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Title: THE EFFECT OF CARMUSTINE (BCNU) ON HEPATIC UDP-GLUCURONYLTRANSFERASE ACTIVITY IN THE RAT

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Abstract approved: _____
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Carmustine (BCNU) is an important anti-tumor agent that is currently enjoying widespread clinical use for the treatment of a wide spectrum of human malignancies. Although deliterous effects of BCNU have been reported on a variety of enzymes, effects on glucuronidation have never been studied. Glucuronidation, an important reaction of phase II drug metabolism, is responsible for the metabolism of many therapeutic agents. Also, the thyroid hormones, sex hormones, and bilirubin, among others, serve as endogenous substrates for UDP-glucuronyltransferase. This study was conducted in order to evaluate BCNU induced changes in glucuronidation, and also to investigate changes that may occur in a membrane bound enzyme system, in order to better understand the biochemical effects of BCNU.

Rats were injected i.p. with a single dose of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) at a dose of 20 mg/kg. Subsequent monitoring of water and food consumption

and body weight over a 22-day period indicated a sharp initial drop in all of these measurements. Between days 5 and 22, food consumption was at control level, but body weight gain remained depressed by 30% as compared to controls. This indicated that food utilization had decreased after BCNU treatment. Determination of hepatic microsomal protein content (mg protein/gram of wet liver) revealed no changes due to BCNU treatment.

UDP-glucuronyltransferase activity, in native microsomes, was found to be significantly elevated by BCNU treatment. The effect was observed with either bilirubin or p-nitrophenol as substrate, occurring by day 3 and lasting as late as 28 days post-treatment. After Triton X-100 activation of the microsomes there was no longer any difference observed in the rate of glucuronidation between control and BCNU-treated animals with either substrate. Kinetic analysis of bilirubin glucuronidation was performed indicating, in native microsomes, an increase in both apparent K_m (90%) and V_{max} (60%) in microsomes from BCNU-treated animals as compared to controls. In activated microsomes, no difference was observed in the rate of glucuronidation between control and treated groups, but normal Michaelis-Menten kinetics were not followed in either control or BCNU-treated groups.

Induction of bilirubin glucuronidation by phenobarbital or p-nitrophenol glucuronidation by 3-methylcholanthrene indicated that BCNU-treated animals were induced

more readily and to a greater degree than control animals.

It may be concluded that single dose administration of BCNU causes a mild and prolonged activation of hepatic UDP-glucuronyltransferase in the rat.

The Effect of Carmustine (BCNU) on Hepatic UDP-glucuronyltransferase Activity in the Rat

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THE EFFECT OF CARMUSTINE (BCNU) ON HEPATIC UDP-GLUCURONYLTRANSFERASE ACTIVITY IN THE RAT

Carmustine (BCNU) is an important anti-tumor agent that is currently enjoying widespread clinical use for the treatment of a wide spectrum of human malignancies. The Nitrosoureas, as a group of compounds, are very unstable under physiological conditions and degrade to give several reactive intermediates (1, 2). The organic isocyanate and carbonium ion are felt to be the most biologically important intermediates and react primarily with proteins (3) and nucleic acids (4), respectively. The reaction with proteins, carbamylation, represents a direct effect of these agents upon enzyme systems and has been demonstrated to occur with a variety of enzymes. Significant effects in vitro and in vivo have been reported on glutathione reductase (5), ribonuclease A (6, 7), cytochrome C (6, 7), and DNA polymerase II (8) among others. The main reaction of the nitrosoureas with nucleic acids, alkylation, has been well documented in the literature. This action of the nitrosoureas could be expected to have a variety of effects upon cellular enzyme systems.

The effects of the nitrosoureas upon enzyme systems have mostly been studied in vitro with isolated non-membrane bound enzymes. The effects of in vivo administration of one of these, 1,3-bis(2-chloroethyl)-1-nitrosourea* upon the membrane bound microsomal enzyme system, cytochrome P_{450},

has been investigated by Lu and Larson (9). They demonstrated a delayed reduction in content and activity of this enzyme system. This effect was not seen until seven days after treatment and reached its maximum at day 21 post-treatment. These studies indicate a significant decrease in phase I drug metabolism subsequent to BCNU treatment *in vivo*.

The purpose of this study was to investigate the effects of BCNU upon UDP-glucuronyltransferase (EC 2.4.1.17), the most important microsomal enzyme system of phase II drug metabolism (10, 11). It has been demonstrated, by purification, that at least two forms of UDP-glucuronyltransferase exist in rat hepatocytes (12). Substrates for the two forms of UDP-glucuronyltransferase have been designated as group I and group II (12). Their metabolism is differentially induced by 3-methylcholanthrene and phenobarbital, respectively (13). p-nitrophenol (group I) and bilirubin (group II) were chosen as substrates and their metabolism was followed over a four-week time course after BCNU treatment in rats. Also, 3-methylcholanthrene and phenobarbital induction was investigated at the time when the effect of BCNU was maximal.

* Abbreviations used: UDP-GT: UDP-glucuronyltransferase; BCNU: 1,3-bis(2-chloroethyl)-1-nitrosourea; UDPGA: UDP-glucuronic acid; Pb: Phenobarbital; MC: 3-methylcholanthrene.

MATERIALS AND METHODS

Chemicals. Bilirubin and UDP-glucuronic acid were purchased from Sigma Chemical Company, St. Louis, Mo. p-nitrophenol was purchased from Eastman Organic Chemicals, Rochester, N.Y. p-nitrophenol-β-D-glucuronide was purchased from Boehringer Mannheim, West Germany. BCNU was obtained from the National Institutes of Health, Bethesda, Md. Triton X-100 was obtained from the J.T. Baker Company, Phillipsberg, N.J.

Treatment of animals. Male Sprague-Dawley rats, reared in the Oregon State University School of Pharmacy colony, were used in all experiments. At the initiation of all experiments, the animals weighed from 240 to 250 grams and were from seven to nine weeks of age.

A single dose of BCNU (20 mg/kg), dissolved in corn oil, was administered i.p. The same dose was used in all experiments based upon earlier dose response studies which revealed this to be an effective, but non-lethal dose, over the time course of these studies. The controls were injected with an equal volume of vehicle alone. The UDP-GT activity was assessed at 3, 7, 13, 21, and 28 days after BCNU treatment as indicated in the text.

Experiments were conducted in which daily measurements of food and water consumption were recorded over a 22-day time course after BCNU treatment. Animals were caged individually, after BCNU treatment, in metabolic cages.

In the induction studies, control and BCNU-treated animals received daily i.p. doses of phenobarbital (80 mg/kg) for four days and were sacrificed on the fifth day. In the BCNU-treated animals, induction

was begun eight days after administration of BCNU. For MC induction, a dose of 40 mg/kg, dissolved in corn oil, was given once i.p. nine days after BCNU treatment. The animals were killed four days after MC treatment. For both inducing agents, UDP-GT activity was measured 13 days after BCNU treatment.

Preparation of microsomal fractions. At the time of sacrifice, the animals were from 8 to 12 weeks old and weighed from 200 to 475 grams. The livers were excised, perfused with 0.25 M sucrose, weighed, and homogenized in 0.25 M sucrose to a final concentration of 375 mg liver/ml. The microsomes were prepared by precipitation with calcium according to the method of Schenkman (14).

Experiments were performed on native and activated microsomes. Optimal activation of the microsomes was achieved in the presence of Triton X-100, at concentrations of 0.1% and 0.2% v/v, for bilirubin and PNP, respectively.

Microsomal protein content was determined by the method of Lowry et al (15) using bovine serum albumin as standard.

UDP-glucuronyltransferase activity with p-nitrophenol as substrate. The assay was performed at 37° for ten minutes in the presence of 0.1 M Tris-HCl (pH 7.4), 0.6 mM PNP, 10 mM $MgCl_2$, and 0.8 to 1.0 mg of microsomal protein. Reactions were started by the addition of UDP-glucuronic acid to give a final concentration of 5 mM. Total incubation volume was 1 ml. In the blanks, UDPGA was omitted. The reaction was stopped by the addition of 10% trichloroacetic acid (0.4 ml). The reaction mixture was cooled and centrifuged in order to pellet the microsomal protein.

The supernatant was analyzed directly using high pressure liquid chromatography. A Waters HPLC was used with a μbondapac C_{18} reverse phase column and model 440 detector using a 313 nm filter. The mobile phase was 50% water: 50% methanol in 1% acetic acid (to prevent ionization of PNP on the column). The flow rate was set at 2 ml/minute.

Both the parent compound and conjugated produce could be detected and quantitated on the same chromatogram. It was demonstrated that no interfering peaks were present by running blanks in which PNP was omitted. Retention times for PNP and PNP-glucuronide were 3 minutes, 24 seconds and 1 minute, 54 seconds, respectively.

Total recovery was consistently between 95% and 105% and all calculations were based on product formation. This is an advantage over previously published methods (16, 17) which measure disappearance of substrate.

UDP-glucuronyltransferase activity with bilirubin as substrate. Bilirubin UDP-GT activity was measured according to the method of Heirwegh et al (18); i.e., a system using diazotized ethyl anthranilate, and in which the excess of unconjugated bilirubin does not react (19). The concentrations in the final reaction mixture (0.66 ml) were: 60 mM Tris-HCl, pH 7.4, 7.6 mM $MgCl_2$, 0.12 mM bilirubin added in bovine albumin (10 mg/ml), 2.6 mM UDPGA and microsomal protein (0.4 to 0.6 mg). Incubations were carried out at 37° for 20 minutes. UDPGA was omitted from the blanks.

RESULTS

Food and water consumption and body weight gains after BCNU treatment. BCNU treatment exerted a pronounced early effect on the nutritional status of the rats. During the first three days post-treatment, food consumption decreased to 60% and water intake to 80% of the averaged values reported for the entire 22-day period (Table 1). This initial drop had completely diminished by day 4 (data not shown). The result of this early effect was manifested as a significant weight loss during that time (Figure 1). After day 5 post-treatment, however, the early weight loss had stopped and continuous weight gain was observed over the remainder of the observation period. Controls followed the same pattern, but the effect was much less pronounced and dissipated more rapidly.

Table 1 shows that over the entire time period there was a reduction in both food and water consumption in the BCNU-treated group. Water consumption, down by 17%, was significantly lower in the treated group ($p < .05$), but food consumption was only down by 7%, which was not significant ($p > .05$) and could partially be accounted for by the reduction seen in the first four days post-treatment.

In order to compare weight gains in control versus BCNU-treated animals, least squares analysis was applied to the data in Figure 1 for days 5 to 22 post-treatment. A comparison of regression lines revealed that there was a 21% lower rate of daily weight gain in the BCNU-treated group. This difference was significant ($p < .05$).

The effect of BCNU on microsomal protein content. The combination of BCNU treatment and subsequent nutritional changes observed had very

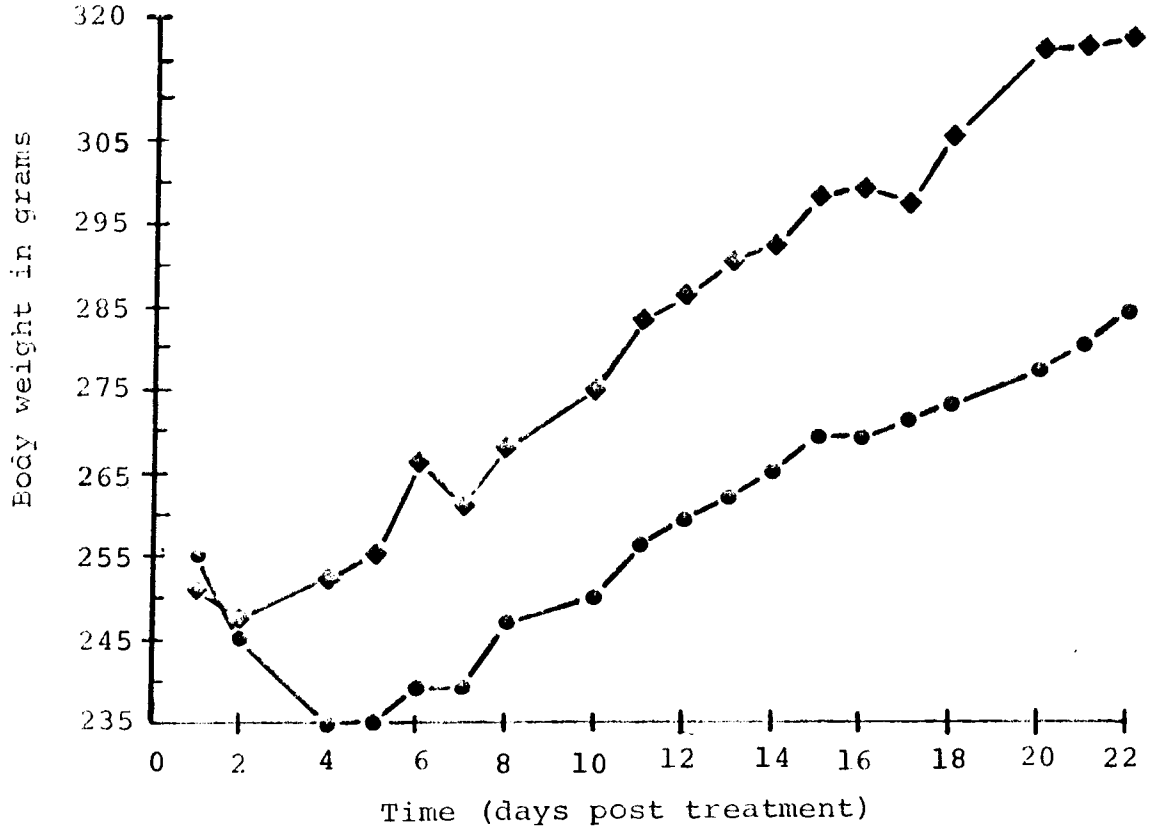

Figure 1. The effect of BCNU administration on body weight gain in the rat. Points for control (◆) and BCNU treated (●) groups represent the average of 3 animals.

Table 1. Effect of *in vivo* BCNU administration on water and food consumption and body weight gain in the rat*

Group	Water consumption a	Food consumption b	Weight gain c
Control	11.2 ±.35	8.38 ±.30	3.56 ±.04
Treated	9.27 ±.48†	7.81 ±.30	2.82 ±.03†

*Results are mean ± S.E.M. for 3 animals and expressed as: (a) ml of water/100 grams of body weight/day (day 0-22 post treatment); (b) grams of food consumed/100 grams of body weight/day (day 0-22 post treatment); (c) body weight gain in grams/day (day 5-22 post treatment).

† $P<0.05$ (one tailed t-test for small independent samples)

little effect on the microsomal protein content. There was no significant difference ($p > .05$) between treated and control groups up to day 21. At this time period there was a 31% reduction in microsomal protein content in the BCNU-treated group and represented the only time where a significant difference ($p < .05$) was observed between the two groups.

UDP-glucuronyltransferase activity with bilirubin as substrate. Bilirubin glucuronide formation, in the native microsomes, was consistently increased in the BCNU-treated group (Table 3). This effect was first significant ($p < .05$) at day 3 and was maximized by day 13 when there was a 54% increase in metabolism in the treated group when compared to controls. This increase in bilirubin glucuronidation seen in the treated group was significant ($p < .05$) at all time periods except day 21.

Triton activation of the microsomes resulted in a 4.4-fold increased activity in the controls and a 3.4-fold increase in the BCNU-treated group. This represented 23% less activation for microsomes from the BCNU-treated animals. After activation of the microsomes, no significant difference existed ($p > .05$) in the metabolic rate between treated and control groups, except at day 3, where the treated group was significantly higher ($p < .05$).

UDP-glucuronyltransferase activity with p-nitrophenol as substrate. PNP metabolism followed very much the same pattern as bilirubin metabolism. In the native microsomes, there was a significant increase ($p < .05$) in the rate of PNP glucuronide formation in the BCNU-treated group at days 3, 7, and 21. The largest difference in activity was

Table 2. Effect of *in vivo* BCNU administration on microsomal protein content in the rat liver*

	Days post treatment					
Group	1	3	7	13	21	28
Control	16.7 ± .14	18.5 ± 2.0	13.1 ± 1.0	16.4 ± 1.3	11.3 ± .27	12.3 ± .29
Treated	16.5 ± .81	18.0 ± 2.2	15.0 ± .30	16.0 ± 2.6	7.79 ± .37†	9.89 ± 1.5

*Results are mean ± S.E.M. for 3 animals and expressed as: mg of micromosomal protein/gram of wet liver.

† $P < .05$ (one tailed t-test for small independent samples)

Table 3. Effect of *in vivo* BCNU administration on bilirubin glucuronide formation in rat liver microsomes*

	Days post treatment					
	1	3	7	13	21	28
Native Microsomes						
Control	.187 ± .01	.193 ± .02	.250 ± .03	.233 ± .01	.190 ± .01	.163 ± .01
Treated	.177 ± .01	.273 ± .01†	.326 ± .02†	.359 ± .01†	.200 ± .01	.197 ± .01†
Activated Microsomes						
Control	.685 ± .11	.736 ± .08	.890 ± .03	.693 ± .04	.840 ± .02	1.01 ± .13
Treated	.592 ± .02	.955 ± .04†	.909 ± .04	.741 ± .07	.808 ± .04	.996 ± .10

*Results are mean ± S.E.M. for 3-6 animals expressed as: nmoles substrate transformed per minute per mg of microsomal protein.

† $P < .05$ (two-tailed t-test for small independent samples)

observed at day 3 when there was a 66% increase in the treated group when compared to controls. Even though PNP glucuronidation was not significantly increased ($p > .05$) at days 13 and 28, activity was still elevated by 18% and 40%, respectively.

Triton activation, when considering all time periods, resulted in a 12-fold increased activity in microsomes from the control group and a 9.5-fold increase in activity in the BCNU group. This 20% greater activation in the control group paralleled the effect seen with bilirubin as substrate. After activation, no significant difference ($p > .05$) was observed in PNP metabolism between treated and control groups, at any of the time periods.

Kinetic measurements of bilirubin metabolism. Kinetic assay of bilirubin conversion was performed on day 13 post-treatment (time of maximal effect). Reaction conditions were the same as previously described (see Methods and Materials section) with UDPGA being held constant at 2.6 mM and bilirubin concentrations being varied from 0.03 mM to .12 mM (see Figures 2 and 3) for both native and activated microsomes. Figure 2 is the resulting Lineweaver-Burk plot for native microsomes. When kinetic parameters were compared at this single concentration of UDPGA (2.6 mM) there was, in the native microsomes, a 90% increase in K_m for bilirubin and a 60% increase in V_{max} in the BCNU-treated group. In the Triton activated microsomes, from both control and BCNU-treated animals, normal Michaelis-Menten kinetics were not observed, although no significant difference ($p > .05$) in the rate of glucuronide formation occurred between the two groups at any of the substrate concentrations used. The change in glucuronidation

Table 4. Effect of *in vivo* BCNU administration on p-nitrophenol glucuronide formation in rat liver microsomes*

	Days post treatment					
	1	3	7	13	21	28
Native Microsomes						
Control	2.01 ± .32	2.48 ± .32	2.14 ± .10	2.77 ± .39	3.05 ± .26	3.13 ± .48
Treated	2.10 ± .17	4.13 ± .57†	3.06 ± .09†	3.27 ± .67	4.56 ± .27†	4.37 ± .62
Activated Microsomes						
Control	26.5 ± .92	27.9 ± 1.9	36.2 ± 1.5	23.5 ± .65	40.1 ± 3.5	36.9 ± 3.2
Treated	24.8 ± 3.4	30.5 ± 3.7	36.1 ± .84	28.3 ± 2.5	51.7 ± 8.5	38.8 ± 4.5

*Results are mean ± S.E.M. for 3 animals expressed as: nmoles substrate transformed per minute per mg of microsomal protein.

† $P < 0.05$ (two-tailed t-test for small independent samples)

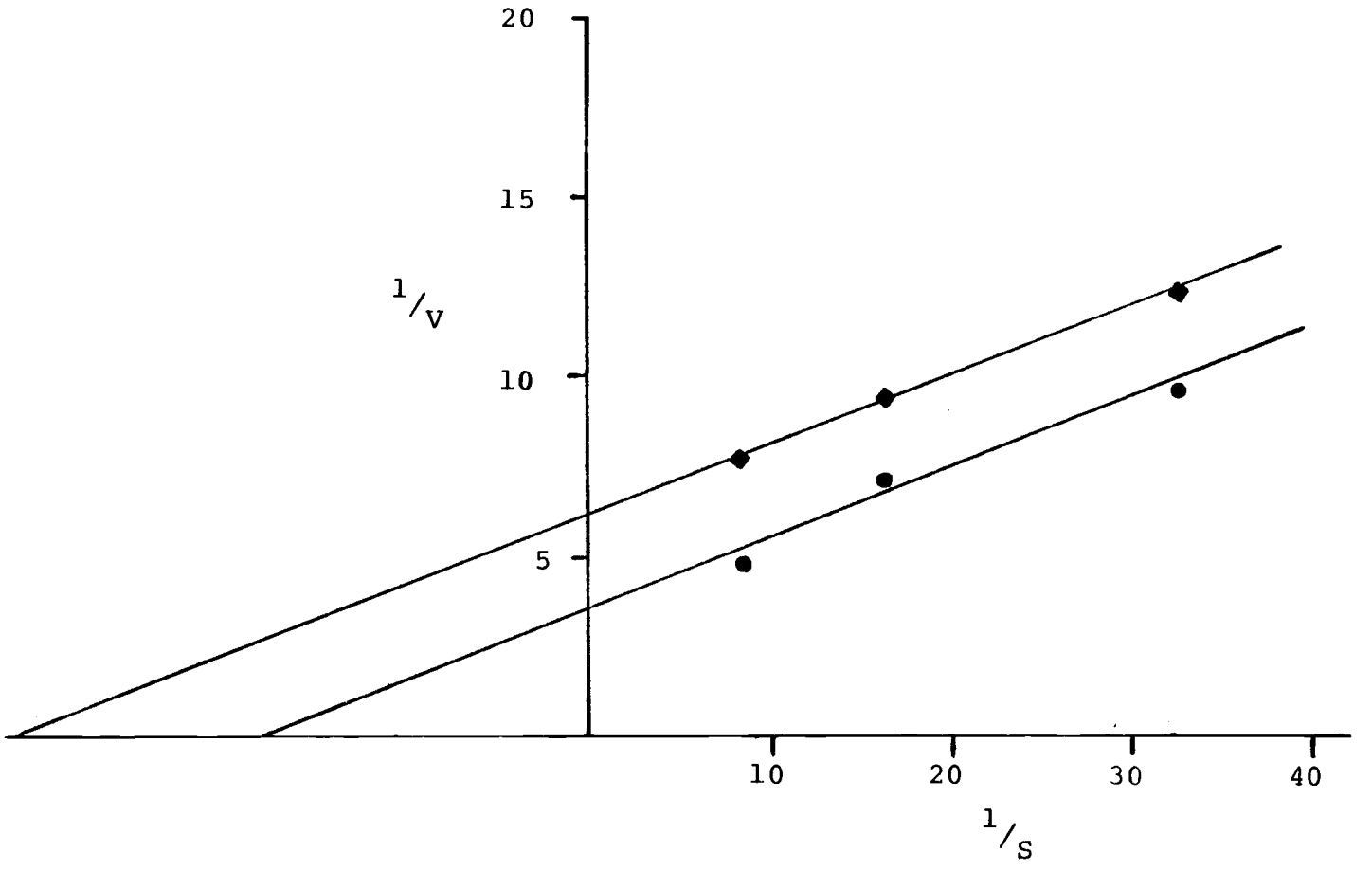

Figure 2. Lineweaver-Burk plot of UDP-glucuronyltransferase with bilirubin as substrate in native hepatic microsomes from control (◆) and BCNU treated (●) rats. Substrate concentration is expressed in mM and the velocity (V) as nmoles substrate transformed per minute per mg of microsomal protein. UDP glucuronic acid concentration was 2.6 mM. Each point represents the average of three animals. Animals were sacrificed at day 13 post-treatment. Straight lines were drawn using least squares analysis.

rate, in the activated microsomes, that occurred by varying the bilirubin concentration is shown in Figure 3.

Metabolism in animals induced with phenobarbital or 3-methylcholanthrene. It was generally observed with either inducing agent, Pb or MC, that induction occurred more readily and to a greater extent in the BCNU-treated group (Table 5). Animals for these studies were sacrificed at day 13 post-treatment.

An increase in bilirubin metabolism, after Pb induction, was observed in both the induced control and induced BCNU-treated groups. There was only 17% increase in glucuronidation in the induced control group. This did not represent a significant increase ($p > .05$) and therefore induction had not occurred. In the induced BCNU-treated group, bilirubin metabolism increased by 48% over the non-induced controls. This represented a significant increase ($p < .05$) in metabolism when compared to both induced and non-induced controls. Therefore, in contrast to the induced control group, induction of bilirubin metabolism was observed in the BCNU-treated group after Pb treatment.

A sharp increase in PNP metabolism was observed in both induced control and induced BCNU-treated groups after MC treatment. In the induced controls, there was a 3-fold increase in PNP metabolism as compared to a 4-fold increase in the induced BCNU-treated group. Although no significant difference ($p > .05$) was observed in the degree of induction between the two groups, induction occurred more readily in the BCNU-treated group. Out of five control animals treated with MC only two were induced, while the remaining three animals stayed

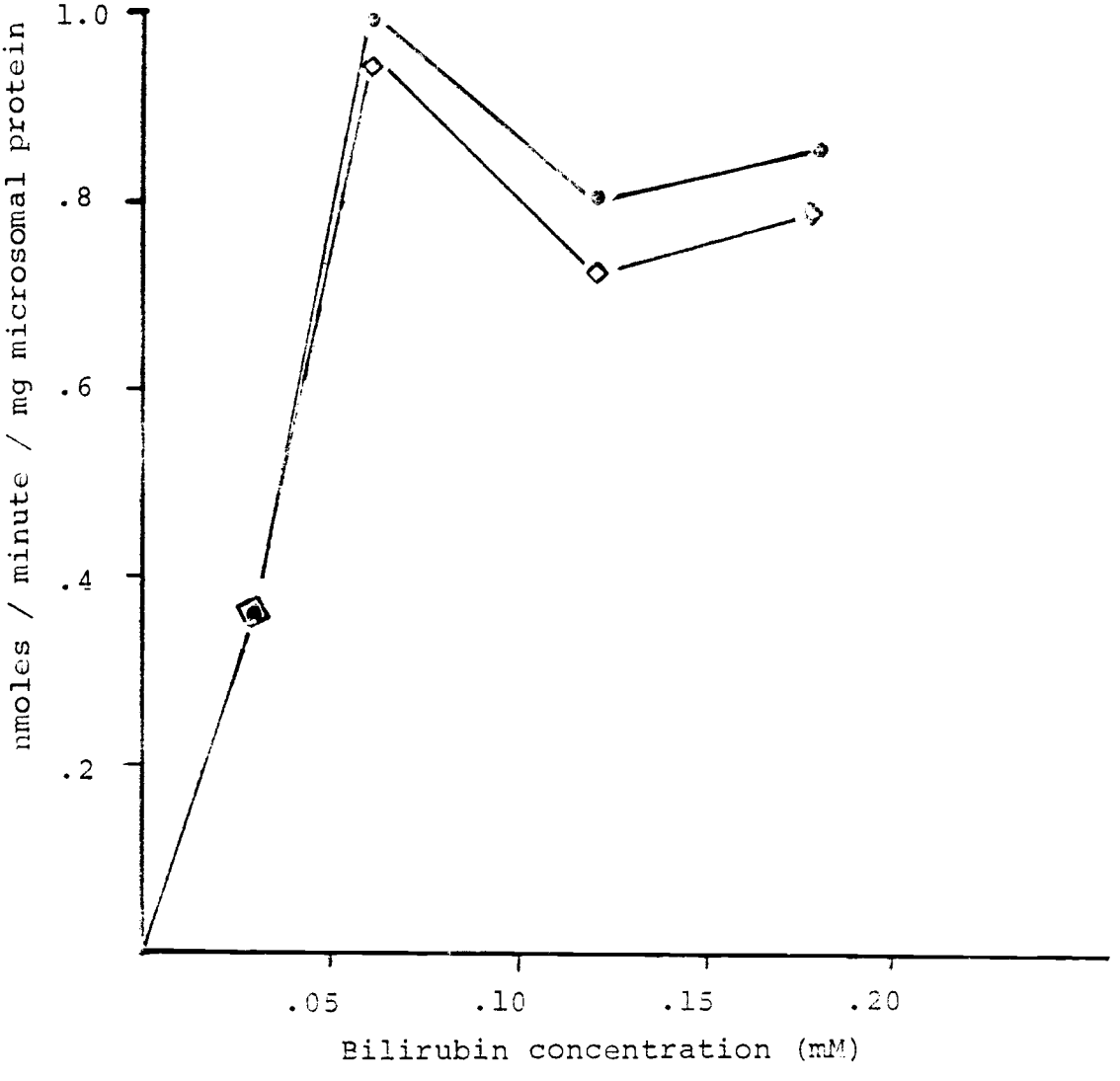

Figure 3. Effect of varying bilirubin concentration on the rate of bilirubin glucuronide formation in activated microsomes of both control (◇) and BCNU-treated (●) rats. UDPG concentration was held constant at 2.6 mM. Animals were sacrificed at day 13 post-treatment.

at the non-induced control level. The value reported in Table 5 for the induced control groups was an average of the two animals that were induced. In the BCNU-treated group, all three animals receiving MC treatment were induced.

Table 5. Hepatic microsomal glucuronidation after induction in control and BCNU-treated rats*

		Percent Stimulation
Bilirubin glucuronidation after phenobarbital induction		
Control	.916 ± .05 (15)	---
Induced Control	1.07 ± .03 (3)	117
Induced BCNU-treated	1.36 ± .06 (3)†	148
p-nitrophenol glucuronidation after 3-methylcholanthrene induction		
Control	53.3 ± 4.5 (3)	---
Induced Control	173 ± 49 (2)	325
Induced BCNU-treated	228 ± 48 (3)	425

*Results are mean ± S.E.M. for the number of animals given in parentheses and expressed as: nmoles of substrate transformed per minute, per mg of microsomal protein. Animals were sacrificed on day 13 post-treatment with BCNU. For the methods of induction, see Materials and Methods section.

† Significantly different from controls and induced controls $p < 0.005$ (one-tailed t-test for small independent samples).

DISCUSSION

BCNU treatment has an effect upon the nutritional status of the rats. A sharp initial drop in water and food consumption and body weight was observed over the first three days post-treatment, but these effects had largely subsided by day 5. Short-lived anorexia after BCNU treatment is well recognized during both animal and clinical studies. Over the rest of the observation period (days 5 to 22) nutritional intake was only decreased 7% by BCNU treatment and did not represent a significant change. Even though food consumption was not changed, we found a 20% reduction in the rate of weight gain in the treated group. Weight gain to food consumption ratios were .42 for controls versus .36 for the treated group. This indicated that while food consumption did not significantly decrease there had been some interference with the animals' ability to utilize their nutritional intake.

Microsomal protein content remained unchanged by BCNU administration or subsequent nutritional deficits for up to three weeks after treatment. It has been demonstrated both *in vivo* and *in vitro* that BCNU can inhibit protein synthesis (20, 21). The half life of microsomal protein has been estimated to be about 48 hours (22); therefore, if an inhibition of protein synthesis were to be reflected in microsomal protein content, this effect should be noted early after treatment. We observed no early changes. Nutritional changes also appeared to have had little effect on microsomal protein content which also would be expected to occur early when changes in the nutritional intake were the greatest. From these observations, any changes in glucuronidation could not be explained by alterations in overall microsomal protein content.

In the native microsomes, bilirubin and PNP glucuronidation was consistently increased after BCNU treatment. With both substrates this effect was first significant at day 3 post-treatment and persisted throughout the entire time period (28 days). UDP-GT operates in a latent or constrained form in the intact liver (23) and in native microsomes (24). It is still debated whether the latent state of the enzyme is better explained by a compartmental (25, 26) and/or a conformational (24) model. Because we were working with a latent form of the enzyme in the native microsomes, the increase in glucuronidation observed could have been due to either partial activation of the enzyme or an increase in the concentration of UDP-GT in the microsomes.

The increased rate of glucuronidation, in the native microsomes, subsequent to BCNU treatment, was no longer observed after Triton activation of the microsomes. This observation was due to a greater degree of activation occurring in microsomes from control versus treated animals. Activation of the latent form can be brought about by a variety of methods that alter microsomal membrane structure (e.g., addition of detergents, ultrasonication, etc.). By regulating the conditions (e.g., detergent concentration), a fully activated form of the enzyme can be obtained which represents total enzyme activity. An increase in the rate of glucuronidation, in the BCNU-treated group, was only observed in the native microsomes. After detergent activation of the microsomes a difference in the rate of glucuronide formation between the two groups no longer existed. Therefore, it was felt that BCNU treatment had caused a mild and prolonged activation of UDP-GT,

instead of an increase in the UDP-GT concentration in the microsomes.

If activation, instead of an increase in enzyme concentration, was responsible for the observed increase in the rate of glucuronidation found in native microsomes it would be reasonable to expect not only an increase in V_{max}, but also an altered K_m in the BCNU-treated animals. It was for this reason that a kinetic assay of bilirubin glucuronidation was conducted on day 13 post-treatment (time of maximal effect). In the native microsomes, we observed an increase in both the apparent K_m (90%) and V_{max} (60%) in microsomes from BCNU-treated animals, when compared to controls. This indicated that a kinetically different form of UDP-GT was present in the native microsomes after BCNU treatment, which is in agreement with the above hypothesis. In the activated microsomes, from both control and BCNU-treated animals, normal Michaelis-Menten kinetics were not followed, but no significant difference in rate of glucuronidation was observed at any of the substrate concentrations used. Kinetic analysis of bilirubin glucuronidation is complicated by a number of factors (e.g., insolubility of bilirubin, etc.) that exist in this in vitro system and problems have been commonly reported in the literature (28, 29, 30). Our studies were conducted at a single UDPGA concentration (2.6 mM). In order to obtain values that most closely approximate the true K_m and V_{max} of in vitro bilirubin glucuronidation, a bisubstrate kinetic analysis would be necessary. The data that we have obtained from the kinetic analysis and metabolic studies indicate that glucuronidation is only altered in the native microsomes. This could be the result of changes in the lipid environment of this latent form of the enzyme which are no longer

observed after activation which is brought about by disruption of the membrane structure.

All of the effects upon metabolism observed were the same with either bilirubin or PNP as substrate. It has long been felt that multiple forms of UDP-GT exist in the rat hepatocyte (27). Recently, Bock et al (12) have purified to apparent homogeneity two forms of UDP-GT designated as enzyme 1 and enzyme 2. PNP appears to be metabolized primarily by enzyme 1 and bilirubin by enzyme 2 (12). It was for this reason that these substrates were chosen in order that a differential effect of BCNU on the isozyme forms could be detected. Our studies indicate that the effects of BCNU are not selective for either isozyme.

Induction of PNP glucuronidation by MC administration, or bilirubin glucuronidation by pretreatment with Pb is well recognized (13). Induction has been shown to be the result of a somewhat selective increase in the synthesis of enzyme 1 (form metabolizing PNP) or enzyme 2 (form metabolizing bilirubin) (12). Induction studies were performed on BCNU-treated animals (day 13 post-treatment) and controls with each of the inducing agents. We found that induction of metabolism of the respective substrates was achieved more readily and reached a higher level in the BCNU-treated animals, demonstrating a greater inducibility following BCNU treatment.

In conclusion, these studies indicate that BCNU treatment causes a significant and prolonged effect upon the lipoprotein interactions governing UDP-GT activity in the endoplasmic reticulum of the rat hepatocyte. The activation that we observed could be due to a general

hepatotoxic effect, as it has been shown that a fully activated form of UDP-GT can exist *in vivo* due to severe membrane injury (31, 32). BCNU is not a powerfully cytotoxic agent to the hepatic parenchymal cell, especially at the dosage employed (33, 34). The other possibility is that there is a specific alteration in lipoprotein metabolism. Studies using methyl nitrosourea have shown, in the rat bladder urothelium, the formation of lipid droplets from the endoplasmic reticulum and changes in its unit structure (35). Also, in similar studies, alterations in the renewal of the luminal membrane by insertion have been demonstrated (36). These observations show that with at least one nitrosourea derivative alterations in lipoprotein metabolism do occur.

As stated earlier, the rationale for undertaking this study was twofold. First, we wanted to evaluate the effects of BCNU on the most important mode of phase II drug metabolism, glucuronidation. The effect we observed was a mild and prolonged activation of UDP-GT. I do not feel that this increase in activity would represent a dramatic alteration in glucuronidation in patients receiving BCNU. It should be emphasized that measurements of the rate of glucuronidation were obtained using an *in vitro* system. In the intact organism, other factors come into play (e.g., substrate transport into the cell, intracellular concentration of UDPGA, and product transport out of the cell), and caution must exercised in extrapolating *in vitro* data.

Secondly, we hoped to gain a better understanding of the biochemical changes that occur in the cell after exposure to BCNU. I feel that the most important finding to come out of this study was the mild and

and prolonged increase in glucuronidation rate, in native microsomes, most likely due to an alteration in the enzyme's lipid environment. As stated above, other investigators have reported changes in membrane metabolism after treatment with nitrosoureas. It has long been recognized that cellular transformation involves changes in the plasma membrane and that cell surface changes can bring about an immune response. Therefore, I feel that changes in membrane metabolism may be an important aspect of the therapeutic and/or toxic effects of the nitrosoureas. Further research stemming from this study, I feel, would most fruitfully be directed toward looking at changes in membrane composition and fluidity, in order to better understand the effects of BCNU on membrane metabolism, which may prove to be an important aspect for understanding the pharmacology of the nitrosoureas.

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