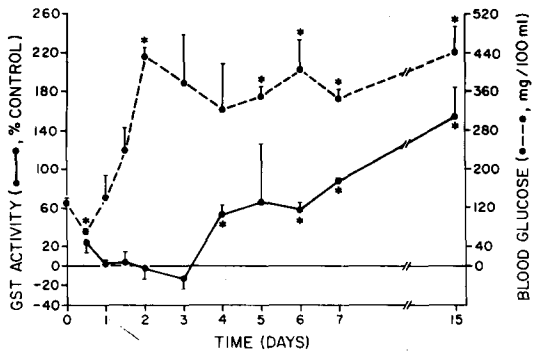

Fig. 1. Glutathione S-transferase (GST) activity of liver cytosol assayed with CDNB (percent above and below control) and blood glucose levels following a single i.p. injection of 200 mg STZ/kg. Each point is the mean ± 1 S.E.M. of determinations from three to four mice (except day 5, where N = 2). The entire time course is a composite of four separate experiments. Control values for both GSH S-transferase activity (1.56 ± 0.11 units/mg protein) and blood glucose (167 ± 8 mg/100 ml) were determined at various points during an experiment and did not differ significantly within groups of mice. Asterisks indicate significantly different from control, $P < 0.05$.

RESULTS

Glutathione S-transferase activity assayed with CDNB was increased 4 days after an injection of STZ (Fig. 1). The activity was about 50% above control on day 4 and rose to about 150% above control on day 15. The blood glucose level reached its maximum value after only 2 days, following an initial decrease to about half the control level 12 hr post-injection.

Since nicotinamide injected just prior to STZ antagonizes the diabetogenic action of streptozotocin [2], mice were injected with nicotinamide before STZ to determine the effect on GSH S-transferase activity. The data in Table 1 demonstrate that, while nicotinamide pretreatment prevented the STZ-mediated increase in blood glucose levels, it did not influence the STZ-induced increase in GSH S-transferase activity. Nicotinamide alone had no effect on either blood glucose or GSH S-transferase activity levels. Alloxan-induced hyperglycemic mice did not exhibit increases in GSH S-transferase activity (Table 2).

Table 1. Effect of nicotinamide pretreatment on STZ-induced increases in GSH S-transferase activity and blood glucose levels*

Treatment	N	Blood glucose (mg/100 ml)	GSH S-transferase activity (units/mg protein)
STZ	2	365.4 ± 14.4†	3.04 ± 0.51†
NICO + STZ‡	5	178.0 ± 11.4	2.89 ± 0.17†
NICO	4	147.7 ± 12.6	1.49 ± 0.21

* Cytosol was prepared from mice 9 days after injection (200 mg STZ/kg, i.p.). Blood glucose and GSH S-transferase activity of cytosol from untreated mice were 153.7 ± 7.3 mg/100 ml and 1.38 ± 0.08 units/mg protein respectively (N = 4). Values are mean ± 1 S.E.M.

† Significantly different from control, $P < 0.05$.

‡ Nicotinamide (500 mg/kg in 0.3 ml sterile distilled water) was injected i.p. 10 min before STZ.

Table 2. GSH S-transferase activity of hepatic cytosol from control and alloxan-treated mice

	GSH S-transferase activity* (units/mg protein)	Urinary glucose† (%)
Control	2.17 ± 0.22	~0
Alloxan‡	2.12 ± 0.18	~0.5

* Values are the mean ± 1 S.E.M. of determinations from three different cytosols.

† Values were estimated with glucose enzymatic test strip (TES-tape); 0.5% represents about 500 mg glucose/100 ml urine.

‡ Alloxan 140 mg/kg in 0.2 ml isotonic saline was injected i.p. Cytosol was prepared 12 days later.

Streptozotocin was added to liver cytosol from a control animal to see if the drug had any direct effect on GSH S-transferase activity. Table 3 shows that there was no difference between the GSH S-transferase activities of cytosol incubated with STZ and control at either pH 4.5 or 6.5. The difference in activities after incubation at these two pH levels may be due to denaturation of the GSH S-transferases at the lower pH.

The calculated apparent K_m from two studies in which [CDNB] was varied is 0.247 ± 0.040 for the control and 0.268 ± 0.004 for the enzyme from STZ-treated mice. These two values are not significantly different. The apparent V_{max} is 0.940 ± 0.040 for the control and 2.04 ± 0.030 for the treatment group. This 2-fold increase is significant (95% confidence limit). The same relationship between the K_m and V_{max} values of control and treatment enzyme was observed when the GSH concentration was varied (K_m = 0.140 for the control and 0.182 for the STZ-treated, V_{max} = 1.52 for the control and 4.72 for the treated).

Aliquots of liver cytosol from control, STZ-treated, and nicotinamide-pretreated mice were applied to an SDS-polyacrylamide gel (Fig. 2). In addition, fractions from the GSH S-transferase activity peak which eluted from a Sephadex G-75 column onto which cytosol from a control mouse was applied were pooled and concentrated. Proteins from

Table 3. Effect of STZ added *in vitro* on GSH S-transferase activity

	GSH S-transferase activity† (units/mg protein)	
pH of Incubation buffer*	Control	STZ
4.5	1.00 ± 0	1.00 ± 0.11
6.5	1.78 ± 0.11	1.78 ± 0.22

* Aliquots of a 300-fold dilution of mouse liver cytosol (2.0 units/mg protein) were incubated with 1 mg STZ/ml on ice at the indicated pH for 3 hr before assaying for GSH S-transferase activity at pH 6.5. Controls were cytosol without STZ.

† Values are the mean ± 1 S.E.M. of duplicate determinations from the same cytosol.

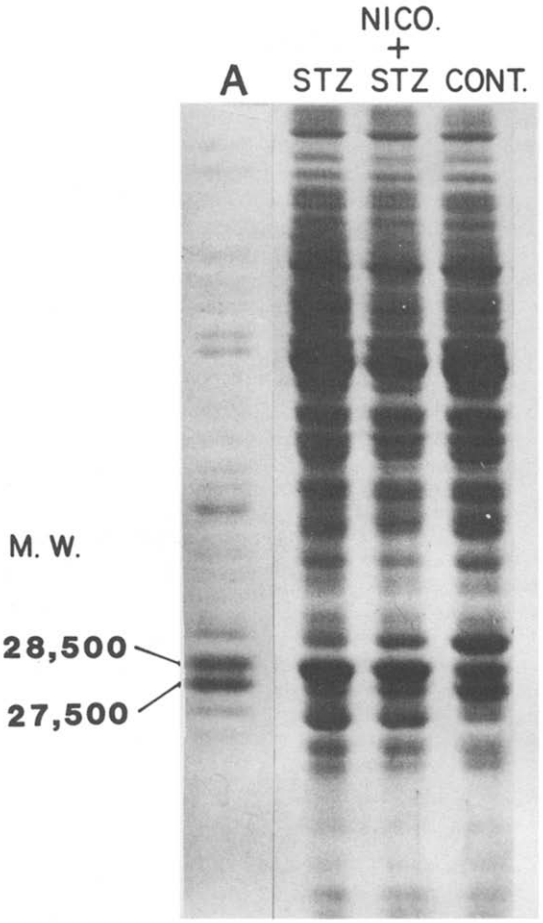

Fig. 2. A representative SDS-PAGE (10% acrylamide) of liver cytosols from mice treated as follows: STZ, streptozotocin-treated (200 mg/kg); NICO. (nicotinamide, 500 mg/kg, injected i.p. 10 min before STZ) + STZ; and CONT., control. Each of these lanes contains about 42 μg of protein. Lane A is an aliquot from the center of the GSH *S*-transferase activity peak eluted from a Sephadex G-75 column onto which cytosol from a control mouse was applied (see Materials and Methods).

the center of this peak had molecular weights of about 58,000. An aliquot of this material was applied to the SDS gel (lane A). Intensely staining bands appeared at molecular weights of about 28,500 and 27,500. The 28,500 molecular weight band stained more intensely in the STZ and nicotinamide pretreatment lanes when compared to the control. The 27,500 molecular weight band was lighter in these two lanes when compared to the control. These band differences were reproduced in two other experiments. Changes in bands other than those discussed above were also observed.

Figure 3 shows the elution pattern of liver cytosol from control mice applied to a CM-Sephadex C-50 cation-exchange column. Three peaks of GSH *S*-transferase activity can be distinguished. The first peak eluted in the initial 30 ml of 10 mM sodium phosphate buffer, pH 7.4. Two peaks of GSH *S*-transferase activity retained by the column were eluted by a 10–80 mM NaCl gradient. The first of these peaks came off the column between 160 and 260 ml, or between 22 and 39 mM NaCl. A smaller peak eluted between 270 and 310 ml, or between 42 and 50 mM NaCl.

Figure 4 is the elution pattern of liver cytosol from STZ-treated mice applied to a CM-Sephadex C-50 column identical to the one used in Fig. 3. Three peaks of GSH *S*-transferase activity eluted from the column in approximately the same volumes and NaCl ranges as those in Fig. 3. However, the first two peaks in Fig. 4 are larger, indicating more total enzyme activity, than the corresponding peaks in Fig. 3. The third peak in Fig. 4 is smaller than its corresponding peak in Fig. 3. These results were reproduced in two other experiments. The same amount of cytosolic protein (210 mg) was applied to each of the columns. The specific GSH *S*-transferase activity of the control cytosol (Fig. 3) was 1.49 units/mg protein, while that of the STZ cytosol (Fig. 4) was 2.02 units/mg protein.

Table 4 is a breakdown of the total units of GSH *S*-transferase activity within each of the peaks shown in Figs. 3 and 4. Peaks 1 and 2 (numbers designate order of elution from columns) from both the control and STZ column each represent about the same percent of the total enzyme activity applied to the columns. However, peak 3 from the STZ column is only 1.4% of that total, while the third peak from the control column is 4.8% of the total.

Aliquots from the peaks shown in Figs. 3 and 4 were applied to an SDS-polyacrylamide gel (Fig. 5). Intensely staining bands with molecular weights of about 28,000 for C2 and S2 (second peaks eluted from control and STZ columns respectively) and 27,500 for C3 and S3 (third peaks to elute) constituted most of the protein in these four lanes. The specific activities of each of these partially purified transferases (assayed with CDNB; units/mg protein) were 7.12 for C2, 7.53 for S2, 1.07 for C3, and 0.95 for S3.

Lanes C1 and S1 each have multiple bands. Aliquots from the first peak of Figs. 3 and 4 were applied to a Sephadex G-100 column to partially purify the transferase(s) present there. A single peak of GSH *S*-transferase activity eluted in each case. Lanes C1a and S1a in Fig. 5, which represent material from the respective GSH *S*-transferase activity peaks of the G-100 column, still have multiple bands, but two intensely staining bands appear at about mol. wt. 28,000 and 26,000.

Aliquots from peaks 1 and 2 of the STZ ion-exchange column (Fig. 4) and peak 3 of the control ion-exchange column (Fig. 3), as well as cytosol from STZ-treated mice, were subjected to isoelectric focusing on polyacrylamide tube gels under non-denaturing conditions (Fig. 6). Three peaks of GSH *S*-transferase activity can be distinguished when the same liver cytosol from STZ-treated mice that was applied to the ion-exchange column was subjected to isoelectric focusing (Fig. 6A). The zeniths of the three peaks represent isoelectric points of about 6.1, 8.2, and 9.2, with the 8.2 peak having most of the enzyme activity. The same pattern of three peaks was observed when cytosol from a control mouse was focused (not shown). Two peaks of GSH *S*-transferase activity were detected from peak 1 of

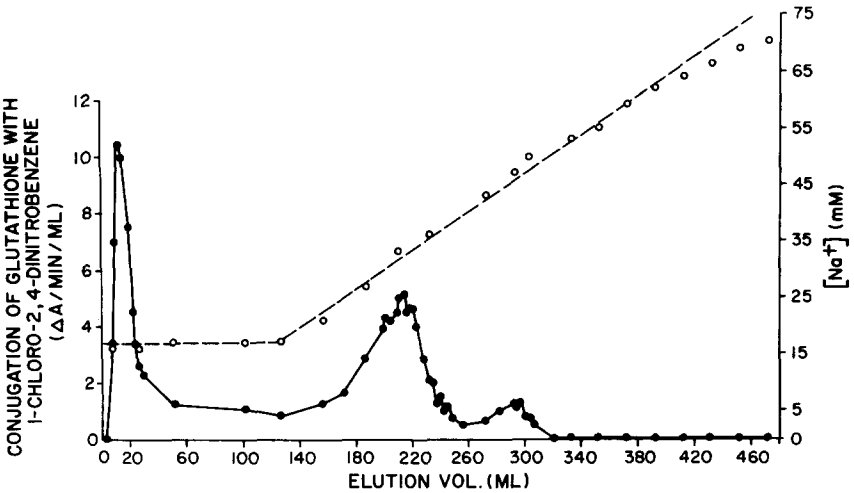

Fig. 3. Elution pattern from a CM-Sephadex C-50 cation-exchange column to which cytosol from control mice was applied. Key: (●——●) GSH *S*-transferase activity, and (○— —○) concentration of sodium in eluting buffer (10 mM sodium phosphate, pH 7.4). Two hundred and ten milligrams (10 ml) of dialyzed cytosol (1.49 units/mg protein) was applied to the column. Sixty-one percent of the enzyme activity applied to the column was recovered. The flow rate was about 16 ml/hr, and 2-ml fractions were collected.

the STZ ion-exchange column (Fig. 6B): one peak having a pI of about 8.2 and the other, larger, a pI of about 6.1. The focused aliquots from peak 2 of the STZ column and peak 3 of the control column (Fig. 6, panels C and D respectively) each gave one peak, with pI values of about 8.2 and 9.2 respectively. When gel slices with the greatest enzyme activity similar to those constituting the peaks in panels C and D of Fig. 6 were soaked in phosphate buffer (pH 6.5) overnight at 4° and aliquots were applied to an SDS-polyacrylamide gel, a single band with a molecular weight of about 28,000 appeared from the gel slice representing the center of the pI 8.2 peak (Fig. 6C). The gel slice from the center of the pI 9.2 peak (Fig. 6D) gave a single band with a molecular weight of about 27,500 (data not shown).

Other nitroso compounds were injected into mice to determine their effects on the GSH *S*-transferase activity of mouse liver cytosol (Table 5). Only CTZ and DEN produced significant increases in enzyme

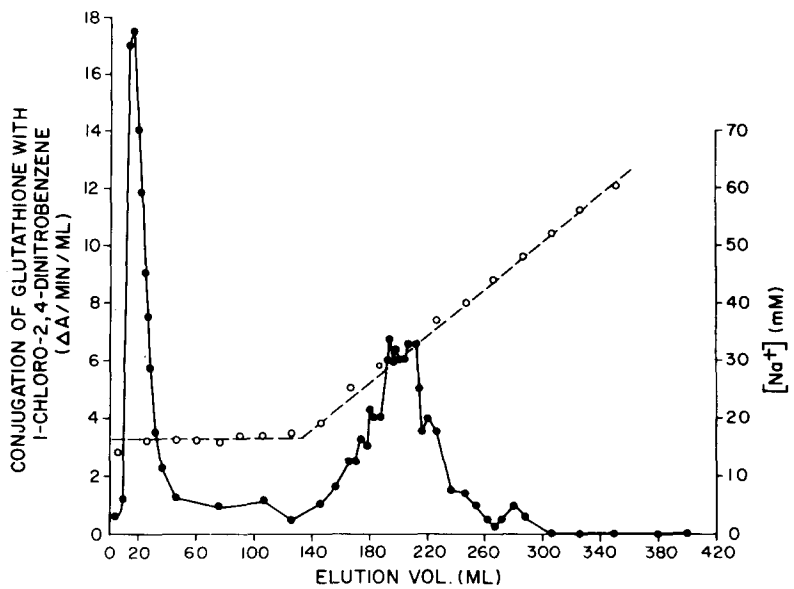

Fig. 4. Elution pattern from a CM-Sephadex C-50 cation-exchange column to which cytosol from STZ-treated mice was applied. Key: (●——●) GSH *S*-transferase activity, and (○— —○) concentration of sodium in eluting buffer (10 mM sodium phosphate, pH 7.4). Two hundred and ten milligrams (10 ml) of dialyzed cytosol (2.02 units/mg protein) was applied to the column. Fifty-seven percent of the enzyme activity applied to the column was recovered. The flow rate was about 16 ml/hr, and 2-ml fractions were collected.

Table 4. Comparison of peaks of GSH *S*-transferase activity from control and STZ cytosols applied to ion-exchange columns

Peak*	GSH *S*-transferase activity† (units)	
	Control	STZ
1	60	94
2	98	133
3	15	6

* Number of peak refers to order of elution from column. Peaks 1 included from 0 to 40 ml and from 0 to 50 ml, peaks 2 from 155 to 250 ml and from 145 to 260 ml, and peaks 3 from 270 to 322 ml and from 268 to 306 ml for the control and STZ columns respectively.

† Two hundred and ten milligrams (10 ml) of each cytosol, containing 312 (control) and 424 units (STZ) of GSH *S*-transferase activity, was applied to CM-Sephadex C-50 columns. The specific activities of the control and STZ cytosols were 1.49 and 2.02 units/mg protein, respectively. Sixty-one and 57% of the initial enzyme activity from the control and STZ cytosols, respectively, were recovered from the columns. These recoveries include the activity from the fractions between peaks 1 and 2 and peaks 2 and 3 from each of the ion-exchange columns.

activity. While CTZ gave a modest increase, DEN was as effective as STZ in elevating GSH *S*-transferase activity.

DISCUSSION

This work confirms the finding of Rouer *et al.* [6] that streptozotocin causes an increase in mouse liver cytosolic glutathione *S*-transferase activity as assessed with 1-chloro-2,4-dinitrobenzene. Attempts to correct the insulin deficiency characteristic of STZ-induced diabetes by either insulin injections or the implantation of insulin-containing miniosmotic pumps were unsuccessful. In an alternative approach, nicotinamide was administered prior to STZ to protect against the hyperglycemia indicative of the STZ-induced diabetic state [2]. Table 1 shows that mice pretreated with nicotinamide did not show the hyperglycemia indicative of the insulin-deficient STZ-treated mice, although their liver cytosolic GSH *S*-transferase activity was elevated. In rats, nicotinamide pretreatment prevents STZ-induced increases in tyrosine aminotransferase and aryl hydrocarbon hydroxylase activity [15, 16]. In contrast to STZ, alloxan, another diabetogenic agent, had no effect on GSH *S*-transferase activity (Table 2). Neither genetically hypoinsulinemic (db/db) nor hyperglycemic (ob/ob) diabetic mice show elevated GSH *S*-transferase activities compared to their respective controls [6]. Thus, insulin deficiency is unlikely to be responsible for the increase in GSH *S*-transferase activity observed after STZ treatment. Rather, STZ may be acting directly on the liver to increase GSH *S*-transferase activity there.

While the long period of time after STZ injection for the increase in CDNB-conjugating GSH *S*-transferase activity to appear (Fig. 1) suggested that the development of the diabetic state might be responsible for this increase, the finding that increased activity occurred even in the absence of apparent diabetes suggests that this effect is related to other actions of STZ. The antitumor activity of streptozotocin, as well as its cytotoxicity, is due to its methylnitrosourea moiety and is independent of its diabetogenic action [17]. Chlorozotocin, BCNU, CCNU, and DEN all caused an increase in GSH *S*-transferase activity (Table 5). The fact that the only nitrosourea to produce a statistically significant increase was CTZ is probably due to the larger treatment groups used. The nitrosamine DEN produced an increase in transferase activity comparable

Table 5. Effects of BCNU, CCNU, CTZ, DEN, and DMN administration on mouse liver cytosolic GSH *S*-transferase activity

Compound	GSH *S*-transferase activity* (units/mg protein)		% Above control
	Control	Treated	
BCNU†	1.16 ± 0.12 (5)	1.55 ± 0.09 (10)	34
CCNU‡	1.16 ± 0.12 (5)	1.42 ± 0.15 (7)	22
CTZ§	1.85 ± 0.10 (18)	2.35 ± 0.14‖ (18)	27
DEN¶	0.56 ± 0.04 (10)	1.50 ± 0.10** (10)	168
DMN††	1.48 ± 0.07 (8)	1.54 ± 0.17 (5)	4

* Values are the mean ± 1 S.E.M. (N = the number in parentheses).

† At 25 mg/kg in 0.2 ml of 10% ethanol in distilled water. Cytosol was prepared 13 days later.

‡ At 30 mg/kg in 0.2 ml of 5% corn oil, 5% ethanol in distilled water. Cytosol was prepared 13 days later.

§ At 20 mg/kg in 0.2 ml citrate buffer, pH 4.5. Cytosol was prepared 15 days later.

‖ Significantly different from control, $P < 0.01$.

¶ At 150 mg/kg in 0.2 ml phosphate-buffered saline. Cytosol was prepared 14 days later.

** Significantly different from control, $P < 0.05$.

†† At 20 mg/kg in 0.2 ml phosphate-buffered saline. Cytosol was prepared eight days later.

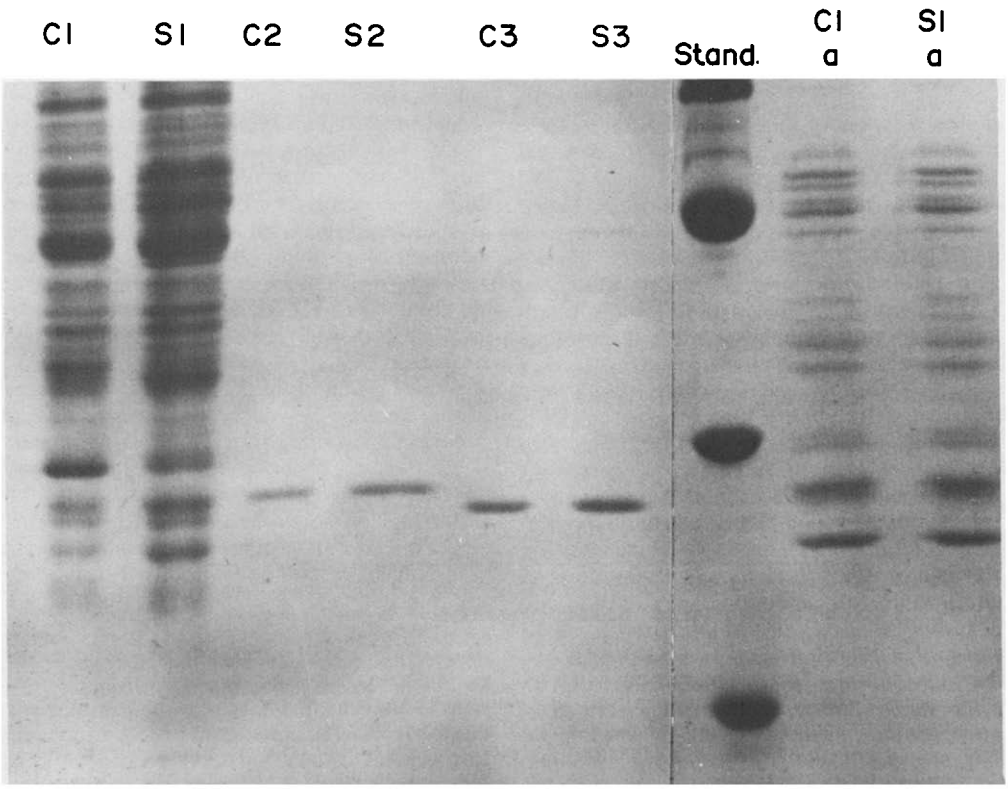

Fig. 5. SDS-PAGE (15% acrylamide) of samples from the peaks in Figs. 3 and 4. C, control peaks (Fig. 3). S, STZ peaks (Fig. 4). Numbers after letters refer to order of elution from ion-exchange columns. Aliquots were taken from the middle of each peak. C1 and S1 (aliquots from the 16 and 18 ml fractions respectively), 8 μl each containing about 30 and 38 μg of protein respectively. C2 and S2 (aliquots from the 214 and 194 ml fractions respectively), 50 μl each containing about 5 and 7 μg of protein respectively. C3 and S3 (aliquots from 295 and 280 ml fractions respectively), 50 μl each containing about 9 and 8 μg of protein respectively. C1a and S1a, aliquots from peaks C1 and S1 (18 and 20 ml fractions respectively), were each applied to a Sephadex G-100 column, and fractions from the GSH S-transferase activity peak eluted in each case were concentrated by ultrafiltration and applied to the gel. C1a and S1a each contain about 15 μg of protein (50 μl of each applied). Stand., standard marker proteins: bovine serum albumin (mol. wt. = 67,000), ovalbumin (mol. wt. = 43,000), carbonic anhydrase (mol. wt. = 30,000), and soybean trypsin inhibitor (mol. wt. = 20,100). The GSH S-transferase activities (units/ml) of the material applied to the gel were; C1, 3.28; S1, 5.47; C2, 1.61; S2, 2.11; C3, 0.39; S3, 0.31; C1a, 0.69; S1a, 0.78.

to STZ (Table 5). Another nitrosamine, DMN, had little effect on enzyme activity (Table 5), but was significantly more toxic than any of the other nitroso compounds tested (C. Agius, unpublished observation). Since these agents are all potent alkylators [18], the increase in GSH S-transferase activity they cause may be nitroso related, and possibly the result of alkylation of DNA. General toxicity is probably not responsible for this increase since the highly toxic DMN was not an effective inducer. However, DMN is also a potent alkylating agent and carcinogen [19], so if alkylation is causing an increase in GSH S-transferase activity, a specific alkylating event(s) may be involved.

The long time course for the STZ-mediated increase in GSH S-transferase activity (Fig. 1) and the fact that STZ had no effect on GSH S-transferase activity *in vitro* (Table 3) suggest that the drug is not exerting its influence by a direct action on the enzymes (i.e. allosteric activation). The doubling of the V_{max} is, therefore, probably a reflection of an increase in the amount of GSH S-transferases.

Although Rouer *et al.* [6] observed an increased affinity (lower apparent K_m) of the substrates GSH and CDNB for the GSH S-transferases from STZ-injected mice over those of the control, we observed no significant difference in apparent K_m values for either substrate between control and STZ-treated mice. The reason for this discrepancy, other than the use of different strains of mice is unknown.

A decrease in GSH levels in livers of STZ-injected rats [1] suggests a role for the GSH S-transferases in the *in vivo* metabolism of this drug. However, this decrease is small compared to the high concentration of GSH found in hepatic cytosol [20] and, if it occurs in the mouse, is unlikely to be responsible for the increased GSH S-transferase activity induced by STZ.

The method of Hayes *et al.* [11] separated what appear to be three distinct transferases (Figs. 3 and 4). The isoelectric focusing pattern of cytosol from an STZ-treated mouse (Fig. 6A) also suggests the presence of at least three transferases. The electrophoretic patterns in Fig. 5 and the specific activi-

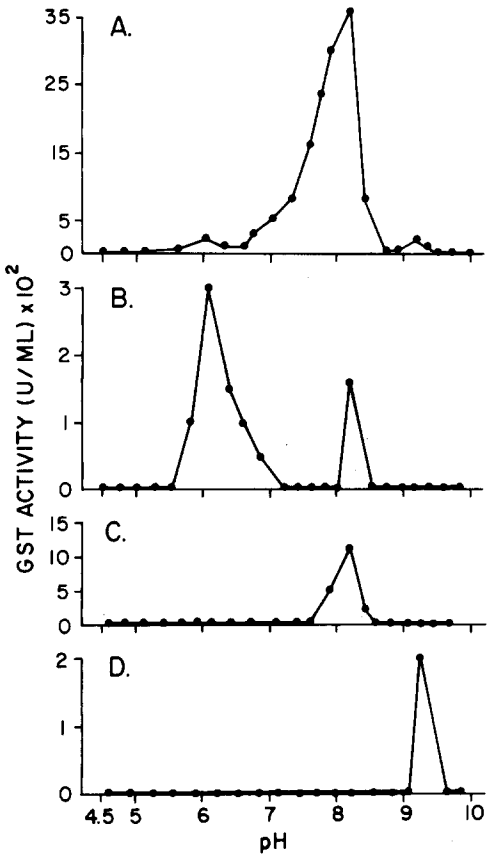

Fig. 6. Plot of pH vs GSH *S*-transferase activity of samples from the peaks in Figs. 3 and 4 which were subjected to isoelectric focusing in polyacrylamide tube gels under nondenaturing conditions. (A) Dialyzed cytosol from STZ-treated mice; 2.2 units of GSH *S*-transferase activity applied to gel, 51% recovered. (B) Peak 1 in Fig. 4; 0.51 units applied to gel, 9% recovered. (C) Peak 2 in Fig. 4; 0.53 units applied to gel, 17% recovered. (D) Peak 3 in Fig. 3; 0.053 units applied to gel, 19% recovered. Peak numbers refer to order of elution from columns. Note that the GSH *S*-transferase (GST) activity scales are different and that C represents more enzyme activity than B or D.

ties with CDNB suggest that C2 and S2 are the same transferase, and distinct from C3 and S3, which appear to represent a single, different transferase. Isoelectric focusing (Fig. 6, C and D) provided evidence that peaks 2 and 3 may have only one transferase associated with each, since only one peak of GSH *S*-transferase activity appeared for each. The native molecular weight of proteins eluting in the GSH *S*-transferase activity peak from the G-75 column is about 58,000. Thus, the two transferases partially purified in this study may each be homodimers. One isozyme had a subunit molecular weight of about 28,000, a pI of about 8.2, and was induced by STZ. The other had a subunit molecular weight of about 27,500, a pI of about 9.2, and was decreased by STZ. The changes observed in the electrophoretic patterns of cytosol from control, STZ-treated, and nicotinamide-pretreated mice in Fig. 2 (28,500 mol. wt. band darker and 27,500 mol. wt. band lighter in STZ and NICO + STZ lanes compared to control) are consistent with the induction of the pI 8.2 transferase and the reduction of the pI 9.2 isozyme by STZ. The fact that STZ had a differential effect on these two transferases suggests that they may be under independent regulation.

These two transferases may be the same as those purified by Pearson *et al.* [21], each homodimers with subunit molecular weights of 25,000, one having a pI of 8.7 and the other a pI of 9.3. The discrepancy between the subunit molecular weights and isoelectric points reported here and those of Pearson *et al.* may be due to technical factors.

At least one other GSH *S*-transferase appears to be induced by STZ (see the first peak in Figs. 3 and 4). Panels A and B of Fig. 6 show a transferase with a pI of about 6.1. More than one transferase may be present in the first peak in Fig. 4 (see Fig. 6B). Benson *et al.* [22] have reported the purification of six GSH *S*-transferases from female CD-1 mice fed 2(3)-*tert*-butyl-4-hydroxyanisole (BHA). Further work is necessary to determine how many GSH *S*-transferases are present in the first peak and which are affected by STZ.

To summarize, STZ increased GSH *S*-transferase activity in mouse liver cytosol by a mechanism independent of its diabetogenic action. The drug increased the concentration of at least one isozyme, while decreasing the amount of another. Other nitroso compounds also increased GSH *S*-transferase activity.

There is evidence to suggest that the nitrosoureas may be metabolized by the GSH *S*-transferases [23, 24]. Since these drugs are used clinically, a better understanding of the role of the GSH *S*-transferases in the metabolism of these drugs, and how these agents are affecting this enzyme system, is warranted.

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