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Cerebral edema in the rat with galactosamine induced severe hepatitis

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Summary. With D-galactosamine hydrochloride severe hepatitis was induced in rats and the water content of cerebrum, cerebellum and brain stem determined. The animals showed a parallel increase in cerebral water content and occurrence of cerebral symptoms.

Patients with endogenous liver coma (acute liver necrosis) may show symptoms that are characteristic for compression of midbrain (such as large pupils, no light reaction of pupils, decerebration rigidity, tachypnea, hyperthermia) and of medulla oblongata (such as apnea, sudden drop in blood pressure, areflexia). None of these symptoms is indicative of elevated pressure of the brain since these signs could also be of metabolic origin[1]. Several authors have found cerebral edema at autopsies on patients who died of endogenous liver coma (table 1)[1-6]. In 1971, Ware et al. observed the presence of cerebral edema in 16 out of 32 patients, 4 of whom exhibited herniation of the cerebellar tonsils or uncal herniation[4]. In 1972, Thölen reported on 16 patients all with cerebral edema, 9 with impression of the tentorium cerebelli and 7 with hernia of the tonsilla cerebelli[1]. Williams et al. found cerebral edema in 13 out of 16 patients[5]. Subsequently, the same authors studied the autopsy reports and discovered cerebral edema in 40 out of 105 cases with massive liver necrosis[6]. Therefore it may be concluded that, in cases of endogenous liver coma, the cause of death is often cerebral edema. Clinically, the question may be raised whether in acute liver necrosis all symptoms of the coma are due to cerebral edema. Further it is of interest to know whether early prophylaxis and treatment of cerebral edema would give the liver cells sufficient time to regenerate.

In the present study, the water content of different parts of the brain (cerebral hemispheres, cerebellum, brain stem) was determined in rats with severe acute hepatitis induced by D-galactosamine hydrochloride. The following questions were investigated: Does cerebral edema occur when liver failure is induced experimentally? Can this be evaluated quantitatively? When and in which part of the brain does it occur? With what symptoms can it be correlated?

Methods. Female albino rats with a weight of 180–200 g were given i.p. 2.5 g D-galactosamine hydrochloride/kg b. wt (solution: 0.45 N, pH value at 7.4 with 1 N NaOH). During the course of the experiment, food and water were not limited. The animals were killed by decapitation 24, 36, 42, 48, 54 h after the injection, and in another experiment the animals were not killed until the onset of severe cerebral symptoms independent of the time of galactosamine injection. The brain was immediately removed and dissected into the different parts: the 2 cerebral hemispheres, cerebellum and brain stem. The water content of the above-mentioned parts was determined by calculating the difference between the wet weight and the dry weight. The specimens were dried in an oven at 100 °C for 24 h. 24 h after the galactosamine injection, a 4-ml blood sample was taken from 10 rats by heart puncture (4 ml blood + 1 ml sodium citrate 3.8%) and the plasma used to determine the prothrombin time (reagent used: activated rabbit brain thromboplastin, DADE).

Results. The average values for the water content of the different cerebral regions removed from untreated control animals are given in table 2. Animals treated with galactosamine:

- A dose of 2.5 g/kg D-galactosamine hydrochloride is lethal for albino rats with a weight of 180–200 g. Preliminary experiments have shown that most of the animals die 40–56 h after the injection.
- From the 36th h on, the animals become increasingly sleepy and inactive.
- Prior to death, the animals passed through phases of various duration of total immobility (no reaction at all to optical, acustic or tactile stimuli). Also violent trembling and hyperactive phases with excessive and uncontrolled motor activity were observed (frequent and sudden jumps with transition to severe convulsive spasms).
- 24 h after the injection, most animals showed an abnormal tendency to bleed.
- 24 h after the injection, the Quick value for all animals investigated was less than 3% (average prothrombin times for untreated animals = 100%).
- The water content of all cerebral regions remained more or less constant over a 24-h period. Only the cerebellum showed a tendency toward water retention 24 h after the injection. 36 h after administration of galactosamine, the water content of the cerebellum was significantly higher, i.e. by 0.45% when compared to the control values (t-test according to Student: $p < 0.01$), after 42 h by 0.77% ($p < 0.001$). For this time period, a significant increase in the water content of the brain stem was also noted: 0.54% ($p < 0.05$). 48 and 54 h after the galactosamine injection, the water content of the cerebellum remained significantly high, whereas cerebral hemispheres and brain stem began to dry out.

The largest water increase in the brain stem was observed in rats which were killed when extremely severe cerebral symptoms were evident (as a rule 43–56 h after injection).

Table 1. Autopsies of patients died in endogenous liver coma

Author	Autopsies	Cerebral edema (number)	Cerebral edema (%)
Ware[4]	32	16	50
Thölen[1]	16	16	100
Williams et al.[5]	16	13	81
Hanid et al.[6]	105	40	38

Table 2. Water content in brain (ml H_2O/100 g wet weight)

		Controls	Galactosamine Time after administration (h)					Rats with most severe cerebral symptoms
			24	36	42	48	54	
Cerebrum left hemisphere	Mean ± SD	78.69 ± 0.24	78.75 ± 0.28	78.80 ± 0.34	78.83 ± 0.21	78.69 ± 0.29	78.68 ± 0.18	78.72 ± 0.31
	(n)	(33)	(16)	(15)	(16)	(17)	(10)	(16)
	Swelling		+ 0.08%	+ 0.14%	+ 0.18%	± 0%	− 0.01%	+ 0.04%
Cerebrum right hemisphere	Mean ± SD	78.69 ± 0.24	78.69 ± 0.28	78.67 ± 0.36	78.77 ± 0.18	78.63 ± 0.29	78.53 ± 0.21	78.68 ± 0.37
	(n)	(34)	(16)	(16)	(16)	(17)	(10)	(16)
	Swelling		± 0%	− 0.04%	+ 0.10%	− 0.08%	− 0.20%	− 0.01%
Cerebellum	Mean ± SD	77.81 ± 0.40	77.99 ± 0.25	78.16 ± 0.32	78.41 ± 0.43	78.17 ± 0.37	78.38 ± 0.31	78.30 ± 0.36
	(n)	(33)	(16)	(15)	(16)	(17)	(10)	(16)
				$p < 0.01$	$p < 0.001$	$p < 0.01$	$p < 0.001$	$p < 0.001$
	Swelling		+ 0.23%	+ 0.45%	+ 0.77%	+ 0.46%	+ 0.73%	+ 0.63%
Brain stem	Mean ± SD	73.67 ± 0.64	73.77 ± 0.60	74.00 ± 0.53	74.07 ± 0.49	73.82 ± 0.50	73.51 ± 0.85	74.10 ± 0.62
	(n)	(33)	(16)	(16)	(16)	(17)	(9)	(16)
					$p < 0.05$			$p < 0.05$
	Swelling		+ 0.14%	+ 0.45%	+ 0.54%	+ 0.20%	− 0.22%	+ 0.58%
Total water content	Mean ± SD	77.55 ± 0.26	77.51 ± 0.36	77.67 ± 0.41	77.75 ± 0.25	77.58 ± 0.32	77.57 ± 0.26	77.61 ± 0.36
	(n)	(34)	(16)	(14)	(16)	(17)	(9)	(16)
					$p < 0.05$			
	Swelling		− 0.05%	+ 0.15%	+ 0.26%	+ 0.04%	+ 0.03%	+ 0.08%

In these cases, a rise in water content of 0.58% ($p < 0.05$) could be determined. The water content of the brain stem was considerably higher in the above-mentioned cases than in animals which were killed 48 and 54 h after injection without considering the severity of cerebral symptoms.

Discussion. With single i.p. administration of D-galactosamine, hepatitis can be induced in rats, which is similar to acute viral hepatitis in men, clinically, biochemically and morphologically[7-9]. Severity and progression of the hepatitis induced with galactosamine depends upon dose and upon the age of the experimental animals[8,10]. Thus, with a high dose of galactosamine (2.5 g/kg in albino rats weighing 180–200 g) a fulminant disease with cerebral signs can be induced which is due histologically to massive liver necrosis[10]. The production of acute liver necrosis in rats with galactosamine seems to be an excellent method for the investigation of cerebral symptoms. Galactosamine, in contrast to other hepatotoxins (e.g. carbon tetrachloride), has essential advantages: selective liver toxicity[7,8,10], all pathological changes easily reproducible, and great similarity to clinical, biochemical and morphological changes occuring with fulminant hepatitis in men[10].

The present study shows that the degeneration of liver tissues due to galactosamine results in an increase in the water content of the cerebellum and the brain stem. It is not known if galactosamine passes the blood-brain barrier and thus interfers with cerebral metabolism. It is unlikely that direct toxic damage to the brain due to galactosamine is responsible for the cerebral edema, because soon after administration, galactosamine is removed from the blood stream and absorbed by the liver[8]; furthermore, the maximum water increase in the cerebrum becomes evident only 42 h after the injection. In addition, charcoal hemoperfusion increases the survival rate of rats with cerebral symptoms induced by galactosamine, at a time when the liver is already severely damaged and no significant amount of galactosamine is circulating in the blood stream[10]. Hanid et al. have observed in animal experiments (species not known) that surgically induced massive liver damage can also lead to cerebral edema. Intracranial pressure, which was recorded by means of pressure probes implanted subdurally, showed, immediately after the surgical procedure, 12.81±2.52 mm Hg which is slightly above the normal value of 8.0±2.5 mm Hg. Until death of the animals 8–16 h later, the pressure continued to increase to an average value of 51.6±11.86 mm Hg, whereas the intracranial pressure of the controls had normalized 8 h after laparotomy[6].

It may be concluded that severe liver necrosis, independent of etiology, can cause early swelling of the brain. Due to liver necrosis, brain damage as a result of haematogenic metabolic toxicity is very likely to occur.

In the present study with galactosamine, cerebellum and brain stem showed significant swelling, whereas the 2 cerebral hemispheres at no time after galactosamine injection showed a significant increase in the water content. Thus it becomes evident that different parts of the brain show varying degrees of sensitivity to acute liver necrosis: cerebellum > brain stem > cerebral hemispheres. Due to liver necrosis, cerebral damage, resulting possibly from metabolically toxic factors, occurs primarily in the cerebellum which shows the greatest amount of water retention. The fact that the different parts of the brain are not equally sensitive to toxins has already been determined, e.g. in cases of i.p. ammonia poisoning in rats where primarily the brain stem is damaged (cerebellum was not considered in that study)[11].

A parallel increase in cerebral swelling and cerebral symptoms was observed in animals 36 h after the administration of galactosamine. The largest increase in water content of the brain stem was found in those rats killed at the time of the most severe cerebral manifestations. The symptoms are identical to those described by Reulen and Baethmann in their study of cerebral edema in rats induced by dinitrophenol. These include: breathing disorders, dizziness to unconsciousness (according to Reulen and Baethmann), slower reflex response[12]. It may be concluded that different degrees of hepatic cerebral disturbances in men and certain animals are due to intracellular water accumulation in different parts of the brain.

The present study on cerebral disorders in rats induced by galactosamine, and the study of Hanid et al. involving surgically induced degeneration of the liver[6], are in agreement with the fact that in fulminant liver necrosis-independant of etiology-early cerebral edema occurs which then leads to a progressive increase in intracranial pressure. Furthermore, if one considers persons dying of endogenous liver coma and showing in a high percent of autopsies cerebral edema, which was frequently also the cause of

death[1-6], then it becomes clear that in cases of acute liver failure early prophylaxis of cerebral edema is necessary, and intensive therapy should be initiated as soon as the first changes in consciousness appear.

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Effect of pretreatment with prednisolone on the phagocytic activity of mouse peritoneal macrophages in vitro

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Summary. The phagocytic activity on in vitro cultured mouse peritoneal macrophages derived from animals treated with 6-alpha-methyl-prednisolone was examined. The statistical evaluation of results showed an increase of phagocytic activity of macrophages derived from treated animals in comparison with controls.

Corticosteroids possess different effects on phagocytic activity. These substances, in fact, inhibit the adhesion of foreign particles and of microorganism on mononuclear phagocyte surface[2] and reduce the avidity of surface receptors for immunoglobulins and complement which are involved in the early stages of phagocytosis[3,4]. These effects can be related with the changes in the cell surface properties induced by prednisolone in epithelial-like cells cultured in vitro[5].

The phagocytic process which follows the recognition and adhesion of foreign particles or bacteria on the cell surface is energy-dependent. The main source of this energy is represented by glycolytic pathway, while the Krebs cycle activity is less important[6].

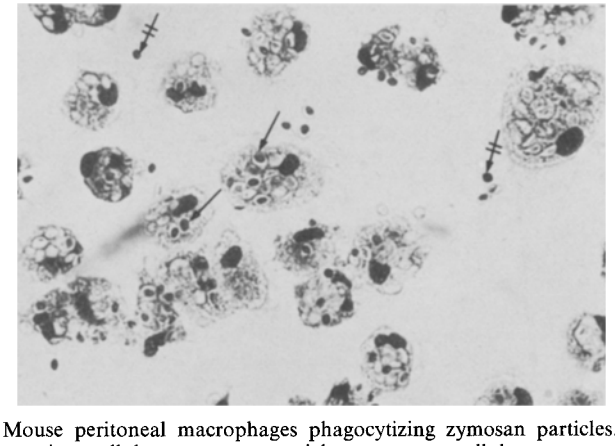
High concentrations of cortisone depress respiration and stimulate glycolysis of in vitro cultured cells, while lower concentrations of this drug stimulate the activity of the respiratory chain[7]. In this paper we have studied the phagocytic activity on in vitro cultured mouse peritoneal macrophages derived from animals treated with 6-α-methyl-prednisolone, in order to obtain further information on the influence of corticosteroids on the phagocytic process.

Materials and methods. Animals. In all the experiments, male albino mice (Swiss strain) 8 weeks old and weighing 38–40 g were used. Prednisolone was administered by i.m. injection at the dose of 2.5 mg/kg/day for 7 days. A batch of animals was treated with plain saline solution as control. This treatment was discontinued for 2 days and then 2 ml of a starch suspension were injected i.p.

Reagents. 6-α-methyl-prednisolone emisuccynate (Hoechst) was dissolved in bidistilled water at the concentration of 0.20 mg/ml. This solution was sterilized by filtration through Millipore GS (diameter 0.22 µm) filters. Sterile potato starch (Merck) was suspended in saline solution (1.5 g/100 ml). Zymosan (ICN Pharmaceuticals) was suspended in Parker's 199 medium (Difco) at the final concentration of 1.5×10^7 particles/ml. This concentration was 10 times higher than the number of the cells in culture.

Cell cultures. 1 day after starch i.p. administration the animals were killed by cervical dislocation. Macrophages were obtained from mouse peritoneum and cultured according to the technique described elsewhere[8] in Leighton tubes containing a cover slip at 37 °C. The nutrient medium consisted of Parker's 199 medium (Difco) supplemented with 15% of fetal calf serum (Microbiological Associates) and 10% of lactalbumin hydrolysate (NBC, enzymatic). The cell suspension was counted and diluted, if necessary, with nutrient medium to a concentration of 1.5×10^6 cells/ml and distributed in the Leighton tubes. 24 h after cell establishment, the nutrient medium was renewed to remove the nonadherent cells. This procedure provided a more uniform cell monolayer and enhanced the macrophages survival during the culture[9].

Experiments. 8-day cell cultures were used for the experiments. The nutrient medium was eliminated from the Leighton tubes and the cell cultures were washed 3 times with medium 199 to remove traces of serum. In a 1st group of experiments, the nutrient medium was replaced by 2 ml of nonopsonized zymosan suspension, while in a 2nd group of experiments, the same amount of opsonized zymosan particles was added to cell cultures. Opsonization of zymosan was carried out by adding to zymosan suspension a 5% dilution of homologous serum in saline solution, both heat-inactivated or not. This mixture was incubated for 30 min



Mouse peritoneal macrophages phagocytizing zymosan particles. ←: intracellular zymosan particles. ⊣⊢: extracellular zymosan particles.