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.

Reagents. A solution of D-galactosamine-HCl (Gal-N) (E. Merck, Darmstadt) in a single dose of 2.25 g/kg b.wt was given i.v. about 48 h after the narcosis. Gal-N was always prepared fresh (solution: 0.4 N in physiological saline, pH adjusted to 6.8[33]). Mannitol was given as a solution of 20% (wt/v).

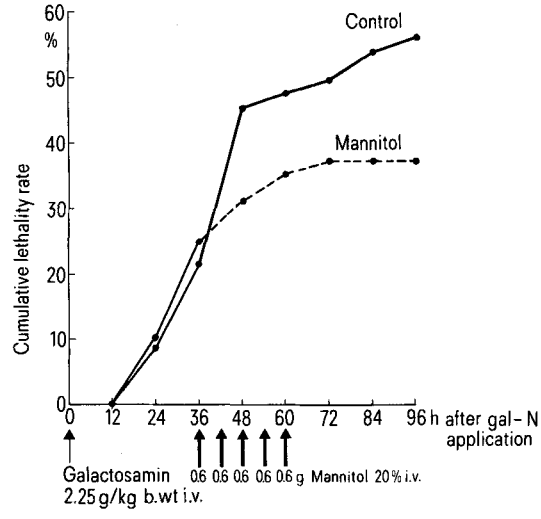
Experimental design. a) Control group (47 rats): insertion of the venous catheter as described, permanent infusion of physiological saline (1 ml/h) over 7 days. Application of gal-N 48 h after anesthesia.

b) Mannitol group (58 rats): insertion of the catheter and gal-N application were identical to the controls. A dose of mannitol (0.6 g/30 min) was infused every 6 h from the 36th to the 60th h after gal-N administration. Animals were continuously observed during the treatment period in order to detect an occasional iatrogenic death defined as serum osmolarity > 360 mosmol (determined immediately after death) and/or oliguria < 5 ml/12 h. These criteria were defined according to observations in preliminary experiments in which these situations led uniformly to death in otherwise healthy animals. Infusion of physiological saline (1 ml/h) was continous except during gal-N and mannitol application. The survival rate was determined after 96 h. Control observations of the liver histology in the acute stage and 4 weeks after gal-N administration were made in 12 animals.

c) In 3 other groups of rats (with gal-N hepatitis with and without mannitol treatment, and healthy animals) prothrombin time, SGOT, SGPT, total protein and albumin were determined. Blood was sampled by heart puncture immediately after killing the animals by ether.

d) Another 96 rats were killed by decapitation 36, 42, or 48 h after gal-N application, or 1 or 6 h after the last mannitol infusion. The brain was removed and dissected into the 2 hemispheres, cerebellum and brain stem. The specimens were dried in an oven at 100 °C for 24 h. The water content of these parts was determined by difference between the wet and the dry weight.

Results. About 30 h after the application of 2.25 g gal-N/100 g b.wt i.v. the rats became immobile. Their body heat fell from 38.1 °C to a minimal value of 35 °C at 32 h. Violent trembling and uncontrolled motor activity were also observed. Most animals showed and abnormal tendency to bleed (subconjunctival hematoma, epistaxis). Some animals of both groups had a paraparesis of the hind legs, due to a hematomyelia, as confirmed by autopsy. A modification of these signs of liver damage under mannitol treatment was not observed, but an improvement of the



Cumulative lethality rate.

Table 1. Cerebral water content (g H_2O/100 g wet weight)

	42 h after gal-N application 1 h after last mannitol dose		6 h after last mannitol dose	
Cerebrum (left hemisphere)	77.95 ± 0.46	78.47 ± 0.37	78.51 ± 0.36	78.55 ± 0.30
	$p < 0.01$***		$p > 0.05$ (NS)	
Cerebrum (right hemisphere)	78.04 ± 0.61	78.45 ± 0.30	78.46 ± 0.35	78.45 ± 0.35
	$p < 0.05$		$p > 0.05$ (NS)	
Cerebellum	77.11 ± 1.10	77.73 ± 0.40	77.54 ± 0.96	77.71 ± 0.56
	$p < 0.01$		$p > 0.05$ (NS)	
Brain stem	72.83 ± 0.70	73.65 ± 0.60	73.68 ± 0.48	73.74 ± 0.70
	$p < 0.004$		$p > 0.05$ (NS)	

* Mannitol-treated rats; ** rats without treatment (control); *** mean ± SD, statistical analysis: Mann-Whitney-test, $p = 0.05$ defined as limit of significance.

Table 2. Serum values in mannitol-treated and control rats with gal-N induced hepatitis and in healthy animals

	gal-N hepatitis Mannitol treatment	No treatment	Normal values
Prothrombin	4.6 ± 0.9%* (13)[a]	6.1 ± 0.9%* (13)	100 ± 1.0% (10)[b]
Prothrombin	50.8 ± 11.4%** (8)[a]	44.9 ± 12.9%** (10)	
SGOT	517 ± 188 U/l** (8)[a]	346 ± 112 U/l** (9)	42 ± 4 U/l (10)[c]
SGPT	841 ± 415 U/l** (8)[a]	482 ± 215 U/l** (9)	31 ± 3 U/l (10)[d]
Total protein	47.1 ± 1.1 g/l** (7)[a]	47.7 ± 1.6 g/l** (10)	60.1 ± 1.3 g/l (10)[b]
Albumin	25.2 ± 0.6 g/l** (6)[a]	25.5 ± 0.7 g/l** (8)	34.9 ± 0.2 g/l (10)[b]

* 24 h after gal-N application; ** 49 h after gal-N application; statistical analysis: unpaired t-test: [a] $p > 0.05$ (NS) (mannitol vs no treatment), [b] $p < 0.001$, [c] $p < 0.01$, [d] $p < 0.025$ (normal values vs values in gal-N hepatitis with or without treatment). All values are mean ± SEM with the number of rat in parentheses.

coma was frequent (analogous to our observations in man[16]).

The overall lethality in the mannitol-treated group was 48.3% (28 of 58 rats), in the control group 57.4% (27 of 47 rats). 20.7% (12 of 58 rats) of the mannitol group rats died between the 12th and the 36th h, i.e. before the beginning of the mannitol application (control: 21.3%) (fig.). According to the criteria defined prior to the experiments (see methods) 10 of 58 rats suffered an iatrogenic death. Excluding animals which died before treatment or through mannitol toxicity, we found a lethality of 16.7% (6 of 36 rats) in the treated and 44.4% (16 of 36 rats) in the control group ($p < 0.025$ Fisher's exact test).

The effect of mannitol on cerebral water content was significant at 36, 42 and 48 h after gal-N application. The decreased brain water was found 1 h after the last mannitol dose. However, 6 h after the last mannitol infusion no reduction in cerebral water content was observed. The average values for the brain water content in rats killed after 42 h are given in table 1. At 36 h and 48 h essentially similar data were obtained.

The liver damage as defined by prothrombin time, serum values of transaminases, total protein and albumin, and histological examination were not significantly different in the 2 groups with gal-N hepatitis (table 2).

4 weeks after gal-N treatment complete restitution of liver structure with only minimal intralobular inflammatory infiltration was histologically seen in both groups with gal-N hepatitis.

Discussion. Gal-N produces an acute liver failure in rats that shows the clinical, histological and biochemical disturbances found in humans with acute liver failure[23-25, 34]. Fulminant liver failure is associated with cerebral edema[16-21]. Therefore, the treatment of cerebral edema has primary relevance to acute hepatic necrosis. The principal rationale of such efforts is to sustain life until the liver regenerates sufficiently to assure survival.

To our knowledge there are no clinical studies which prove the effectiveness of any kind of therapy in fulminant hepatic failure. In animal models, charcoal hemoperfusion improved the survival significantly in rats with gal-N hepatitis[12]. Alpha-tocopherol as a biological antioxidant, and hydrocortisone have shown an improvement of morphological changes of the liver, but the lethality rate has not been determined[35,36]. Hanid et al. showed a significant increase of intracranial pressure in pigs after hepatic devascularization. Despite a lowering of intracranial pressure by methylprednisolone, given at the moment of the devascularisation, survival was not improved[37]. This result could be explained by irreversible liver damage.

In the present study cerebral edema associated with necrosis of the liver was treated with mannitol. The diminished lethality can be attributed to the reduction of the cerebral water content. Because the effect of mannitol on brain water content is transient, the infusions must be given in intervals of less than 6 h. The high iatrogenic lethality seen in our study may be due to the fixed mannitol dose. By adjusting individual dosages according to serum osmolarity and intracerebral pressure, side effects can be avoided[38].

Mannitol is not metabolized and is excreted unchanged in the urine[28,30]. Mannitol did not modify the liver damage defined by prothrombin time, serum controls of transaminases, protein and histological examination. Therefore, metabolic improvement of the liver damage or cerebral function by mannitol is very unlikely.

Our results with mannitol treatment and Chirito's experience with charcoal hemoperfusion[12] suggest that a combined application of these 2 measures in acute hepatic failure may be the most effective approach. Clinical trials have to confirm this impression.

1 Acknowledgments. We thank Mr Zarko Rajacic for expert technical help and Professor Werner Stauffacher for discussion of the manuscript and critical comments.

2 Requests for reprints: W. Zimmerli, Divisions des Maladies infectieuses, Hôpital cantonal, CH-1211 Genève, Switzerland.

3 C. Trey and C.S. Davidson, in: Progress in Liver Diseases, vol. 3, p. 282. Ed. H. Popper and F. Schaffner. Grune and Stratton, New York 1970.
4 B. Lucké and T. Mallory, Am. J. Path. *22*, 867 (1946).
5 G. Klatskin, in: Diseases of the Liver, p. 604. Ed. L. Schiff. J.B. Lippincott, Philadelphia 1975.
6 D. Vergani, G. Mieli-Vergani, A. Alberti, J. Neuburger, A.L.W.F. Eddleston, M. Davis and R. Williams, New Engl. J. Med. *303*, 66 (1980).
7 J.C. Schultz, J.S. Adamson, Jr, W.W. Workman and T.D. Norman, New Engl. J. Med. *269*, 999 (1963).
8 R.D.K. Reye, G. Morgan and J. Baral, Lancet *2*, 749 (1963).
9 A.G. Redeker, I.L. Schweitzer and H.S. Yamahiro, New Engl. J. Med. *294*, 728 (1976).
10 European Association for the study of the liver (EASL), Gut *20*, 620 (1979).
11 A.G. Redeker and H.S. Yamahiro, Lancet *1*, 3 (1973).
12 E. Chirito, B. Reiter, C. Lister and T.M.S. Chang, Artificial Organs *1*, 76 (1977).
13 S.L. Newman, D.B. Caplan and P.A. Ahmann, New Engl. J. Med. *299*, 1079 (1978).
14 D.A. Trauner, F. Brown, E. Ganz and P.R. Huttenlocher, Ann. Neurol. *4*, 275 (1978).
15 B.A. Shaywitz, J.M. Leventhal, M.S. Kramer and J.L. Venes, Pediatrics *59*, 595 (1977).
16 H. Thölen, Klin. Wschr. *50*, 296 (1972).
17 R.C. Pirola, J.M. Ham and R.G. Elmslie, Gut *10*, 898 (1969).
18 A.J. Ware, A.N. D'Agostino and B. Combes, Gastroenterology *61*, 877 (1971).
19 D.B.A. Silk, P.N. Trewby, R.A. Chase, P.J. Mellon, M.A. Hanid, M. Davies, P.G. Langley, P.G. Wheeler and R. Williams, Lancet *2*, 1 (1977).
20 A. Hanid, R.L. Mackenzie, P.J. Mellon, M. Davis and R. Williams, Clin. Sci. molec. Med. *52*, 22 (1977).
21 U.B. Gröflin and H. Thölen, Experientia *34*, 1501 (1978).
22 R. Williams, Br. med. J. *1*, 213 (1971).
23 D. Keppler, R. Lesch, W. Reutter and K. Decker, Exp. molec. Path. *9*, 279 (1968).
24 K. Decker and D. Keppler, in: Progress in Liver Diseases, vol. 4, p. 183. Ed. H. Popper and F. Schaffner. Grune and Stratton, New York 1972.
25 B.L. Blitzer, J.G. Waggoner, E.A. Jones, H.R. Gralnick, D. Towne, J. Butler, V. Weise, I.J. Kopin, I. Walters, P.F. Teychenne, D.G. Goodman and P.D. Berk, Gastroenterology *74*, 664 (1978).
26 W. Reutter, B. Hassels and R. Lesch, in: New Trends in the Therapy of Liver Diseases, p. 121. Ed. A. Bertelli. Karger, Basel 1975.
27 M. Grün, H. Liehr and U. Rasenack, Acta hepatogastroenterol. *23*, 64 (1976).
28 R.A. Fishman, New Engl. J. Med. *293*, 706 (1975).
29 B.L. Wise, R.K. Perkins, E. Stevenson and K.G. Scott, Exp. Neurol. *10*, 264 (1964).
30 B.L. Wise and N. Chater, J. Neurosurg. *19*, 1038 (1962).
31 F.D. Brown, L. Johns, J.J. Jafar, H.A. Crockard and S. Mullan, J. Neurosurg. *50*, 423 (1979).
32 A. Engberg, Acta physiol. scand. *75*, 170 (1969).
33 D. Horton, in: The Amino Sugars, vol. I A, p. 3. Ed. R. Jeanloz. Academic Press, New York 1969.
34 J. Terblanche, R. Hickman, D. Miller, et al., in: Artificial Liver Support, p. 163. Ed. R. Williams and I. Murray-Lyon. Pitman, London 1975.
35 A.A. Mihas, W. Doos, R.G. Gibson and B.I. Hirschowitz, Ala. J. med. Sci. *13*, 384 (1976).
36 St.K. Bartsokas and D.G. Papadimitriou, Endokrinologie *69*, 321 (1977).
37 M.A. Hanid, R.L. Mackenzie, R.E. Jenner, R.A. Chase, P.J. Mellon, P.N. Trewby, I. Janota, M. Davis, D.B.A. Silk and R. Williams, Gastroenterology *76*, 123 (1979).
38 C.P. MacGraw, Johns Hopkins med. J. *143*, 169 (1978).