1 Acknowledgments. We are grateful to Sandoz Ltd for a gift of LL 21-943 and to I.C.I. for gifts of propranolol and atenolol.
2 T. Meinertz, H. Just, W. Kaspar, F. Kersting and K.-H. Brening, Lancet *1*, 270 (1979).
3 W.H. Frishman, B. Weksler, J.P. Christodoulou, C. Smithen and T. Killip, Circulation *50*, 887 (1974).
4 A.M. Michelakis and R.G. McAllister, J. clin. Endocr. *34*, 386 (1972).
5 F.A. Oski, L.D. Miller, M. Delwaria-Papactopoulos, J.H. Manchester and J.C. Shelburne, Science *175*, 1372 (1972).
6 B. Kristensen, E. Steiness and J. Weeke, J. clin. Pharmac. Ther. *23*, 624 (1978).
7 N. Emmelin, Experientia *21*, 57 (1965).
8 G. Kunos, B. Robertson, W.H. Kan, H. Preiksaitis and L. Mucci, Life Sci. *22*, 847 (1978).
9 J.H. Botting and A. Gibson, Lancet *1*, 875 (1979).
10 H. Boudoulas, R.P. Lewis, R.E. Kates and G. Dalamangas, Ann. intern. Med. *87*, 433 (1977).
11 J.A. Pantano and Y.C. Lee, Archs intern. Med. *136*, 867 (1976).
12 J.H. Myers and L.D. Horwitz, Circulation *58*, 196 (1978).
13 A.E.G. Rain and E.M. Vaughan Williams, Br. J. Pharmac. *70*, 205 (1980).
14 B.J. Clark, K. Saameli and F. Troxler, Br. J. Pharmac. *52*, 123P (1974).
15 G. Glaubiger and R.J. Lefkowitz, Biochem. biophys. Res. Commun. *78*, 720 (1977).
16 O. Pathomvanich, Ph.D. Thesis, University of London 1970.
17 J.R. Womble, D.R. Larson, J.G. Copeland, B.R. Brown and D.H. Russell, Proc. 8th int. Cong. Pharmac., 1981.
18 N. Himori, T. Ishimori and N. Taira, Archs int. Pharmacodyn. Ther. *242*, 115 (1979).

Manganese prolongation of pentobarbital hypnosis in the male rat[1]

M.J. Deimling and R.C. Schnell

Department of Pharmacodynamics and Toxicology, College of Pharmacy, University of Nebraska Medical Center, Omaha (Nebraska 68105, USA), 13 March 1981

Summary. Following manganese treatment, pentobarbital hypnosis was prolonged in male rats. The maximal effect occurred from 1 to 3 days following manganese treatment and the threshold dose was found to be 3 mg Mn^{++}/kg (i.p.).

Manganese is a physiologically essential trace metal in both man and animals, forming an integral part of the metalloenzymes, pyruvate carboxylase[2] and mitochondrial superoxide dismutase[3]. However, chronic or acute exposure to high levels of this metal can lead to various toxic syndromes[4] including hepatic damage[5,6].

The administration of other trace metals, such as lead[7] and cadmium[8], produces hepatic effects such as inhibition of drug biotransformation which leads to an altered responsiveness to several drugs including the barbiturates. This study was undertaken to examine the effect of acute manganese administration on drug response in the male rat.

Methods. Male, Sprague-Dawley rats, weighing 140–160 g, were obtained from Sasco, Inc. (Omaha, NE) and housed in community cages for at least 1 week prior to use. Animals were maintained in environmentally controlled rooms at approximately 22 °C under a 12/12 h (L:06.00–18.00 h) alternating light-dark cycle with free access to food (Purina Rat Chow, Ralston Purina Company, St. Louis, MO) and tap water.

Manganese ($MnCl_2 \cdot 4\ H_2O$) and pentobarbital Na solutions for injection were prepared using distilled, deionized water such that each animal received 1 ml/kg b. wt, i.p. The duration of hypnosis was defined as the time from loss to recovery of the righting reflex.

Statistical analyses were performed using an analysis of variance (ANOVA) followed by Duncan's New Multiple Range test[9] where appropriate. The acceptable level of significance was established at $p=0.05$.

Results. Preliminary studies in this laboratory showed that a manganese dose of 10 mg Mn^{++}/kg (i.p.) significantly prolonged the duration of pentobarbital hypnosis. Therefore, this dose was used to examine the time-course of manganese effect on drug response. Animals were treated with manganese at various time periods, ranging from 1 to 10 days, prior to

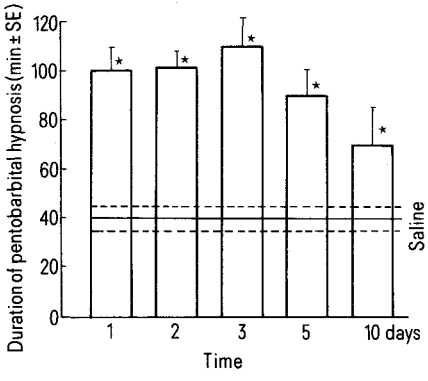

Figure 1. Time-course of manganese prolongation of pentobarbital hypnosis in male rats treated with manganese (10 mg Mn^{++}/kg, i.p.). At the specified time intervals animals received pentobarbital Na (35 mg/kg, i.p.) and the duration of hypnosis was determined. Controls received normal saline ten days prior to pentobarbital. *Significantly different from control animals ($p < 0.05$).

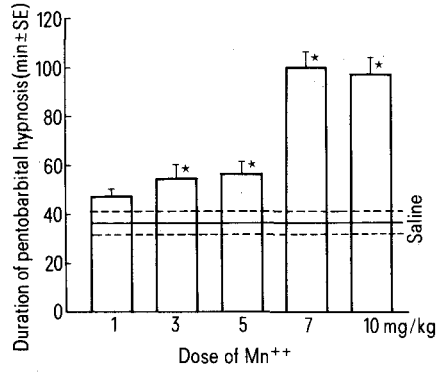

Figure 2. Dose-response of manganese prolongation of pentobarbital hypnosis in male rats. Male rats were treated with manganese (1–10 mg Mn^{++}/kg, i.p.) and 3 days later animals received pentobarbital Na (35 mg/kg, i.p.) and the duration of hypnosis was determined. *Significantly different from control animals ($p < 0.05$).

receiving pentobarbital Na (35 mg/kg, i.p.) and the subsequent determination of hypnotic duration. Control animals received normal saline 10 days prior to pentobarbital.

The results of this experiment demonstrated a significant prolongation of hypnosis in manganese-treated animals as compared with controls at all time periods examined (fig. 1). Maximal effect on drug response was observed 1-3 days following metal administration, with a slight decrease toward control values on days 5 and 10.

Next, the dose-response of manganese effect on drug response was examined. Manganese, in doses ranging from 1 to 10 mg Mn^{++}/kg (i.p.), was administered to male rats and 3 days later animals received pentobarbital Na (35 mg/kg, i.p.) and the duration of hypnosis was determined.

The results of this experiment indicated that manganese doses as low as 3 mg Mn^{++}/kg could significantly prolong the duration of pentobarbital-induced hypnosis (fig. 2). A manganese dose of 1 mg Mn^{++}/kg was without significant effect.

Discussion. The results of the present study indicate that manganese, at a threshold dose of 3 mg Mn^{++}/kg (i.p.), can alter drug response in the male rat. Furthermore, following a dose of 10 mg Mn^{++}/kg (i.p.), drug response is significantly altered for at least 10 days, with a maximal effect observed from days 1-3.

Several other metal ions have been shown to alter drug response. Included are arsenic and beryllium[10], cadmium[8], lead[7], and the methylmercuric ion[7,11].

Metal-induced alterations of drug response most likely result from decreased hepatic biotransformation as a result of decreased levels of cytochrome P-450[12]. Since Maines and Kappas[13] demonstrated that manganese can also decrease hepatic cytochrome P-450, this may explain the results observed in the present study. Further experimentation is required before this can be accurately determined.

1 Acknowledgments. This work was supported by NIEHS Research Grant ES-02425. These data were submitted as part of a Ph.D. thesis to Purdue University, West Lafayette, Indiana, by M.J. Deimling.
2 M.C. Scrutton, M.F. Utter and A.S. Mildvan, J. biol. Chem. *241*, 3480 (1966).
3 R.A. Weisinger and I. Fridovich, J. biol. Chem. *248*, 3582 (1973).
4 G.C. Cotzias, Physiol. Rev. *38*, 503 (1958).
5 D.J. Baxter, W.O. Smith and G.C. Klein, Proc. Soc. exp. Biol. Med. *119*, 966 (1965).
6 D. Wasserman and M. Wasserman, Environ. Res. *14*, 379 (1977).
7 A.P. Alvares, S. Leigh, J. Cohn and A. Kappas, J. exp. Med. *135*, 1406 (1972).
8 W.M. Hadley, T.S. Miya and W.F. Bousquet, Toxic. appl. Pharmac. *28*, 284 (1974).
9 D.B. Duncan, Biometrics *11*, 1 (1955).
10 H.A. Riberio, Proc. West. pharmac. Soc. *13*, 13 (1970).
11 A.P. Alvares, J. Cohn and A. Kappas, Drug Metab. Dispos. *2*, 259 (1974).
12 M.D. Maines and A. Kappas, Science *198*, 1215 (1977).
13 M.D. Maines and A. Kappas, Biochem J. *154*, 125 (1976).

Mannitol treatment of cerebral edema in rats with galactosamine-induced severe hepatitis[1]

W. Zimmerli[2], Ch. Grubinger, H. Thölen, M. Oberholzer and L. Bianchi

Department of Medicine, Kantonsspital, University of Basel, CH-4031 Basel (Switzerland), and Institute of Pathology, Kantonsspital, University of Basel, CH-4031 Basel (Switzerland), 26 February 1981

Summary. Severe hepatitis is induced with D-galactosamine hydrochloride in rats. Animals develop brain edema which was treated with mannitol. 1 h after the last mannitol infusion brain water content decreased, but it was not decreased 6 h after treatment. The therapy reduced lethality ($p < 0.025$). The rigorous brain edema therapy improved the prognosis of fulminant hepatic failure in the rat.

Fulminant hepatic failure in man is highly lethal[3]. Its etiology may be viral hepatitis[4], toxin or drug-induced massive hepatic necrosis[5,6], or fatty degeneration seen with excessive tetracyclin[7], and Reye's syndrome[8].

Several attempts have been made to diminish the lethality of acute hepatic failure. It is difficult, however, to test the efficacy of any kind of therapy, because 1. fulminant hepatic failure is a rare event, 2. the course of the disease in an individual patient is unpredictable, and 3. there are numerous etiologies[4-15].

In liver failure, several authors have observed a high incidence of cerebral edema proved by autopsy[16-20]. Gröflin found a parallel increase in cerebral water content and in the occurrence of cerebral symptoms in an animal model; galactosamine (gal-N) induced severe hepatitis[21]. These observations suggest the importance of supportive care in the therapy of the acute liver failure. Since the liver has an extraordinary capacity for regeneration[22], we postulate that the prognosis of fulminant hepatic failure is mainly determined by the efficient treatment of brain edema in the acute stage[13-15]. To test this hypothesis we have developed an animal model with gal-N hepatitis. Gal-N is a selective hepatotoxin[23-25]. The pathogenesis of the gal-N hepatitis is characterized by well-defined biochemical alterations[26,27].

Among the different kinds of cerebral edema[28], brain edema in fulminant hepatic failure shows characteristics of the cytotoxic type. In this situation osmotherapy is believed to be most effective[28]. Accordingly, we selected a 20% mannitol solution to establish an osmotic gradient from blood to cerebrospinal fluid[29]. Mannitol has been shown to reduce intracranial pressure, as documented by clinical trials[13-15], and in animal models[30,31].

In the present study we investigated whether rigorous treatment of brain edema could ensure survival until liver regeneration. We showed that mannitol lowers brain water content and reduces lethality in rats with gal-N induced hepatic failure.

Materials and methods. Animals. We used inbred female albino rats (KSBK 60, origin Wistar strain) weighing 180–220 g. All animals were allowed free access to food (Nafag®, Gossau, Switzerland) and water throughout the experiments. They were housed individually in metabolic cages at 25 °C under optimal hygienic conditions.

Infusion system. A polyethylene tube with an inner diameter of 0.4 mm (Portex) was introduced in neuroleptanalgesia (Hypnorm®, Duphar, Amsterdam) by a modified technique described by Engberg[32]. A perfusion pump (UNITA I, Braun Melsungen AG) allowed a precise dosage of drugs through the permanent access to the venous system.