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INHIBITION OF DRUG-METABOLIZING ENZYMES IN THE RAT AFTER BACILLUS CALMETTE–GUÉRIN TREATMENT*

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Abstract—Drug-metabolizing enzyme activities and protein content of hepatic microsomal preparations from adult Sprague–Dawley rats were examined at 3, 6, 10 and 14 days after intravenous, subcutaneous and intradermal administration of two different strains of Bacillus Calmette-Guérin (BCG). Pasteur liquid strain BCG, injected intravenously at 6×10^8 organisms/m^2 in normal saline, caused 20–45 per cent reduction of aniline hydroxylase (AH) and 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide demethylase (DICD) activities and the cytochrome P-450 content of microsomes from female rats but only 10–25 per cent decrease in these parameters in males. Microsomal protein content was decreased 30–40 per cent in both sexes on days 10–14. Administration of BCG by the subcutaneous route caused only 5–25 per cent impairment of AH and DICD activities in female rats, while intradermal administration of the adjuvant was without discernible effect on the rat hepatic microsomal drug-metabolizing system. The duration of pentobarbital-induced narcosis was increased up to 70 per cent in both male and female rats after intravenous administration of BCG. Tice strain lyophilized BCG at the same dose elicited similar but lesser effects. BCG added *in vitro* had no effect on AH and DICD activities. Livers of rats treated intravenously contained numerous granulatomous lesions throughout the parenchyma; the damage was variable and less severe in the subcutaneously treated animals. Intradermally treated rats showed normal liver histology.

Nonspecific augmentation of host immunity with bacterial adjuvants has yielded encouraging therapeutic response in a number of experimental and human neoplasias [1]. Since immunotherapy is generally considered to be maximally effective for the eradication of low tumor burdens, the antigenic stimulant is usually administered subsequent to, or concomitant with, conventional cytoreductive therapy. The most extensively investigated immunoadjuvant is Bacillus Calmette-Guérin (BCG) a strain of *Mycobacterium bovis*. In current experimental clinical studies, BCG is often used as an adjunct to chemotherapy. Gutterman *et al.* [2] have demonstrated that the addition of BCG to 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DIC) significantly prolongs remission duration and survival of patients with metastatic malignant melanoma. The same investigators have shown [3] that BCG increases the duration of chemotherapy-maintained complete remissions in adults with acute myelogenous leukemia. These and other [4–7] promising results indicate that immunotherapy added to chemotherapy has an important role in the management of disseminated malignancies.

Toxic manifestations of BCG therapy depend on the route and the frequency of administration. A high incidence of hepatic dysfunction has been reported [8–10] in patients with melanoma treated by intralesional BCG. Similar side-effects have been documented [10] in patients after intradermal administration of the adjuvant. It has been suggested [10] that the overall incidence of hepatic dysfunction in BCG-treated patients may be much higher than recognized, since granulatomatous lesions are usually not apparent by serological tests or by routine clinical evaluation. Alteration in hepatic function assumes paramount significance in patients receiving chemotherapy because the biological properties of many drugs are greatly modified by hepatic enzymes. The NADPH-dependent mixed-function oxidase system located in the endoplasmic reticulum plays a major role in the metabolism of several clinically important antitumor agents including cyclophosphamide [11], DIC [12], procarbazine [13], 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) [14], 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea [14, 15] (CCNU), mercaptopurine [16], nitrogen mustard [17] and, probably, adriamycin.† Adjuvant-induced changes in mixed-function oxidase activity can affect the rate of metabolism of such drugs and, conceivably, alter their duration of action and toxicity. We have, therefore, examined the influence of BCG treatment on the activity of hepatic drug-metabolizing enzymes in the rat. We now report on the effects of two different strains of BCG, administered subcutaneously (s.c.), intravenously (i.v.) or intradermally (i.d.) on the activities of the mixed-function oxidase system. A preliminary account of this work has appeared [18].

MATERIALS AND METHODS

In the present study, aniline and DIC were used as model substrates for drug metabolism *in vitro*. The

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former compound undergoes ring-hydroxylation to give *p*-aminophenol, while the latter compound undergoes [12] oxidative *N*-demethylation to yield formaldehyde, 4-aminoimidazole-5-carboxamide and other products. Cytochrome P-450, the central component of the electron-transport chain responsible for drug metabolism, was also monitored.

Chemicals. The following drugs and reagent grade chemicals were used: NaDP sodium salt (Sigma), glucose 6-phosphate disodium salt (Sigma), glucose 6-phosphate dehydrogenase (Sigma), aniline (Eastman), semicarbazide hydrochloride (Fisher), sodium dithionite (Fisher), ethylenediamine tetraacetic acid (EDTA) (Mallinckrodt), phenobarbital sodium (Mallinckrodt), and pentobarbital sodium (Baker). DIC was provided by the Drug Development Branch of the Cancer Chemotherapy National Services Center, National Cancer Institute. DIC-dimethyl[^{14}C] (15.25 mCi/m-mole) was synthesized in 72 per cent yield from 5-diazoimidazole-4-carboxamide and dimethylamine[^{14}C] [prepared from dimethylamine[^{14}C] hydrochloride (15.25 mCi/m-mole; New England Nuclear)] by the method of Shealy *et al.* [19]. The chemical purity of the DIC-dimethyl[^{14}C] was established by spectrophotometric, chemical and chromatographic methods. The radiochemical purity, determined chromatographically in three different solvent systems, was greater than 99 per cent. It was stored as a solid in the dark at -20°.

Animals. Adult male and female Sprague Dawley rats (Sprague–Dawley Laboratories, Madison, Wis.) weighing 200–250 g at the start of the experiments were used. They were housed in metal cages in groups of four over hardwood bedding in an air-conditioned room (21–23°) with alternate periods of 12 hr light and 12 hr dark. Purina Lab Chow and water were provided freely. Both treated and control animals were weighed at the time of injection and daily thereafter.

BCG. Two strains of BCG were used: a liquid strain obtained from the Pasteur Institute, Paris, and a lyophilized strain (Tice) obtained from the Chicago Research Foundation, Chicago, Ill. Both preparations were suspended in physiologic saline and administered to rats as a single dose of 6×10^8 viable organisms/m^2 in a volume of 0.2 ml either i.v. into the tail vein, or s.c. or i.d. in the right axillary area. The BCG dosage approximates [20] that administered [2] to man by scarification in the immunotherapy of malignant melanoma. Control rats were treated with sterile physiologic saline, since it was found that administration of the culture media alone at dosages equivalent to those administered with the BCG had no effect on any of the parameters measured in this study. The composition of the culture media/0.2 ml of BCG suspension was as follows: (1) Pasteur BCG: dipotassium hydrogen phosphate, 10 μg; asparagine, 5 μg; citric acid 1.25 μg; magnesium sulfate, 1.25 μg; ammonium ferric citrate, 0.125 μg; and glycerol, 0.15 μl; and (2) Tice BCG: dipotassium hydrogen phosphate, 3.125 μg; asparagine, 25 μg; citric acid, 12.5 μg; magnesium sulfate, 3.125 μg; ammonium ferric citrate, 0.375 μg; glycerol, 0.375 μl; and lactose, 3.75 μg. At 3-, 6-, 10-, and 14-day intervals after adjuvant administration, four experimental rats together with their respective controls were killed by decapitation and exsanguinated for 10 sec. The activity of the hepatic drug-metabolizing enzymes was assayed as described below.

Preparation of microsomes. A 25% liver homogenate in 0.1 M potassium phosphate buffer, pH 7.4, was centrifuged at 10,000 *g* for 20 min at 4°. The supernatant fraction was aspirated and centrifuged at 105,000 *g* for 60 min at 4°. The microsomal pellet was washed by resuspension in ice-cold 1.15% KCl 10 mM EDTA followed by resedimentation at 105,000 *g* for 30 min. The final pellet was reconstituted in 0.1 M potassium phosphate buffer, pH 7.4, such that each ml of suspension contained microsomes from 250 mg wet wt of liver (approximately 5–10 mg of microsomal protein/ml).

Microsomal protein content was determined by the method of Lowry *et al.* [21] using crystalline bovine serum albumin as the reference standard.

Aniline hydroxylase (AH) assay. Each incubation mixture consisted of 5 μmoles aniline, 2 μmoles NADP, 25 μmoles glucose 6-phosphate, 3.5 units of glucose 6-phosphate dehydrogenase, 25 μmoles magnesium chloride and 1.0 ml of microsomal suspension in a total volume of 5.0 ml buffered at pH 7.4 with 0.1 M potassium phosphate. Reaction was initiated by the addition of microsomes. Incubations, conducted in triplicate, were carried out for 20 min at 37° in open-necked 25-ml Erlenmeyer flasks in a Dubnoff shaker (120 oscillations/min). Reaction was terminated by the addition of 2 ml of 20% trichloroacetic acid. Precipitated protein was removed by centrifugation, and aliquots of the clear supernatant were assayed spectrophotometrically for *p*-aminophenol according to the method of Kato and Gillette [22].

5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide demethylase (DCID) assay. The method is a modification of the procedure of Mizuno and Humphrey [23]. The incubation mixture consisted of 1.5 μmoles DIC-dimethyl[^{14}C] (5 μCi, 6.67 mCi/m-mole), 0.24 μmole NADP, 3 μmoles glucose 6-phosphate, 0.4 units of glucose 6-phosphate dehydrogenase, 3 μmoles magnesium chloride, 5 μmoles semicarbazide hydrochloride and 0.2 ml of microsomal suspension in a total volume of 0.6 ml buffered at pH 7.4 with 0.1 M potassium phosphate. Incubations were conducted in triplicate in 10.0-ml Erlenmeyer flasks protected from light. Reaction was initiated by the addition of microsomes and allowed to proceed for 30 min at 37°. Under these conditions, the production of formaldehyde was linear with protein concentration and time. Reaction was terminated by the addition of 0.2 ml of 15% $ZnSO_4 \cdot 7\ H_2O$ followed by 0.2 ml of saturated $Ba(OH)_2 \cdot 8\ H_2O$. After standing for 5 min, the precipitated protein and $BaSO_4$ were removed by centrifugation at 10,000 *g* for 10 min. Aliquots (0.5 ml) of the clear supernatant were transferred to 10-ml test tubes fitted with Teflon-lined screw-caps; 0.5 ml of 0.03% formaldehyde solution was added followed by 1.0 ml of 0.15% 2,4-dinitrophenylhydrazine in 1 N HCl. The tubes were heated at 85–90° for 3 min and then cooled to room temperature for 30 min. Benzene (2.0 ml) was added and the mixture was agitated on a vortex shaker for 20 sec. After a brief centrifugation at 2000 *g* for 5 min to separate the phases, the benzene layer was aspirated and washed with 2.0 ml of 0.05 N HCl. The benzene layer was again separated

by centrifugation, and aliquots (0.2 ml) were transferred to counting vials. PCS scintillator (Amersham/Searle Corp.) (11.0 ml) was added and the solutions were counted in triplicate. The counting efficiency was 70–72 per cent; quenching was corrected with automatic external standards. The radioactivity contained in the benzene extracts was shown by thin-layer chromatography to be entirely associated with formaldehyde-2,4-dinitrophenylhydrazone. Recovery of liberated formaldehyde was 91 per cent as determined by adding known amounts of [^{14}C]formaldehyde to incubation mixtures containing nonradioactive DIC and repeating the above procedure. Enzyme activities were calculated on the assumption that only one of the methyl groups of DIC was converted into formaldehyde.

Cytochrome P-450. The cytochrome P-450 content of microsomes was determined by the dithionite-difference method of Omura and Sato [24]. The molar extinction coefficient of the reduced P-450–CO complex was taken as 91 cm^{-1} mM^{-1}.

Sleeping times. Sodium pentobarbital, 0.35% in physiologic saline, was administered i.p. at a dose of 35 mg/kg to male rats and 30 mg/kg to female rats. Sleeping time was defined as the time between the loss and the recovery of the righting reflex.

All experiments were repeated at least twice to document the reliability and reproducibility of the data. Control values were determined at each time interval. Experimental and control groups were evaluated by Student's *t*-test.

Histology. Freshly excised livers were sliced and immediately fixed in 10% formalin. Sections were cut from paraffin-embedded blocks and stained with hematoxylin-eosin and methyl green-pyronine. Ziehl–Neelsen staining was used to test for acid-fast organisms. Tissue sections were examined under a light microscope.

RESULTS

The effect of i.v. administration of Pasteur strain BCG on the activities of AH, DICD, and on the cytochrome P-450 content of hepatic microsomes from male and female rats over 14 days is shown in Fig. 1.

Compared to controls, the AH activity/mg of microsomal protein in male rats was marginally reduced over the observation period (Fig. 1a), solid line), inhibition being significant only on day 3. By comparison, the AH activity in female rats was significantly reduced at all time intervals after BCG administration (Fig. 1d). The variation in DICD activity over the same time period (Fig. 1, panels b and e) was closely similar to that of AH in either sex. The cytochrome P-450 content of microsomes (Fig. 1, panels c and f), however, did not correlate closely with AH and DICD activities in either males or females. A clear sex difference was nevertheless apparent, P-450 levels being significantly depressed in males only on day 10, but in females on days 6, 10 and 14.

Expressed g wet weight of liver (Fig. 1, broken lines) rather than mg of microsomal protein, all three parameters were reduced to a greater extent in both sexes at all times after BCG treatment. The difference is due to decreased microsomal protein in the treated groups (Table 1), a consequence primarily of BCG-induced hepatomegaly. Over the duration of the

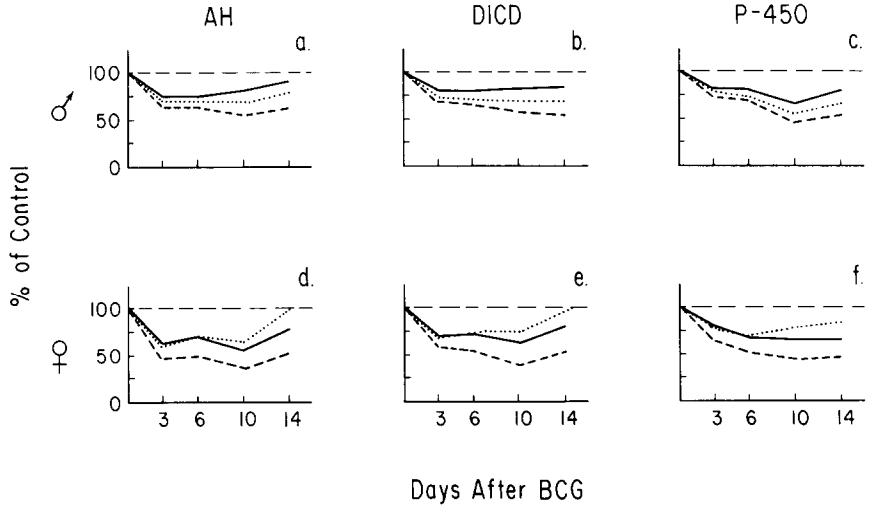

Fig. 1. Aniline hydroxylase (AH) and DIC demethylase (DICD) activities, and cytochrome P-450 content per mg of microsomal protein, per G wet wt equivalent of liver and per whole equivalent in male and female Sprague–Dawley rats after an i.v. injection of Pasteur liquid strain BCG (6×10^8 organisms/m^2). Key: (—) per mg of microsomal protein; (– – –) per g wet wt equivalent of liver; and (····) per whole liver equivalent. Results are expressed as a percentage of control. Each point represents the mean value of observations from four rats. Average control values of AH (nmoles *p*-aminophenol/mg of protein/min), DICD (nmoles formaldehyde/mg of protein/min) and P-450 (nmoles cytochrome P-450/mg of protein)/mg of microsomal, per g wet wt equivalent of liver, and per whole liver equivalent are as follows. (Males) AH: 0.76 ± 0.02, 13.41 ± 0.24, 144.3 ± 5.5; DICD: 0.167 ± 0.008, 3.02 ± 0.15, 31.7 ± 1.5; P-450: 0.97 ± 0.02, 17.1 ± 0.4, 184.4 ± 8.1. (Females) AH: 0.67 ± 0.02, 13.74 ± 0.55, 94.3 ± 2.7; DICD: 0.161 ± 0.009, 3.25 ± 0.20, 22.7 ± 1.3; P-450: 0.68 ± 0.02, 13.9 ± 0.4, 95.9 ± 3.1.

Table 1. Body weight, liver weight, and protein content of hepatic microsomes of adult male and female Sprague–Dawley rats after an i.v. injection of Pasteur liquid strain BCG (6×10^8 organisms/m^2)

Day after BCG	Body wt* (g)		Liver wt (g)		Liver wt* / Body wt (10^3)		Hepatic microsomal protein† (mg/g liver)		Total hepatic microsomal protein† (mg)	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
3 Control	239 ± 5	217 ± 2	9.2 ± 0.1	7.1 ± 0.3	38.4	32.7	17.4 ± 0.4	18.7 ± 0.7	159.4 ± 6.1	132.5 ± 6.5
Treated	236 ± 5	217 ± 6	9.7 ± 0.3	8.8 ± 0.4	41.0 (108)	40.6‡ (124)	15.4 ± 0.2 (89)	15.1 ± 1.0 (81)	149.1 ± 4.3 (94)	132.3 ± 8.2 (100)
6 Control	257 ± 4	224 ± 6	9.4 ± 0.2	7.0 ± 0.4	36.4	31.1	18.4 ± 0.3	19.3 ± 0.8	172.0 ± 3.2	133.4 ± 2.7
Treated	248 ± 3	222 ± 3	9.7 ± 0.2	9.3 ± 0.3	39.1 (107)	41.9‡ (135)	15.9 ± 0.4 (86)	14.5 ± 0.7‡ (75)	154.0 ± 4.2‡ (90)	134.2 ± 3.4 (101)
10 Control	284 ± 2	225 ± 2	11.4 ± 0.5	6.6 ± 0.3	40.1	29.4	18.7 ± 0.7	21.4 ± 0.2	212.3 ± 7.5	141.5 ± 5.8
Treated	286 ± 6	237 ± 4	13.9 ± 0.8	11.7 ± 0.6	48.5‡ (121)	49.3‡ (168)	12.9 ± 0.3‡ (69)	14.4 ± 0.3‡ (67)	179.8 ± 6.3‡ (85)	167.4 ± 5.3‡ (118)
14 Control	321 ± 6	233 ± 6	13.4 ± 0.6	7.1 ± 0.3	41.7	30.3	16.2 ± 0.6	22.3 ± 0.1	216.7 ± 14.3	157.2 ± 6.6
Treated	311 ± 5	241 ± 2	16.3 ± 0.2	12.3 ± 0.6	52.5‡ (126)	51.0‡ (168)	11.0 ± 0.6‡ (68)	16.2 ± 0.4‡ (73)	180.4 ± 12.9‡ (83)	200.5 ± 15.3‡ (128)

* Average body weights of male and female rats on day 0 were 221 ± 6 g and 206 ± 5 g.
† Numbers in parentheses represent percentage of control values.
‡ Significantly different from control: $P < 0.05$.

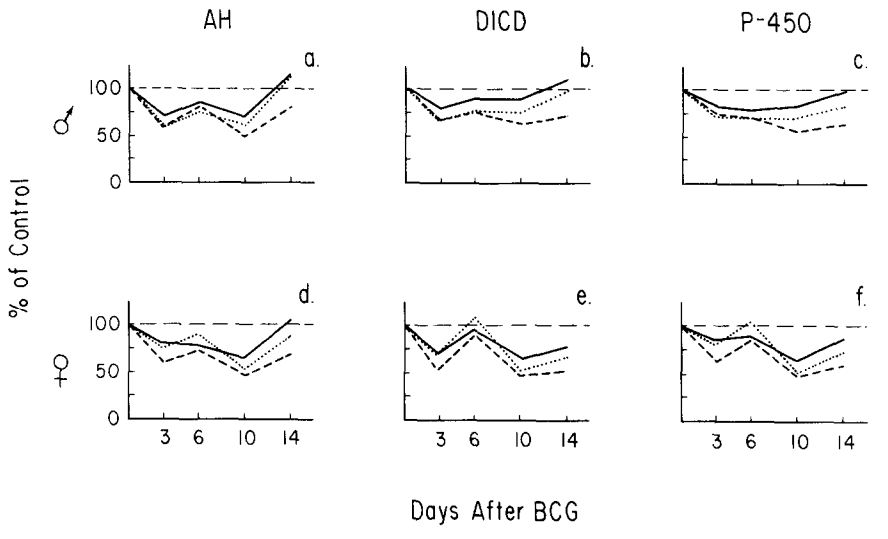

Fig. 2. Aniline hydroxylase (AH) and DIC demethylase (DICD) activities, and cytochrome P-450 content per mg of microsomal protein, per g wet wt equivalent of liver and per whole liver equivalent in male and female Sprague–Dawley rats after an i.v. injection of Tice-lyophilized BCG (6×10^8 organisms/m^2). Key: (—) per mg of microsomal protein; (– – –) per g wet wt equivalent of liver; and (····) per whole liver equivalent. Results are expressed as a percentage of control. Each point represents the mean value of observations from four rats. Average control values of AH (nmoles *p*-aminophenol/mg of protein/min), DICD (nmoles formaldehyde/mg of protein/min) and P-450 (nmoles cytochrome P-450/mg of protein) per mg of microsomal, per g wet wt equivalent of liver, and per whole liver equivalent, respectively, are as follows. (Males) AH: 0.73 ± 0.03, 13.53 ± 0.57, 142.1 ± 5.9; DICD: 0.192 ± 0.012, 3.56 ± 0.23, 37.4 ± 2.4; P-450: 1.09 ± 0.04, 20.20 ± 0.74, 212 ± 10.4. (Females) AH: 0.74 ± 0.03, 13.33 ± 0.53, 113.8 ± 4.6; DICD: 0.180 ± 0.019, 3.22 ± 0.32, 27.5 ± 3.1; P-450: 0.91 ± 0.05, 16.39 ± 0.90, 139.9 ± 7.7.

experiment, the liver weight body weight ratio increased to 126 per cent of control values in male rats and to 168 per cent of control values in female rats (Table 1); no difference was apparent in the rate of body weight gain between the control and the treated groups during this period (Table 1). Since the increase in liver weight was not matched by a corresponding increase in total hepatic microsomal protein, the content of microsomal protein unit wet weight of liver was reduced in the treated groups (Table 1). Despite the greater relative liver weight gain in females, the per cent decrease in microsomal protein concentration was similar in both sexes throughout the observation period; the rate of microsomal protein synthesis was therefore greater (and/or the rate of degradation slower) in females compared to males after BCG treatment. This difference is reflected in the total microsomal protein content of liver; by day 14, levels decreased to 83 per cent of control values in male rats but increased to 128 per cent of control values in females (Table 1). The net result of the sex-related differences in enzyme-specific activities and total hepatic microsomal protein was that the AH and DICD activities of whole liver were reduced to a comparable extent in males and females on days 3, 6 and 10 after BCG administration (Fig. 1, dotted lines). On day 14, AH and DICD activities were still significantly reduced in males but recovery to control levels was apparent in females.

Tice strain BCG administered i.v. at a dose equal to that of the Pasteur strain elicited qualitatively similar although quantitatively different results (Fig. 2). Female rats again were generally more susceptible to the inhibitory effects of the organism than male rats, and reduction of all three parameters was greatest in females 10 days after BCG treatment. A biphasic response was additionally evident, particularly in female rats. As with the Pasteur strain BCG, the sex-related differences in activity tended to diminish when the results were expressed in terms of the drug-metabolizing capacity of the whole liver although, overall, the correlation was less marked. Comparison of the relative magnitudes of the responses elicited by the two strains of BCG indicated that the Pasteur organism was generally the more inhibitory.

The duration of pentobarbital-induced narcosis after i.v. administration of Pasteur strain BCG is shown in Table 2. With the exception of female rats on day 14, both sexes slept longer at all time intervals after BCG treatment. Relative to controls there was no apparent sex difference in sleeping times. Of the three indices of drug-metabolizing activity *in vitro*, the prolongation of sleeping times correlated most closely with the drug-metabolizing capacity of the whole liver.

Administration of BCG by the s.c. route produced erratic and poorly consistent responses. Although none of the three parameters was significantly reduced in male rats, slight (15–25 per cent) but significant ($P < 0.05$) decreases in AH and DICD activities were evident in female rats on day 10. Pentobarbital-induced sleeping times were slightly increased (30 per cent) at this time but the difference was not statistically significant ($P = 0.18$).

Table 2. Effect of an i.v. injection of Pasteur liquid strain BCG (6×10^8 organisms/m^2) on the pentobarbital-induced sleeping time of Sprague Dawley rats*

Days after BCG	Sleeping time, min (mean ± S. E.)			
	Males		Females	
	Control	BCG	Control	BCG
3	56 ± 5	85 ± 4† (152)‡	110 ± 5	174 ± 10† (158)
6	56 ± 5	76 ± 5† (136)	104 ± 2	135 ± 7† (130)
10	52 ± 3	88 ± 5† (169)	114 ± 2	193 ± 21§ (169)
14	58 ± 1	86 ± 11§ (148)	111 ± 7	142 ± 12 (128)

* Eight animals per group.
† Significantly different from the control; $P < 0.01$.
‡ Numbers in parentheses represent percentage of control.
§ Significantly different from the control; $P < 0.05$.

Administration of BCG by the i.d. route had no perceptible effect on the hepatic microsomal drug-metabolizing enzyme system of the rat.

Direct addition of BCG to the incubation mixtures at a dose of 10^7 organisms/g equivalent of liver caused no change in the rates of metabolism. Addition of BCG to whole liver slices prior to homogenization likewise had no effect on the reaction rates.

Histological examination of liver sections from rats treated i.v. revealed numerous granulatomous lesions throughout the parenchyma and pronounced infiltration of the portal fields by lymphohistiocytes; the intensity of the response was generally greater in female rats than in males. In the s.c.-treated group, livers of male rats were histologically normal; however, in females a mild, although occasionally severe, granulatomous response was evident. No pathological abnormalities were observed in the i.d.-treated group. All of the examined tissues were free of acid-fast organisms of Ziehl–Neelsen staining.

DISCUSSION

The results presented in this report demonstrate that i.v. administration of BCG to Sprague–Dawley rats causes a sex-related, time-dependent impairment of the hepatic microsomal drug-metabolizing enzyme system; s.c. administration of the agent produces similar though much weaker effects. Impairment of the drug-metabolizing enzyme system by BCG was not due to the systemic toxicity of the agent; treated rats gained weight at the same rate as controls. Moreover, adjuvant-induced arthritogenic disease [25], a condition in the rat frequently associated with repression of the drug-metabolizing system [26–29] was absent in the BCG-treated animals. Competitive inhibition of enzyme activity by binding of lipophilic components of BCG to microsomal protein seemed an unlikely mechanism, since direct addition of BCG to the incubation mixtures produced no change in reaction rates. Reduced enzyme activity is most probably a consequence of damage to some component of the electron-transport chain; cytochrome P-450, however, appeared not to be the critical target site since its levels did not correlate closely with enzyme activities.

The more pronounced inhibitory effect of BCG treatment on drug-metabolizing enzymes from female rats is surprising, since a number of pathological or abnormal physiological conditions including hyperthyroidism [22], adrenalectromy [30], X-irradiation [31], and starvation [22] cause a reduction in enzyme activity in male rats without significantly affecting, or even increasing, enzyme activity in female rats. Moreover, conditions that alter enzyme activity in both sexes, e.g. diet [32] and tumors [33], generally affect males to a greater extent. These differences have been attributed [30, 31] to depletion of androgens in males under stress and the ensuing loss of the normal hormonal stimulation on the drug-metabolizing enzyme system. The present observations, however, are not consistent with this interpretation.

A plausible mechanism for the inhibition of the drug-metabolizing enzyme system by BCG involves elaboration of components of the immune effector arm. After i.v. injection bacterial adjuvants are cleared mainly in the liver and the spleen by phagocytosis [34]. A complex sequence of immune reactions ensues, finally resulting in stimulation of the reticuloendothelial system (RES) with hepatosplenomegaly. Activated macrophages constitute an important component of the stimulated RES [35]. In the mouse [36–41], the activator macrophage response reaches maximum intensity 5–20 days after BCG administration. It is conceivable that in the rat the macrophage response damages some component of the electron-transport chain of drug-metabolizing enzymes. The increased elaboration of superoxide anion [42] and hydrogen peroxide [35] by actively phagocytizing macrophages and the enhanced oxidative activity [35] of stimulated macrophages are particularly relevant in this context because it has been well established [43, 44] that the drug-metabolizing enzyme system is readily impaired by peroxidative degeneration of microsomal membrane phospholipids. Many of the principal features of our findings are consistent with an immune-mediated mechanism. First, the delayed onset of maximal enzyme inhibition is in keeping with the latent period required for macrophage activation [36–41]. Second, the greater magnitude of enzyme inhibition in females is reminiscent of the finding of Halpern *et al.* [39] that i.v. administration of BCG to mice produces greater stimulation of the RES in females than in males. Third, the return of enzyme activity to near control levels by day 14 is commensurate with the rather rapid loss of macrophage activity that normally follows stimulation

[36-41]. Fourth, the more pronounced inhibitory properties of Pasteur BCG than of Tice BCG conform with the finding of Mackaness *et al* [45] that in mice the former organism is a more potent stimulator of the RES. Finally, good qualitative agreement is observed between the magnitude of enzyme inhibition and the intensity of the hepatic granulomatous response.

The clinical implications of adjuvant-induced impairment of drug-metabolizing enzyme activity with regard to the efficacy and toxicity of cancer chemotherapeutic agents require further investigation. However, it is likely that changes in drug efficacy will depend upon the pharmacokinetics of the individual drugs and whether metabolism is an activation or a detoxification process. For drugs which are activated by first-order kinetics and eliminated mainly as metabolites, e.g. cyclophosphamide, the total amount of metabolite(s) will probably not change as a result of changes in the rate of metabolism; accordingly, the therapeutic properties of such drugs are not expected to change. On the other hand, for drugs that are mainly cleared as the unchanged parent compound, e.g. DIC, it is likely that reduced rates of biotransformation will lead to suboptimal levels of the active metabolite(s). For drugs that are detoxified by microsomal enzymes, the effect of reduced metabolic activity is more predictable: here, the prolonged duration of action will produce enhanced toxicity.

In addition to the above, BCG may modify pharmacologic response by altering the level of activity of enzymes involved in conjugation, distribution and excretion. Finally, investigators concerned with the chemoimmunotherapy of transplantable tumors or the immunoprophylaxis of chemically induced tumors should be alerted to the possibility of drug–adjuvant interactions. The use of adjuvant dosages in such studies typically 10- to 100-fold in excess of those employed during the present investigation renders this a particularly cogent consideration.

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