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The Effect of Dimethyl Sulfoxide on Cholesterol and Bile Acid Metabolism in Rats (42604)

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Abstract. The effect of DMSO on cholesterol and bile acid metabolism was studied in rats. Male Sprague–Dawley rats were randomly assigned to one of two groups and given either tap water or 2% DMSO (v/v) in tap water to drink for 9 days. Both food (stock rat diet) and water were available *ad libitum*. Animals in both groups gained weight equally throughout the study. They also had similar liver weights (g/100 g body wt) at the end of the study (control: 5.0 ± 0.1 ($N = 6$) vs DMSO: 4.9 ± 0.1 ($N = 6$)). The activity of hepatic cholesterol 7α-hydroxylase (pmole/mg/min), the rate-limiting enzyme of bile acid biosynthesis, was significantly ($P < 0.005$) reduced in the treated animals (control: 9.7 ± 1.0 ($N = 6$) vs DMSO: 4.3 ± 0.7 ($N = 6$)). Plasma cholesterol (mg/dl) was significantly ($P < 0.005$) elevated in the treated animals (control: 90 ± 3 ($N = 6$) vs DMSO: 107 ± 4 ($N = 6$)), a finding consistent with the reduced CH-7α hydroxylase activity in this group. DMSO treatment did not affect either microsomal cholesterol content or hepatic glutathione content. Thus, this study has shown that DMSO treatment per se can affect cholesterol and bile acid metabolism. However, the precise mechanisms whereby DMSO exerts the observed effects are not known.

Several studies have shown that dimethyl sulfoxide (DMSO), a colorless, polar organic solvent, can affect the severity of experimentally induced atherosclerosis (1, 2). Layman *et al.* (1) showed that severity of atherosclerotic lesions in cholesterol-fed rabbits was reduced in animals receiving 2% DMSO in their drinking water. Kedar and Sohar (2) obtained similar results with cholesterol-fed rabbits treated with 22 g DMSO/kg/day. Interestingly, in both studies, plasma cholesterol was unaffected by DMSO treatment.

In an attempt to determine the mechanism for the effect of DMSO on atherosclerotic lesions, Layman *et al.* (1) and Alam and Layman (3) showed that the uptake and accumulation of low-density lipoprotein (LDL) cholesterol by cultured fibroblasts could be significantly reduced by 2% DMSO in the medium. They suggested that the effect of DMSO on atherosclerosis in cholesterol-fed rabbits might be due to a DMSO-induced inhibition of uptake of LDL cholesterol by tissues and cells (1).

If the above hypothesis is correct, then DMSO treatment might also affect cholesterol metabolism by the liver since the liver is known to be a major site for the uptake and catabolism of LDL (4). Additionally, since the liver is the major organ involved in cholesterol homeostasis (5), then changes in hepatic cholesterol metabolism might also be expected to affect plasma cholesterol levels.

Even though, in the previous studies, plasma cholesterol was unaffected by DMSO (1, 2), it is entirely possible that subtle changes in plasma cholesterol had been masked by the large dietary cholesterol "load" used in those studies (1, 2). Thus, the objective of the present study was to examine the effect of DMSO treatment on cholesterol metabolism in the rat fed stock diet without added cholesterol. Particular emphasis was placed on studying the changes in hepatic cholesterol 7α-hydroxylase (CH-7α hydroxylase, EC 1.14.13.17), the rate-limiting enzyme in the conversion of cholesterol to bile acids (6), since the catabolism of cholesterol to bile acids is quantitatively the most important pathway of elimination of cholesterol from the body (7).

Materials and Methods. *Chemicals.* 4-^{14}C-Cholesterol (New England Nuclear, Boston, MA) was mixed with unlabeled cholesterol (99+% pure, Sigma Chemical Co., St. Louis,

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MO) and purified by thin-layer chromatography (TLC) on preparative (500 m thick) TLC plates (Analtech Inc., Newark, DE) developed in benzene/ethyl acetate (2:3, v/v) (8). The specific activity was 2.2×10^{12} dpm/mole. For CH-7α hydroxylase assay, each tube contained 190,000 dpm of the purified 4-^{14}C-cholesterol. NADPH, nicotinamide, sulfosalicylic acid, *O*-dinitrobenzene, sulfanilamide, (N-[1-napthyl]) ethylene diamine dihydrochloride, and glutathione *S*-aryl transferase were all obtained from Sigma Chemical Co. All other chemicals and solvents were of reagent grade and used as supplied.

Animals and treatment. Male Sprague–Dawley rats weighing between 60 and 70 g were obtained from Harlan Sprague–Dawley, Inc. (Indianapolis, IN), randomly assigned to one of two groups and group housed (three/cage) in polypropylene cages with stainless steel wire floors. Since DMSO treatment is known to induce diuresis (9), this housing arrangement prevented the animals from coming in contact with the adsorbent bedding. DMSO was provided in tap water as a 2% (v/v) solution. The level of DMSO selected was based on a preliminary study using 5% DMSO which was found to be toxic based on body weight gain and liver weight. Control animals received plain tap water. Both food and water were provided *ad libitum*. Water intake, for a 24-hr period, was *estimated* five times during the study (as shown in Fig. 1). It was estimated as the difference in total weight of the water bottle over a 24-hr period and normalized to 100 g body weight as shown below:

$$\frac{\text{bottle wt at time 0} - \text{bottle wt after 24 hr}}{\text{total body wt of rats in the cage}} \times 100.$$

On Day 9 of the study, the animals were anesthetized with a subcutaneous injection of ketamine and xylazine (100 and 13 mg/kg body wt, respectively) (10), weighed, and exsanguinated by cardiac puncture. EDTA was used as the anticoagulant (1 mg/ml blood). The livers were rapidly removed, weighed, and processed as described below. Unless otherwise specified, all subsequent procedures were carried out between 0 and 4°C.

Estimation of hepatic glutathione (GSH) content. A portion of the liver (between 0.4 and 0.6 g) was precisely weighed and homogenized in 5 ml of chilled 4% sulfosalicylic acid using a Potter–Elvehjem homogenizer equipped with a Teflon pestle. The homogenate was centrifuged at 20,000*g* in a Sorvall RC5B refrigerated centrifuge for 20 min, and the protein-free supernatant was removed and used for the estimation of hepatic GSH content as described by Asaoka and Takahashi (11). Hepatic GSH content was expressed as micromoles per gram of liver (wet wt).

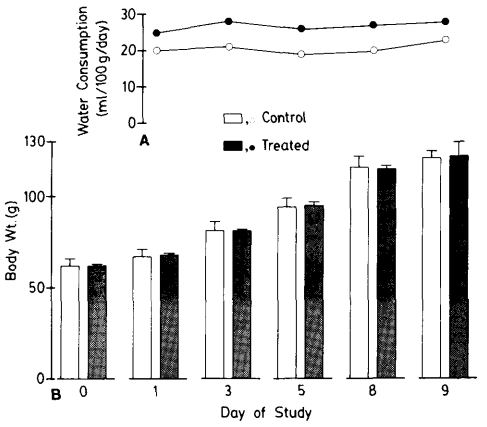

FIG. 1. Water intake (A) by the animals and changes in their body weight (B) during the study. The data for body weight are shown as the mean values of seven animals in the control group and six animals in the DMSO group. The bars represent the standard deviation. Since water intake could not be assessed on Day 0 of the study, those data points have been skewed to the right so as to coincide with the appropriate day of the study.

Hepatic CH-7α hydroxylase activity. The remainder of the liver was homogenized (30%, w/v) in 0.1 *M* potassium phosphate buffer, pH 7.4, containing 5 m*M* $MgCl_2$, 30 m*M* nicotinamide, 1 m*M* EDTA, and 30 m*M* NaF as described above, and the microsomal fraction was obtained as described by Mitropoulos and Balasubramaniam (12). The microsomal pellet was resuspended in the original volume of the above buffer without EDTA, and CH-7α hydroxylase activity was assayed as described previously (8) based on the method of Shefer *et al.* (13). Microsomal protein was measured by the Hartree (14) modification of the Lowry procedure (15), and CH-7α hydroxylase activity was expressed as picomoles of 7α-hydroxy-

cholesterol formed/milligram of microsomal protein per minute.

Estimation of plasma and hepatic microsomal cholesterol. Hepatic microsomal cholesterol content was estimated as described previously (8) based on the procedure of Morin and Elms (16) for plasma cholesterol. An aliquot of the microsomal suspension (500 μl) was saponified with 1 *N* ethanolic NaOH and the nonsaponified neutral sterols were extracted with petroleum ether. Cholesterol in the petroleum ether extract was quantitated by gas–liquid chromatography using stigmasterol as the internal standard. Analysis was carried out in a Hewlett–Packard (Cupertino, CA) Model 5790A gas–liquid chromatograph equipped with a DB1701, 30 m $\times$ 0.25 mm $\times$ 0.25-μ (film thickness) capillary column (J and W Scientific, Inc., Folsom, CA) and a Hewlett–Packard Model 3390A integrator/plotter. Hydrogen was used as the carrier gas at a linear velocity of 48 cm/sec, and the split ratio was 40:1. The operating temperatures were as follows: column, 275°C; injector, 285°C; detector (FID), 300°C. Microsomal cholesterol content was expressed as micrograms per milligram of microsomal protein.

Plasma cholesterol content was analyzed by the clinical pathology laboratory of the College of Veterinary Medicine using a Hitachi/Boehringer Mannheim (Indianapolis, IN) Model 705 Autoanalyzer and expressed as milligrams per deciliter.

Statistical analysis. The data on water consumption was subjected to weighted regression analysis using the number of animals in each group as the weighting factor, and tested for coincidence as described by Seber (17). All other data from control and treated animals were compared using Student's *t* test for unpaired means. A P value < 0.05 (two-tailed test) was considered as significant.

Results and Discussion. Water intake by the animals and changes in their body weight during the course of the study are shown in Fig. 1. Dimethyl sulfoxide-treated animals drank significantly ($P < 0.001$) more water than controls (control: 19.7 $\pm$ 0.3 vs DMSO: 25.2 $\pm$ 0.4; ml/100 g body wt/day; mean $\pm$ SEM). Increased water consumption by the DMSO-treated animals was not surprising in view of the well-documented diuretic effect of DMSO (9). Thus, the treated animals merely increased their water consumption to compensate for the increased urinary loss of water.

Animals in both groups showed a similar weight gain (Fig. 1) throughout the study. Additionally, there was no difference in liver weight (g/100 g body wt) at the end of the study (control: 5.0 $\pm$ 0.1 ($N = 6$) vs DMSO: 4.9 $\pm$ 0.1 ($N = 6$)). Based on the average water intake (Fig. 1), the animals were receiving between 4.9 and 5.6 g DMSO/kg body wt/day. It must be pointed out that this range of DMSO intake represents an *average range* because of the manner in which water intake was estimated. While there may have been individual variations in DMSO intake (due to individual variations in water intake), the similarity in the rate of weight gain (Fig. 1) and final liver weight between the two groups suggests that toxic doses of DMSO were not consumed. Furthermore, this level of DMSO treatment is well below the published estimates of toxic levels of DMSO in the rat (9). However, toxic effects of DMSO treatment were observed in a preliminary study in rats treated with 5% DMSO. Based on the estimated average water intake, those animals were receiving 22 g DMSO/kg body wt/day, which is within the toxic range for the rat (9). Interestingly, a similar dose of DMSO had no effect on weight gain in rabbits (2).

The effects of DMSO treatment on hepatic CH-7α hydroxylase activity and plasma cholesterol are shown in Fig. 2. CH-7α hydroxylase activity was significantly ($P < 0.005$) lower in the treated animals relative to that in controls. Consistent with the reduced CH-7α hydroxylase activity was the finding of significantly elevated ($P < 0.005$) plasma cholesterol in the treated animals.

Since a previous study showed that DMSO treatment might affect tissue cholesterol (1) and that hepatic GSH content is believed to affect CH-7α hydroxylase activity (18, 19), it was important to determine whether DMSO treatment had affected hepatic microsomal cholesterol and GSH content. As shown in Fig. 3, DMSO treatment had no effect on either hepatic microsomal cholesterol or GSH content.

The mechanism for the profound inhibition of CH-7α hydroxylase activity in DMSO-

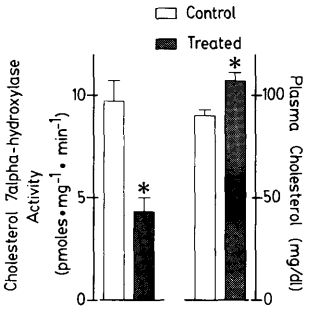

FIG. 2. Hepatic CH-7α hydroxylase activity and plasma cholesterol in control ($N = 6$) and DMSO-treated ($N = 6$) animals (means ± SEM). The animals were given either tap water or 2% DMSO in water to drink. On the ninth day after starting the treatment, the animals were killed for the assay of hepatic microsomal CH-7α hydroxylase activity and total plasma cholesterol. *Significantly different ($P < 0.005$) from corresponding control value.

treated animals is not known. Clearly, the inhibitory effect was not mediated by changes in microsomal cholesterol content or GSH content. While DMSO is known to have a variety of effects on membrane structure and function (20, 21), such effects are not necessarily predictive of specific changes in activities of membrane-bound enzymes (22–24). Further work, particularly with purified CH-7α hydroxylase-specific cytochrome P-450, is necessary to determine the basis for the effect of DMSO on GH-7α hydroxylase activity.

The lack of effect of DMSO on hepatic GSH content in the present study is interesting in view of a previous report where the hepatic GSH content was higher in mice treated acutely with DMSO (8 g/kg body wt twice) compared to *saline-treated controls* (25). The reason for the discrepancy between the two studies may lie in the differences in experimental protocol between these studies. In the previous study, DMSO was administered 20.5 and 8.5 hr before hepatic GSH content was determined. It is not clear if the animals had access to food during the time interval between dosing and GSH determination. This is important because hepatic GSH content can be reduced by short-term food deprivation (26). Since DMSO is a well-known scavenger of free radicals (27), a very important function also of hepatic GSH (28), it is entirely possible that the higher hepatic GSH content in the DMSO-treated animals (relative to saline-treated controls) was due to the GSH "sparing" effect of DMSO. In the present study, the animals were not subjected to acute DMSO administration or changes in feed intake. In addition, they were all killed at the same time (between 8:00 and 10:00 AM). Thus, under these conditions, the levels of DMSO used in this study did not affect hepatic GSH content in the rat.

The hypercholesterolemia observed in the treated animals can be explained by either or both of the following mechanisms. DMSO has been shown to inhibit the uptake of LDL by cultured fibroblasts (3). If DMSO had similar effects on other cells *in vivo,* then a reduction in the clearance of LDL from the circulation would tend to increase plasma cholesterol levels. It is well established that liver has the most important role in the clearance of LDL from the circulation (4) and that the receptor-mediated pathway of LDL uptake is by far the most important mechanism of uptake of LDL by the hepatocyte (4). In the rat, the major proportion of plasma cholesterol is transported by the high-density lipoprotein (HDL) fraction of plasma lipoproteins (29). However, the hepatocyte receptor for LDL, which is regulated by the metabolic demand for cholesterol (4), also binds HDL (30). Thus, if DMSO interferes with the binding of LDL to its receptor, then it is entirely plausible that it also interferes with the binding of HDL to the same receptor. Thus, reduced clearance of HDL from rat plasma would lead to hypercholesterolemia.

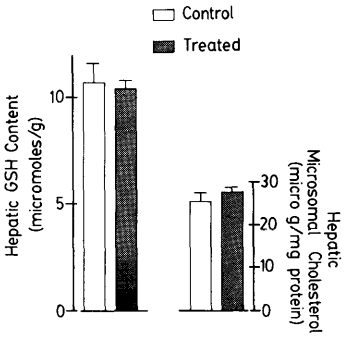

FIG. 3. Hepatic GSH content and microsomal cholesterol content in control and treated animals (mean ± SEM). The animals were treated as described in Fig. 2 following which hepatic GSH content and microsomal cholesterol content were determined in six animals from each group.

The other mechanism which could have contributed to the hypercholesterolemia is the inhibition of CH-7α hydroxylase activity by DMSO treatment. A reduction in CH-7α hydroxylase activity implies a reduction in the utilization of cholesterol for bile acid biosynthesis. Reduced catabolism of cholesterol combined with no change (or an incomplete compensatory reduction) in cholesterol biosynthesis would also lead to hypercholesterolemia.

In summary, this study has shown that DMSO treatment per se can induce profound changes in cholesterol metabolism. The use of high levels of dietary cholesterol in previous studies (1, 2) may have masked these changes in cholesterol metabolism induced by DMSO. Based on the findings of this study, use of DMSO in the treatment of atherosclerosis would be contraindicated. Detailed *in vivo* investigation is warranted to ascertain specifically how DMSO affects cholesterol and bile acid metabolism.

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