elastin bears compressive loads in the radial direction, i.e., that elastin contributes to the resistance of the wall to thinning. Histologic examination of several vessels after treatment with elastase disclosed decreased uptake of Verhoff's elastica stain and occasional broken elastic lamellae.

Collagenase experiments. The wall thickness of 106 arterial segments under control conditions was 0.110 ± 0.001 cm, whereas the wall thickness after treatment with collagenase was 0.104 ± 0.001 cm. This 5.5% reduction was statistically significant ($p < 0.5$). Figure 2 presents radial compressive stresses for the 106 pairs of arterial segments plotted as a function of wall compression. These data show that degradation of wall collagen did not significantly alter wall compressive stress (p = NS), in spite of the reduction in wall thickness. These data suggest, but do not prove, that collagen does not bear a significant portion of radial compressive loads. Histologic examination of several vessels after treatment with collagenase disclosed decreased uptake of Masson's trichrome stain.

Pre-extended arterial sheets. The above studies were carried out on arterial segments that were not stretched in the circumferential or longitudinal directions to avoid multidirectional interactions. However, studying unstretched vessels permitted the connective tissues to retract, possible reorienting them into the radial direction. Elastic lamellae may be observed to retract and reorient in this fashion in vessels that are fixed while undistended[5]. In order to avoid such retraction, 21 additional pairs of vessel segments were pre-stretched simultaneously 50% in both the circumferential and longitudinal directions, and then subjected to stepwise compression. These expriments showed that treatment with elastase (N = 8) significantly decreased radial compressive stress ($p < 0.05$), but that treatment with collagenase (N = 13) did not do so (p = NS). Therefore, it was concluded that in both unstretched and prestretched vessels, treatment with elastase, but not collagenase, decreased radial stress elicited in response to wall compression.

Specificity of enzymes. Use of enzymatic agents raises questions of specificity. Ideally one would employ mammalian granulocyte elastase and mammalian collagenase, but unfortunately neither of these enzymes are commercially available. Therefore, the present study used Worthington ESFF elastase derived from porcine pancreas, and Worthington CLSPA collagenase derived from *Clostridia histolyticum*. Both of these agents are among the most purified and most specific commercially available (Worthington Biochemical Manual, Freehold, New Jersey, USA), but both may act on a variety of substrates besides pure elastin and pure undenatured collagen[6,7]. Moreover, there are some amino acid sequences that are common to both elastin and collagen[8] raising the possibility that some degradation of both connective tissues may have occurred with either enzyme. However, in the present expriments it was found that treatment with the commercially available enzymes produced 1) different histological effects, 2) opposite changes in wall thickness, and 3) different mechanical responses. These observations suggest that, for the most part, mutually exclusive responses were obtained.

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Effect of coenzyme-A, NAD, alpha lipoic-acid and cocarboxylase on survival of rats with galactosamine-induced severe hepatitis

H. Thölen, W. Zimmerli and Z. Rajacic

Dept Innere Medizin and Dept Forschung, Kantonsspital, CH-4031 Basel (Switzerland), 26 July 1984

Summary. Galactosamine, a selective hepatotoxin, produces in rats histologic alterations, which show the characteristics of severe human viral hepatitis. In the present study the efficacy of two different cofactor regimens (coenzyme A, NAD, alpha lipoic-acid, cocarboxylase) in rats with fulminant galactosamine hepatitis were tested. The results showed an improvement of the short-term survival with a short-term treatment and definitely better survival with a long-term regimen with cofactors.
Key words. Liver failure; hepatitis; galactosamine; coenzyme A; NAD.

Introduction. Fulminant liver failure induces important disturbances of liver function including decreased synthesis of essential substances, reduced detoxification of metabolites and alteration of the intermediary metabolism. We previously reported alterations in the decarboxylation of pyruvate with increased blood levels of pyruvate, lactate, acetoin and 2,3-butylene glycol in patients with fulminant hepatitis[1,2]. Pyruvate has to be decarboxylated and oxidized in order to enter the tricarboxylic acid cycle[3]. These key reactions require coenzyme A, NAD, alphalipoic acid and cocarboxylase as cofactors[4]. The lack of one or several of these cofactors might be responsible for the metabolic disorders in patients with acute liver disease. On the basis of this hypothesis, we conducted an uncontrolled clinical study with cofactor treatment[5,6]. The study was performed prior to the general availability of intensive care unit facilities, mannitol treatment and hemoperfusions in the treatment of fulminant hepatitis. Ten out of 26 (38.5%) comatose patients – 20 in stage IV and 6 in stage III – survived[6]. In a later cooperative international study without cofactor treatment only 10/100 (10%) patients with liver coma survived[7].

Since the uncontrolled study indicated a benefit of the cofactor treatment, we were encouraged to check this effect in an animal model and chose the recently described galactosamine (gal-N) hepatitis rat model for this purpose[8–11]. The ideal animal model for cofactor treatment in acute liver failure has to show the histologic characteristics of human disease, namely a combination of necrotic, damaged and intact hepatocytes; the alterations in the intermediary metabolism mentioned above are induced by damaged and not by necrotic liver cells. Gal-N, a selective hepatotoxin, fulfills this requirement by imitating the histologic alter-

Serum values in cofactor treated and control rats with gal-N induced hepatitis and in healthy animals

	gal-N Hepatitis Cofactor treatment* 2 d**	No treatment 2 d	6 d	15 d	Normal values
SGOT	306 ± 100 (17)***[a]	547 ± 156 (16)[b]	226 ± 22 (5)[c]	218 ± 20 (6)[c]	117 ± 27 (8)
SGPT	346 ± 138 (18)[a]	892 ± 350 (15)[b]	42 ± 9 (6)[a]	67 ± 11 (6)[a]	45 ± 14 (8)
Alkaline phosphatase	222 ± 32 (19)[b]	164 ± 17 (16)[a]	136 ± 13 (6)[a]	128 ± 11 (6)[a]	123 ± 14 (10)
Prothrombin	56 ± 7 (19)[e]	49 ± 9 (16)[e]	77 ± 6 (6)[c]	95 ± 1 (6)[a]	99 ± 0.6 (10)
Albumin	22.3 ± 0.6 (19)[e]	23.7 ± 0,8 (16)[c]	30.8 ± 2.2 (6)[a]	31 ± 2.2 (6)[a]	27.2 ± 1 (10)
Urea	5 ± 0.3 (19)[d]	5.7 ± 0.3 (16)[d]	6.3 ± 0.4 (6)[a]	8 ± 0.6 (6)[a]	7.7 ± 0.6 (10)
Creatine	78 ± 2.9 (9)[a]	75 ± 3.9 (8)[b]	91 ± 5.1 (6)[a]	80 ± 2 (6)[a]	86 ± 4.5 (10)

* Cofactor treatment: Infusion of 6 mg CoA, 5.2 mg NAD, 1.6 mg alpha-lipoic acid and 3.3 mg cocarboxylase per 24 h started 24 h after induction of gal-N hepatitis. Statistical analysis (unpaired t-test): No significant difference between treated and untreated rats for values at 2 d. ** Interval in days between an intravenous dose of 2.25 g/kg b.wt gal-N and blood sampling. *** All values are mean ± SEM with the number of rats in paranthese. [a-d]Statistical analysis (comparison with normal values) with unpaired t-test: a) nonsignificant ($p < 0.05$); b) $p < 0.05$; c) $p < 0.01$; d) $p < 0.005$; e) $p < 0.0005$.

ations of severe viral hepatitis[12]. In the present study we tested the efficacy of two different cofactor regimens in rats with fulminant gal-N hepatitis. We found an improvement of the short-term survival with a short-term treatment and a definitely better survival with a long-term regimen with cofactors.

Methods. Animals. First experiment: Inbred female albino rats (KSBK 60, origin Wistar strain) weighing 180–220 g. Second to fourth experiments: Inbred female albino rats (Wistar Hannover) weighing 180–220 g. The strain Wistar Hannover was used, because there was cannibalism in the strain KSBK 60 during breeding after the first experiment, with unpredictable susceptibility to gal-N and with increased concentrations of coagulation factors. 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, under neuroleptanalgesia (INNOVAR-VET, Pitman-Moore, Washington), by a modified technique described by Engberg[13, 14]. A perfusion pump (Unita I, Braun Melsungen AG, Germany) allowed a precise dosage of drugs into the right jugular vein.

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 narcosis. Gal-N was always freshly prepared (solution: 0.4 N in physiological saline, pH adjusted to 6.8)[11]. Cofactors: Alpha lipoic acid, Fluka, Buchs (SG), Switzerland; Coenzyme A, free acid, Boehringer, Mannheim, Germany; Nicotinamide-adenine-dinucleotide, free acid, Boehringer, Mannheim, Germany; Cocarboxylase, Berolase®, Hoffman-La Roche, Basel, Switzerland. The solutions of the cofactors were freshly prepared for each infusion.

Experimental design. First experiment: Control group (40 rats KSBK 60, origin Wistar strain): insertion of the intravenous catheter as described, permanent infusion of physiological saline (1 ml/h) over 3 days. Application of gal-N 48 h after anesthesia. Treatment group (46 rats KSBK 60, origin Wistar strain): insertion of the catheter and gal-N application were identical with the controls. A continuous infusion of 12 mg coenzyme A, 10.4 mg NAD, 3.2 mg alpha lipoc acid and 6.6 mg cocarboxylase per 24 h was given over a period of 52 h, starting 24 h after gal-N application. Second experiment: Control group (21 rats strain Wistar Hannover): insertion of the catheter and gal-N application as above, permanent saline infusion (1 ml/h) over 9 days. Treatment group (27 rats strain Wistar Hannover): insertion of the catheter and gal-N application as above. A continuous infusion of 6 mg coenzyme A, 5.2 mg NAD, 1.6 mg alpha lipoic acid and 3.3 mg cocarboxylase per 24 h was given over a period of 220 h, starting 24 after gal-N application. The survival rate in all groups was determined each day during 28 days.

Third experiment: In 10 rats (strain Wistar Hannover) prothrombin content was determined 24 h after gal-N application, i.e. prior to cofactor treatment and compared to control values in 10 healthy rats. Blood was sampled by tail vein puncture.

Fourth experiment: Sampling of blood in five other groups of rats, strain Wistar Hannover (2 d after gal-N application and at the end of a 24 h infusion with cofactors in the composition given for the 2nd experiment; 2 d, 6 d and 15 d after gal-N

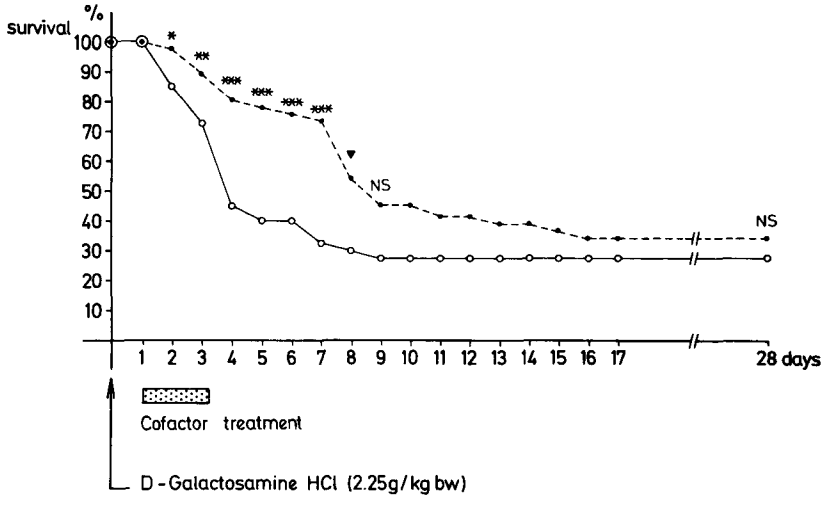

Figure 1. Survival of cofactor-treated and control animals (KSBK60, origin Wistar strain) with fulminant hepatitis. Cofactor treatment: 12 mg CoA, 10.4 mg NAD, 3.2 mg alpha-lipoic acid and 6.6 mg cocarboxylase per 24 h. The arrow indicates the induction of hepatitis with D-galactosamine-HCl. 46 rats were treated with cofactors during 52 h (●——●), and 40 rats received saline (○——○). Statistical analysis with Fisher's exact test: *$p < 0.05$; **$p < 0.005$; ***$p < 0.0005$; $p < 0.025$; NS, nonsignificant.

application, respectively, but without cofactor treatment; healthy animals) and determination of prothrombin content, albumin, GOT, GPT, alkaline phosphatase, urea and creatinine. Blood sampled by subconjunctival puncture immediately after killing animals by ether.

In animals of the third and fourth experiments no catheter was inserted.

Results. The clinical symptoms after gal-N treatment were similar to the ones previously described[9,10]. Figure 1 shows that during 52 h of treatment with cofactors (12 mg CoA/24 h) the daily death rate of control animals exceeded that of treated rats. This difference was seen despite the fact that 4/46 treated rats – included in the statistical analysis – died with anuria 48–72 h after gal-N application. The survival of treated rats was significantly better (Fisher's exact test) than that of control rats during 7 days. At this time the difference decreased due to a higher late death rate in treated animals.

This regimen seemed to be too short, since animals continued to die during 15 days. The table shows that prothrombin levels were normalized only 15 days after induction of hepatitis. We therefore performed a second series of experiments with a cofactor-treatment during 220 h. Furthermore, in order to test the dose-response effect of CoA, we treated these rats with a lower dose of cofactors (6 mg CoA/24 h). Figure 2 shows that with this dose the survival of the treated group was higher at a nonsignificant level during 5 days and at a significant level from the 6th day of treatment until the end of the observation period, 28 days after gal-N application. These differences were seen despite the fact that 5/27 treated rats, which were included in the analysis, developed anuria and died during the first two days of treatment.

Prothrombin content, as a parameter of liver damage, was 8.7 ± 2% 24 h after gal-N application, as compared to 99 ± 2% in healthy control rats. The table shows that prothrombin content was still reduced at the 6th day, but normal 15 days after gal-N application.

In order to detect a possible beneficial effect of cofactor treatment on the liver function we determined several parameters for necrosis and for metabolic activity. As shown in the table, we were not able to detect significant differences between untreated and treated rats with acute liver failure due to gal-N.

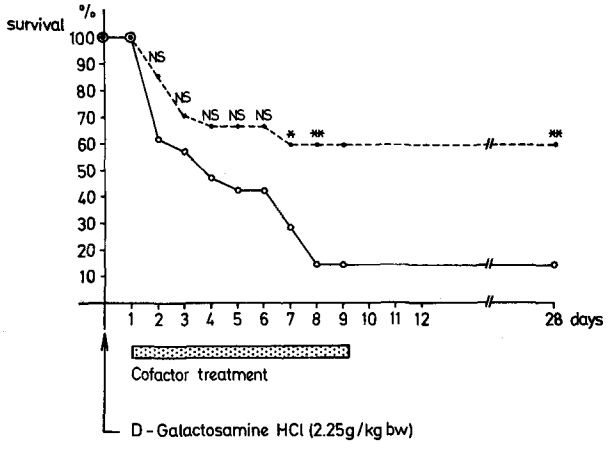

Figure 2. Survival of cofactor-treated and control animals (Wistar Hannover strain) with fulminant hepatitis. Cofactor treatment: 6 mg CoA, 5.2 mg NAD, 1.6 mg alpha-lipoic acid and 3.3 mg cocarboxylase per 24 h. The arrow indicates the induction of hepatitis with D-galactosamine-HCl. 27 rats were treated with cofactors during 220 h (●——●), and 21 rats received saline (○——○). Statistical analysis with Fisher's exact test: *$p < 0.05$; **$p < 0.005$; NS, nonsignificant.

Discussion. Fulminant hepatic failure in man is still highly lethal. The currently best documented treatment for this disease is hemoperfusion[15] and symptomatic treatment of brain edema with mannitol[11,16]. The present study was performed with the hypothesis that metabolic disturbances could trigger the fatal sequence of pathologic events in fulminant hepatitis. We previously reported disturbances of the transformation of pyruvate to acetyl-coenzyme A in patients with acute hepatic failure due to different etiologies[1,2]. This observation is the rationale for treating acute hepatic failure with the cofactors of this metabolic pathway. A previous uncontrolled clinical study with intravenous application of cofactors showed better survival in treated patients than in patients reported in the literature[5,6]. Such a treatment is only rational when it can be shown that intravenously injected cofactors penetrate liver cells and have an effect on the metabolism of hepatocytes. We have demonstrated that these requirements are fulfilled[17,18]. Coenzyme A is increased in liver cells but not in brain tissue after intravenous injection in rats[18]. Furthermore, our group showed that the addition of either of the cofactors to incubated liver slices from rats increases their oxygen consumption[17]. These observations imply that cofactors may also change liver cell metabolism in vivo.

In a previous publication we described a rat model of gal-N induced severe acute liver failure (confirmed by biochemical parameters and histological findings), in which we were able to apply intravenous drugs over a prolonged period[11,13]. With this model we have now investigated the effect of the treatment with cofactors on the survival of rats with acute hepatic failure. The beneficial short-term effect of high doses of cofactors during the first 52 h indicates that the better survival must be treatment-related. The loss of the favorable effect shortly after stopping treatment at a time at which gal-N hepatitis was still active is further important evidence. The prolongation of treatment with cofactors up to the 9th day after gal-N application confirmed their protective effect, since it allowed a significantly better survival up to the end of the observation period after 4 weeks. The fact that the low dose treatment (6 mg CoA/24 h) was less protective during the first two days than the high dose treatment (12 mg CoA/24 h) provides a further argument for the presumption that the beneficial effect on survival was indeed due to cofactors.

There was cannibalism in the rat strain KSBK 60, origin Wistar, during breeding after the first experiment, with unpredictible susceptibility to gal-N and with increased concentrations of coagulation factors. Therefore it was necessary to continue our experiments with another strain, Wistar Hannover. These two strains differ in the base line of the surviving rats without cofactor treatment, 30% for KSBK 60 and 15% for Wistar Hannover. This is of no importance, because in our experience there is also a difference in the base line of the surviving rats in the same strain, KSBK 60, origin Wistar: 30% in this study and 43% in the study published in 1981[11]. Little is known about side effects of cofactor application. It is surprising that in our animal model only cofactor treated rats died with anuria, which could be a treatment-related side effect. Angielski et al.[19] offer an explanation for this toxicity, demonstrating that CoA and NAD, which are metabolized to adenosine, induce acute renal failure in rats[19].

The mechanism of the beneficial effect of cofactors on survival remains unknown. The causes of death in human acute liver failure are brain edema, hemorrhage and infection. We previously observed brain edema in rats with gal-N hepatitis[9,10] and could demonstrate the protective effect of mannitol treatment in these animals[11]. Canalese et al. could recently confirm this experimental data in a clinical study[16]. It is supposed that damaged liver cells produce toxic substances like ethanthiol which inhibit cerebral Na-K-ATPase[20,21]. It is therefore conceivable that brain edema could be prevented, if cofactors prevent the production of toxic substances. We are currently investigating this intriguing possibility.

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A comparison of the effects of the optical isomers of isoproterenol on energy metabolism in a mouse sarcoma

G. R. N. Jones and R. Thatcher[1]

Department of Surgery, King's College Hospital Medical School, The Rayne Institute, 123 Coldharbour Lane, London SE5 9NU (England), and King's College Hospital Medical School, Denmark Hill, London SE5 8RX (England), 6 June 1984

Summary. Disturbance to energy production in the S180 sarcoma (CB) by optical isomers of isoproterenol was assessed from altered adenine nucleotide levels at 1 h. The L-isomer almost halved the ATP level and lowered the energy charge significantly; the D-isomer was inactive. Dependence of tumor injury on cytochrome P-450 activity appears unlikely.
Key words. ATP; D-isoproterenol; energy charge; L-isoproterenol; S180 sarcoma (CB); tumor injury.

In 1957 it was reported that near-lethal doses of a number of hormones and drugs caused hemorrhage and necrosis in the murine S37 sarcoma[2]. Most of the active substances have now been examined[3-5] for their ability to interfere with energy production in the S180 sarcoma (ICRF), a variant of the tumor provided by the Imperial Cancer Research Fund, London, England. Vasopressin[3,5], hydralazine and L-isoproterenol[3-5] have been shown to be very effective in this respect at extremely low doses, although the catecholamines were not as active, even in more toxic amounts[3].

The question whether anti-tumor activity resides in one or in both isomeric forms of the catecholamines and isoproterenol has subsequently arisen; the present study has been restricted to examining the case of the sympathomimetic.

Materials and methods. D- and L-isomers of isoproterenol (3,4-dihydroxy-α-[(isopropylamino)methyl]-benzyl alcohol) D-bitartrate were purchased from Sigma London, England, and Sigma München, FRG, respectively. Enzymes and pyridine nucleotide cofactors were supplied by Boehringer, Mannheim, FRG.

Adult female mice (BALBc) were purchased from OLAC 1976 Ltd, England, and were implanted at 6–8 weeks of age with small pieces (3–5 mg wet wt) of the S180 sarcoma (CB) in the right groin by means of a trochar. The tumor was generously provided by Dr Dorothea Connell, Institute for Cancer Research, London SW3, England. Mice received s.c. injections of the isomeric forms of the sympathomimetic in 0.15 M pyrogen-free saline; control animals received vehicle alone (8 ml/kg). All mice were killed 1 h later. Tumors were freeze-stopped as described[6] within 8.7 ± 1.9 s. Enzymic methods[7,8] were employed to measure adenine nucleotides in neutralized (pH 6.5–7) $HClO_4$ extracts of the sarcoma[6].

Results. The S180 sarcoma (CB) showed dissimilarities with the ICRF variant used in previous studies[3-6,9]. For example, the CB tumor grew much faster, while histological examination revealed much more spontaneous background necrosis. In earlier work with the ICRF form, L-isoproterenol lowered the ATP level by 80% (dose, 2 mg/kg)[4] and by 71% (dose, 1 mg/kg)[5].

The table shows that the response to the L-isomer in this instance was not as marked, the recorded fall in the ATP level being only 45%. Nonetheless, the rise and fall seen in the levels of AMP and ATP respectively were highly significant. In contrast, the D-isomer was completely without effect, all measured parameters remaining substantially unaltered after treatment.

Discussion. The possibility that the changes in energy metabolism brought about by L-isoproterenol develop as a consequence of decreased blood flow through the tumor mass cannot be dismissed out of hand in the absence of direct measurements, but would nonetheless appear to be remote for the following reasons. Upset to energy production caused by the sympathomimetic in the S180 sarcoma (ICRF) can be largely prevented by pretreatment with indomethacin[9]; according to the above argument, protection conferred by the antiinflammatory agent would necessarily entail relative constancy of blood flow

The effects of L- and D-isoproterenol on the adenine nucleotide content of the S180 sarcoma (CB) in BALBc mice 1 h after an s.c. injection of 1 mg base/kg b.wt

Treatment	Metabolites, μmoles/g wet weight*			Energy charge[13]
	ATP	ADP	AMP	
L-Isomer	0.58 ± 0.22**	0.46 ± 0.08	0.43 ± 0.13**	0.54 ± 0.10**
D-isomer	1.07 ± 0.15	0.49 ± 0.04	0.10 ± 0.01	0.79 ± 0.02
Saline control	1.06 ± 0.13	0.48 ± 0.12	0.14 ± 0.07	0.78 ± 0.03

Each group comprised four tumors. Energy charge[13] = (ATP + ½ ADP) ÷ (ATP + ADP + AMP).
* Values represent means ± SD. ** $0.01 > p > 0.002$ (Student's t-test); comparisons made with saline control.