A gravimetric technique to evaluate brain oedema in pigs with acute liver failure: effect of L-Ornithine Phenylacetate treatment

5th year project

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Contents

Note:

Our project was part of a large study designed and investigated by Dr. Christopher Rose and Dr. Lars Marius Ytrebø. Their goal was to explore the pathophysiological mechanisms associated with intracranial hypertension in acute liver failure. Dr. Ytrebø`s well characterized large animal (porcine) model of acute liver failure was used for the study. Many different parameters were measured, analysed and described in this project including the beneficial effects of L-ornithine phenylacetate, a novel ammonia-lowering strategy.

Our project focused on measuring the degree of brain water in different brain regions of pigs with acute liver failure treated with saline vs L-ornithine phenylacetate.

Abstract

Background: Intracranial hypertension is a serious complication in patients with acute liver failure (ALF) which leads to brain herniation and 30% mortality. An increase in intracranial pressure (ICP) is accompanied by an increase in brain water (cerebral oedema) in which hyperammonemia is believed to play a major role. The aim of this study was to measure the amount of brain water in different regions of the brain in pigs with ALF treated with and without an ammonia-lowering compound, L-ornithine phenylacetate (OP).

Methods: 24 Norwegian Landrace pigs were used in this study. ALF was induced by hepatic devascularisation (portacaval anastomosis + hepatic artery ligation) and followed by either treatment with OP (ALF+OP) or saline (ALF). A sham operated control group was included. Animal were sacrificed after 8 hours of ALF, the brain was removed and dissected into 3 white matter and 3 grey matter regions. A sensitive gravimetric technique using gradient columns of specific gravity was used for measuring brain water.

Results: A significant increase in brain water was found in all three grey matter regions (frontal and cerebellar cortex and thalamus) in pigs with ALF vs SHAM ($p<0.05$). ALF pigs treated with OP demonstrated a significant decrease in brain water in the frontal and cerebellar cortex vs ALF ($p<0.05$). A significant increase was found in frontal white matter and brainstem in ALF vs SHAM ($p<0.05$). No change was found in cerebellar white matter. OP-treatment resulted in a significant lowering of brain water in frontal white matter and brainstem vs saline-treated ALF pigs ($p<0.05$)

Conclusion: ALF causes an increase in brain water content in 5/6 brain regions compared to SHAM demonstrating the brain does not swell homogeneously. OP-treatment attenuated ALF-induced brain water content in 4/5 regions demonstrating an association between brain oedema and hyperammonemia in those regions.

1 Background

1.1 Acute liver failure

1.1.1 Definition

Acute liver failure (ALF) is defined as the development of encephalopathy in the presence of acute liver injury of less than 8 weeks duration with no pre-existing liver failure or injury. (1) The major cause of death is brain herniation due to intracranial hypertension.

1.1.2 Frequency and causes

It is estimated that 5 of 6000 hospital admissions are due to ALF (1) and in the United States 2000 people are admitted to hospital every year with this condition. The causes are numerous, but viral hepatitis and drug-induced liver injury are the most common, accounting for respectively 60-80% and 10-20%. This does not apply for all countries because of regional variations in both frequency and etiology. In developing nations viral hepatitis is the principal cause of ALF, whereas in industrialized countries paracetamol toxicity and other drug-induced injuries are the leading cause (2).

1.1.3 Clinical features

ALF affects the physiologic status of virtually all organs in the human body and can rapidly progress to multiorgan dysfunction. The clinical findings that appear under the development of the disease all reflect a decrease in the functionality of different organs, for example jaundice, coagulopathy, decreased vascular resistance (hypotension), renal and electrolyte disturbances, hyperventilation and infections. The clinical hallmark of ALF is the development of hepatic encephalopathy (HE).

Hepatic encephalopathy: The current definition of ALF is dependent on the development of HE, a neuropsychiatric syndrome that develops in patients with liver disease. The clinical manifestations are; personality changes, memory loss, attention deficits, decreased level of

consciousness, asterixis and coma. Specifically in patients with ALF, a rise in intracranial pressure (ICP) develops which is associated with cerebral oedema (3).

1.1.4 Prognosis

The clinical course of ALF in a patient has one of tree outcomes: 1. Liver function recovery following medical treatment. 2. Liver transplantation. 3. Death. Intracranial hypertension secondary to cerebral oedema is the major cause of death in 50-80% of patients with ALF (4).

1.2 Cerebral oedema

1.2.1 Classification

Different types of cerebral oedema:

1. Cytotoxic: The oedema is caused by cell swelling as a result of changes in osmolarity.

2. Vasogenic: The oedema is due to an increased permeability in cerebral vessels.

3. Interstitial: The oedema is caused by increased concentrations of osmotically active solutes in the interstitium.

1.2.2 Relationship between ICP and cerebral oedema

The intracranial space is occupied by four entities: cerebral blood volume, cerebrospinal fluid (CSF), interstitial and cellular compartments. The volumes of these compartments are constantly being regulated to maintain a stable intracranial volume. Because the adult cranium is a rigid compartment there is very poor compliance and very little room to accommodate any increase in intracranial volume. However, due to compensatory mechanisms (ex. reduction in CSF) in the brain, the relationship between brain volume and intracranial pressure (ICP) is not linear (Fig. 1). When the increasing brain volume within the skull cannot be compensated for, a small increase in the intracranial volume causes a marked increase in ICP. In patients with ALF the rise in ICP is a result of cerebral oedema, heterogeneity of regional swelling within the brain and/or increase in cerebral blood flow. Because the relationship between brain volume and ICP is not linear, the amount of brain water/oedema

cannot be calculated directly from ICP, but instead has to be measured with specific methods (2).

1.2.3 Pathophysiology of HE and cerebral oedema

The precise etiology and pathology of HE are not known. One theory is that neurotoxic substances elaborated by the gut are not detoxified by the liver. The neuropsychiatric syndrome that develops in ALF patients is probably due to several factors acting synergistically however the most important factor is ammonia (5). Many studies, both in animals and humans, have demonstrated a close association between blood ammonia and increased intracranial pressure (6, 7, 8, 9). Cerebral oedema and the resulting increase in ICP are two major complications of ALF. The pathogenesis is not clear, but rapid accumulation of ammonia in the brain is regarded as one of the most important factors (Fig. 2). The increasing ammonia can cause astrocytic swelling via an osmotic effect with an increase in astrocytic glutamine concentration or by inhibition of glutamate removal from extracellular space. These changes could be a result of altered expression of genes in the brain, which code for proteins in the central nervous system, such as the glutamate transporter (GLT-1) (10).

Cerebral oedema leading to cerebral herniation is a common cause of death in patients with ALF which is believed to be due to high levels of arterial ammonia (7).

1.3 Therapeutic strategies at lowering ammonia

Ammonia is regarded as one of the most important factors in the development of HE in patients with ALF. Therefore many treatments for HE have focused on lowering ammonia. There are two principal strategies for lowering of circulating ammonia: reduce ammonia production in the gut or increase ammonia removal capacity. Traditional treatment has tried to reduce the production of ammonia in the gut through the use of lactulose, which causes osmotic diarrhoea and reduces the time in which bacteria can metabolise proteins and produce ammonia. Also oral administration of non-absorptive antibiotics reduces the amount of ammonia producing bacteria in the gut. However, recent studies have shown that these strategic approaches are unsatisfactory (11, 12). One way of increasing ammonia removal is a combination of L-ornithine and phenylacetate, administered as L-ornithine phenylacetate (OP) (13).

Ornithine: Ammonia is normally eliminated through the urea cycle which only found in the liver and also by glutamine synthetase (GS), an enzyme found in the liver, brain and muscle. GS activity under physiological conditions in muscle is low, however during liver failure there is up-regulation of GS gene and protein expression along with an increase in GS activity (14). This means that skeletal muscle could be a therapeutic target for the treatment of hyperammonaemia. Increasing glutamate, a substrate for GS, would produce glutamine and detoxify ammonia (Fig. 3). Glutamate is provided to the muscle cell in the form of ornithine, which is rapidly transaminated to glutamate, because ornithine is transported into muscle cells whereas glutamate is not.

Phenylacetate: Phenylacetate is used to lower arterial ammonia in patients with urea cycle enzyme deficiencies. Phenylacetate covalently combines with glutamine derived from glutamate to make phenylacetylglutamine, which is excreted in the urine by the kidneys. The binding of glutamine to phenylacetate prevents an ammonia-rebound effect since glutamine can be metabolised to glutamate and ammonia by phosphate-activated glutaminase (PAG) in the kidneys and intestine (Fig. 3). This gives the advantage that the ammonia is trapped as glutamine and will not return to the circulation through ammoniagenesis.

Hypothesis: The administration of OP (ornithine and phenylacetate) in liver failure stimulates an increase in ammonia removal (ornithine) through amidation of glutamate to glutamine and the glutamine conjugates with phenylacetate preventing glutamine metabolism and an ammonia rebound effect (13) (Fig 3).

2 Aim of study

Our aims were numerous. We first set out to determine whether brain oedema was observed in all 6 different brain regions measured in ALF pigs. To also see if there was a difference in brain water content between grey and white matter. Lastly, to see if the ammonia-lowering effect of OP had an impact on brain water in all 6 brain regions.

3 Methods

3.1 Animal preparation

Animals: 24 Norwegian Landrace pigs (25-30 kg) were randomised into three groups of 8 animals each using sealed envelope system. Group 1: Sham operated (SHAM). Group 2: Hepatic devascularisation (portacaval anastomosis + hepatic artery ligation) (ALF). Group 3: Hepatic devascularisation followed by OP treatment (ALF+OP).

The pigs were kept in the animal department for at least 2 days before the experiments. The conditions in the animal room were strictly controlled at a temperature of 21 ± 1 °C, a relative humidity of 55% $\pm$ 10%, and a 12:12-hr light/dark cycle. The pigs were premedicated with an intramuscular injection of ketamine (20mg/kg) and atropine (1 mg). Anaesthesia was induced with an intravenous bolus of 10 mg/kg pentobarbital and 10 mg/kg fentanyl and maintained with a central venous infusion of 4 mg/kg/hr pentobarbital, 0.02 mg/kg/hr fentanyl, and 0.3 mg/kg/hr midazolam. The pigs were tracheostomized and intubated. Ventilation was maintained with an air-oxygen mixture (F_iO_2= .05) on a volume-controlled respirator. Anaesthesia was stopped after the liver was devascularised. If there were clinical signs of light sedation, small doses of fentanyl and midazolam were given as bolus. Sham operated animals received continuous anaesthesia during the experimental period and received equal amounts of intravenous fluids. Tidal volume was adjusted by means of repeated arterial blood gas analyses to achieve a PCO_2 within the range of 4.5-5.0 kPa. After t = 0 hrs, no adjustments of ventilation were performed. Core body temperature was maintained at 38.5 $\pm$ 1°C with a heating pad and blankets. All animals received 500 ml 0.9% NaCl containing 625 mg of glucose as a preoperative load in order to prevent any preoperative dehydration. During the experiment, 0.9% NaCl was infused at a rate of 3 ml/kg/hr. 50% glucose and 20% human albumin (albumin 200mg/ml) were continuously infused from t = 0 hrs at rates of 0.6048 ml/kg/hr and 0.66 ml/kg/hr, respectively.

Performing an end-to-side portacaval anastomosis followed by ligation of the hepatic arteries induced ALF. 2500 IU heparin was given intravenously to all pigs at the start of the experiment. After t = 0 hrs heparin was given to keep ACT > 100 sec. The pigs were killed with an overdose pentobarbital and potassium chloride after t = 8 hrs.

3.2 OP treatment

L-ornithine was administered intravenously at a dose of 0.05g/kg/hr and phenylbutyrate (pro-drug for phenylacetate) was administered intraduodenally at a dose of 0.07g/kg/hr. Medication was administered as a continuous infusion for the duration of the experiment starting at T=0 and ending at T=8hrs. Physiological saline (9mg/ml) was used as vehicle in both the ALF and sham groups. All groups received equal amount of fluids.

3.3 Brain collection

At the end of experiment brain samples were taken from the pig for measurement of brain water in the tissue.

With the use of a circular cranial saw the brain was removed within 5 minutes. Small cubes (1.5cm x 1.5cm) of brain tissue were dissected from different regions in the sacrificed brain and stored at 4°C until time of further dissection within 5-50 minutes. From this point we worked with only one cube from one specific region until brain tissue measurement was done, before starting with the next cube/region. Grey matter was dissected from white matter (only in frontal lobe and cerebellum) and dissected into very small samples (2mm x 2mm). This dissection was done on Petri dishes placed on ice (15).

Brain water measurements were completed within 55 minutes after the small cubes of tissue were placed in the fridge.

3.4 Gravimetric technique for measurement of cerebral oedema

A widely used gravimetric technique using gradient columns of specific gravity was used for measurement of brain water. By this technique, linear and repeatable density gradients were prepared from which values of tissue specific gravity could be determined (15). With use of specific gravity of solid/dry brain tissue in pigs known from 2002 and the gravimetric technique, percent water per gram brain tissue was calculated (6).

3.4.1 Specific gravity of mixtures

Two mixtures of kerosene (K) and bromobenzene (BB) were used in the preparation of the gradient. In mixture A, the proportion of the solvents was adjusted to equal a specific gravity (sp.gr.) of 0.9750. In the more dense solution (B), the specific gravity was adjusted to 1.0650. The specific gravity of BB = 1.49716 and of K = 0.78734. (15)

The following procedure was used for mixing X millilitre quantities of stock A and B solutions. The volumes of kerosene and bromobenzene for the desired specific gravity of X millilitre of stock A and B were computed using *Equation 1:* (15)

$$ml\ of\ BB = \frac{desired\ sp.gr. - sp.gr.\ of\ K}{sp.gr.\ of\ BB - sp.gr.\ of\ K} \times X \qquad \text{Eq. 1}$$

ml of K = X − ml of BB

Because of error in volume measurements, the specific gravity of the stock solutions was usually not equal to the desired specific gravity, and had to be corrected. To reduce the error, we mixed large quantities (500-2500 ml, depending on numbers of pigs per week) and never used stocks solutions older than one week. To adjust the specific gravity of the stock solution, the following rules were used:

If the measured specific gravity was less than the desired value, we removed Y ml of stock and replaced it with an equal amount of bromobenzene; Y is given by *Equation 2:* (15)

$$Y = \frac{desired\ sp.gr. - sp.gr.\ of\ stock}{sp.gr.\ of\ BB - sp.gr.\ of\ stock} \times X \qquad \text{Eq. 2}$$

If the measured specific gravity was greater than the desired value, we removed Z ml of stock and replaced it with an equal amount of bromobenzene; Z is given by *Equation 3:* (15)

$$Z = \frac{desired\ sp.gr. - sp.gr.\ of\ stock}{sp.gr.\ of\ K - sp.gr.\ of\ stock} \times X \qquad \text{Eq. 3}$$

3.4.2 Preparation of gradient system

See figure 4. At the bottom an empty 100 ml graduated cylinder is placed and filled with the gradient solution used to measure % of brain water in tissue. 40cm above the bottom of the 100 ml graduated cylinder is flask B containing 100 ml of stock B (sp.gr. = 1.0650) and is placed on a magnetic stirrer. The magnetic stirrer must be set on low speed to avoid making air-bubbles. Flask A containing the lighter mixture (sp.gr. = 0.9750) was placed 43cm above flask B. Flask A was then connected to flask B with a polyethylene tubing (PP-60, length 65cm, outer diameter = 1.22mm, inner diameter = 0.76mm), and flask B to the cylinder with two tubing, both equal to the tubing between flask A and flask B. The two tubings were connected side by side with a thin copper thread at each end, and with three small pieces of tape between, keeping the tubing connected all the way. The tape must not contact the stock solutions. Distances are measured from the surface of each liquid (15).

We withdrew the solution into the tubing and clamped the tubing when the solution exited out the end. The clamping must be done carefully, not to pinch the tubing and decrease the inner diameter of the tubing. The tubing was not straight but curly allowing the tubing between flask B and the cylinder to lie against the inner wall of the cylinder. The solution therefore ran against the inner wall, preventing it from dripping into the column and disturbing the gradient. The tubing between flask A and B was taped to the top of flask B, so it dripped directly into the stock B solution and did not run down the inside of the flask. The last step in making the gradient cylinder was to release both clamps (one clamp closed both tubing from stock A to the cylinder, and one between stock A and B) at the same time. During the filling of the columns, the end of the double outflow was maintained 10mm above the surface by gradually lowering the cylinder 2mm at a time using a lift, as the fluid level increased. When the cylinder volume of 100 ml was reached the tubes were clamped at the same time, and capped to prevent air from reacting with the gradient. The filling process took about 20 minutes. The gradient was then calibrated with standards made up of potassium sulfate (K_2SO_4) of known specific gravity. (15)

By this technique, the specific gravity at the bottom of the graduate cylinder is theoretically equal to that of stock B, while at the top of the cylinder it's equal to the average of the specific gravity of the stock A and B (1.020).

3.4.3 Quality control

To check for consistent flow we measured the left over volume of stock A and AB (the mixture between A and B contained in flask B after the cylinder reached 100 ml), and the specific gravity of mixture AB (≈ 1.020 depending on the exact specific gravity of stock A and B) after the cylinder is made. Together, this defines the flow rate and may explain deviations between the columns.

We made 6 columns (cylinders) for each pig, one for each part of the brain.

3.4.4 Preparation of specific gravity standards for gradient calibration

We used reagent grade anhydrous K_2SO_4 to make solutions with concentrations (g/100ml) 6.64, 5.99, 5.34, 4.70, 3.40 corresponding to specific gravities of 1.050, 1.045, 1.040, 1.035 and 1.025. One drop of each standard was gently placed in the column using a pipette. The end of equilibration was recorded at the end of 1 minute. To prevent temperature difference interfering with the specific gravity of K_2SO_4 we stored it at $4\,^{\circ}C$ and took it out of the fridge 3 hours before placing them in the column (15).

3.4.5 Preparation of samples

We studied six different parts of the brain; 3 white matter (frontal lobe white matter, cerebellar white matter and brainstem), and 3 grey matter (frontal cortex, cerebellar cortex and thalamus). Surgical instruments were used to obtain small samples (2mm x 2mm) from the brain. The brain was placed and dissected on a glass plate packed with ice underneath. The samples were carefully placed in the column with the gradient solution using a syringe and dissecting tweezers and the equilibration depth was recorded at the end of 2 minutes. The equilibration depth represents the specific gravity of the sample and the percentage of water in the tissue could be calculated using equation 4 below.

The preparation of the liquid gradients by this technique resulted in a virtually linear relationship between specific gravity and graduate division (Fig. 5).

3.4.6 Measurement of brain water

From an earlier study we know the solid specific gravity of solid matter (sp.gr. $_s$). These are constants (6). From the liquid gradient we get the specific gravity of wet tissue (sp.gr.$_w$). With these two parameters known, gram water per gram tissue can be calculated by *equation 4:* (15)

$$g\ H_20/g\ tissue = 1\text{-}((sp.gr.\ _w\ \text{-}1)/(1\text{-}1/sp.gr.\ _s)sp.gr.\ _w) \hspace{2cm} Eq.\ 4$$

3.5 Source of errors

3.5.1 Weighing errors/ solution errors.

Weighing errors, together with dehydration of the tissue, are probably the biggest source of errors affecting several parts of the experiment. The scale was each day checked by measuring a known volume of distilled water (Sp. Gr. of distilled water = 1.00), and if differences were detected, a correction-number was used during all weighing.

- Making of the K_2SO_4-solutions.
  - Inaccurate scale when weighing dry K_2SO_4.
  - Inaccurate pipetting or inaccurate pipette when checking the specific gravity of the standard solutions.
  - Temperature: To avoid this interfering with the standard curve we kept the solutions at 4 °C for storing and in room temperature the last 3 hours before use. When comparing the specific gravity of K_2SO_4-solutions at 4°C and 22°C we found no differences between 4°C and 22°C (Fig. 6 and table 1).

- Making stock A and B.
  - Inaccurate scale.
  - Inaccurate measuring.[1]

[1] Small differences in stock A and B will shift the gradient dramatically, so it's extremely important to have the correct specific gravity of these and corrected as close as possible to the theoretically value. To reduce this error as much as possible:

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- Make big amounts. We made between 500 ml and 2500 ml.
- When checking the specific gravity of the stock solutions use big volumes. We started with a 1 ml pipette. This is not recommended because the solutions have high viscosity and small volume differences will change the result dramatically. We ended up with using 5 ml pipette. This gave us more accurate measurements.
- Shake the stock solution before every experiment.

3.5.2 The stability of the gradient

We tried to investigate how stable the gradient was over time, and we found that some columns were stable, while other columns changed (Fig. 7 and 8). However we could clearly see that columns made the same day, always behaved in the same way. They were either stable, or fell the same amount. To explain this we tried different theories:

- Light: There is no difference between columns kept in the dark or light during the night.
- Temperature: When columns kept in fridge (4°C) for 15 hours the gradient shifted. When new K_2SO_4 solution was added to the column they increased with 5-18 divisions in the columns kept in the fridge, and the columns kept in room temperature fell 0.5-1.5. When columns kept in water bath with 25°C for 15 hours they fell 1.5-4 divisions, while the columns in room temperature fell 2-3. This could maybe explain some of the changes.
- We compared columns with and without a cap and found no difference between them when checked every day for the following 3 days (Fig. 9)

3.5.3 The accuracy of the gradient.

- There are only small variations in the columns made each day. To get them as accurate as possible it's important to measure the correct specific gravity of stock A and B (see weighing errors), and make sure the flow is stable.

- To increase the sensitivity of the gradient we reduced the range of the gradient. We made stock A with the specific gravity of 1.014 and stock B with the specific gravity of 1.050. 1 division in the new column would with these specific gravities be 0.4 divisions in the old column. The coefficient of determination (R^2) of the new columns was not as good as usual (about 0.9900 compared to 0.9960 in the original columns). In the original columns the tissue stopped after 2 minutes, but in the new columns the tissue kept falling at significant speed. The sensitivity of the column was measured as the difference between the highest and lowest tissue with the ratio 1:0.4. Fig. 10 shows that the new gradient was not as accurate as the original ones.

3.5.4 Possible errors in flow and set up

- The clamping of the tubes might damage the tubes.
- The binding of the double tube with a tread might damage the tubes.
- Dripping from the double tubing into the cylinder.
- Tube A not dripping into flask B, but running down the inside of the flask or the double tubing.
- The unclamping of the single and double tubes were not done at exactly the same time.
- Displacement of flask B with the result that the magnet stopped.
- Double tubing submerged in the solution during filling of the column.
- High speed on the magnet might cause bubbles in the stock.
- Tubing system not properly placed in the bottom of the flasks.

3.5.5 Possible errors with the brain samples

- Tissue samples placed into the cylinder with different force or speed.
- Desiccating/dehydration of the samples: If dissection or immersing the samples in the cylinder took too long, the samples desiccated, becoming sticky and most important got a lower specific gravity. We put 5 samples in the cylinder first, waited for equilibration after 2 minutes, and put the last 3 samples in. It's very difficult to do more than 5 at the time because you can easily lose track of the different tissue

samples. We could clearly see that the first 5 samples had higher specific gravity than the last 3 (Fig. 11 and 12). To decrease this source of error we kept the tissue in the fridge as often as possible, the dissection was done on ice, and kept the cap on the Petri dish as often as possible.

3.5.6 Statistical analysis

Results are expressed as mean±SEM. Significance of difference between groups was tested by unpaired Student t test. $P<0.05$ was considered statistically significant. GraphPad Prism 4.0 (GraphPad Software, San Diego CA) was used.

4 Results

4.1 Exclusions of animals

Data from frontal cortex in pig 2 (ALF) was excluded because of abnormal results. Results from pig 5 (ALF+OP) was excluded because of failed OP treatment. No brain measurements from pig 1 (SHAM) and 6 (ALF) were done because of inaccurate gradient in columns.

4.2 Cerebral oedema

4.2.1 Grey matter

Fig. 13 shows percent brain water in frontal cortex, cerebellar cortex and thalamus in the 3 groups: SHAM, ALF and ALF+OP. In all three grey matters there is a significant ($p<0.05$) higher brain water content in pigs with ALF compared to SHAM. In frontal and cerebellar cortex there is a significant ($p<0.05$) lower brain water content in pigs treated with OP compared to pigs with ALF. There is no significant difference in percent brain water between ALF and ALF+OP in the thalamus.

4.2.2 White matter

Fig. 14 shows percent brain water in frontal white matter, cerebellar white matter and brainstem in the 3 groups: SHAM, ALF and ALF+OP. In frontal white matter and brainstem there is a significant ($p<0.05$) higher brain water content in pigs with ALF compared to SHAM. In frontal white matter and brainstem there are a significant ($p<0.05$) lower brain water content in pigs treated with OP compared to pigs with ALF. There is no significant difference in brain water content between the three groups in cerebellar white matter.

4.3 ICP

During this project co-workers measured ICP in the pigs during the experiment. They found that pigs with ALF had significant higher ICP compared to both SHAM and ALF+OP after 1 hour of ALF induction, which persisted until the end of the experiment (8 hours). No significant difference in ICP was found between SHAM and ALF+OP (Fig. 15).

5 Discussion

5.1 Results

We found that pigs with ALF had higher brain water content compared to the control group (SHAM) in every part of the brain except from cerebellar white matter. ALF pigs treated with OP had a significant lower brain water content compared to ALF pigs treated with saline in every part of the brain except thalamus and cerebellar white matter.

The brain does not swell homogenously. Changes in brain water were different in all 6 regions studied following 8 hours of ALF, with or without OP treatment. From our results we can see no clear pattern in the changes in brain water, and can't conclude that neither grey or white matter have a bigger change in brain water content than the other. Why it seems like there is no clear pattern could be the results of either anatomical or physiological factors, or the fact that we only did experiments on 6 brain regions. If we had examined more white and grey brain regions it would be easier to detect a pattern between white and grey matter if there is any.

In both cerebellar white matter and thalamus we can see differences in brain water content similar to the changes in the other parts of the brain, but these differences are not significant. We had few animals in each group. There is a possibility that larger number of animals could give us more data that would make our results more accurate, and maybe significant differences here too, if these tendencies are real.

There is a known fact that arterial ammonia correlates with brain oedema and ICP. OP treatment lowers ammonia, and should therefore also lower ICP (Fig. 15). This could possibly explain why pigs treated with OP have lower brain water content in several regions than pigs with ALF.

5.2 Source of errors

During the experiment we encountered different sources of errors, both theoretical and practical. Our main challenge was to make a stable and accurate gradient. To examine

possible errors we investigated external factors that could affect both the solutions and the columns:

- Temperature – No difference in specific gravity of K_2SO_4-solutions at 22°C vs. 4°C. The columns/gradients however were influenced by temperature.
- Light – No difference between columns kept in dark and light environment.
- Shrinking of the gradient – This made the gradient more inaccurate with lower coefficient of determination. The tissue kept falling at significant speed at the time of measurement (2 min). This time was calculated based on the original protocol. To reduce the gradient the time of measurement must be adjusted. The depth of equilibration was not reached after 2 minutes. If the time of measurement is correct this could be a more accurate technique.

The biggest sources of errors were probably dehydration of the brain samples and inaccurate weighing. Accurate weighing is essential to get correct concentrations and specific gravity of different solutions. To reduce this error we made large amounts of solutions enough for 3 days of experiments (18 columns). We also investigated the dehydration of the brain tissue. We found out that the first group of tissue samples had higher brain water content than the last group, probably due to dehydration. We tried to reduce this by dissecting on ice, keeping the cap on the Petri dish and we kept the tissue in the fridge until used in the experiment.

6 Conclusion

ALF results in an increase in brain water content in 5/6 brain regions. This shows the brain does not homogeneously swell during ALF and that grey matter areas may be more susceptible to swelling than white matter areas. Our results also reveal cerebellar white matter is more resistant to oedema. OP treatment lowered brain water content in 4/5 oedematous brain regions induced by ALF. These results demonstrate an association between hyperammonemia and brain oedema in those 4 brain regions which responded to OP treatment. The thalamus did not respond to OP treatment and therefore its brain water content is not associated with ammonia.

7 Acknowledgement

First of all we must thank Dr. Christopher Rose and Dr. Lars Marius Ytrebø for allowing us to participate in their project. We appreciate all their help and enthusiasm during the project. Specially thanks to Mr. Rose for guidance and positivity during the writing process.

8 Tables

Table 1:

Specific gravity	At 22 ℃	At 4 ℃	Difference	Numbers of data
1.050	35,875	35,75	0,125	4
1.045	47,875	47,875	0	4
1.035	73,125	73,5	-0,375	4
1.025	95,375	95,75	-0,375	4

Comparison of average specific gravity of K_2SO_4-solutions at 22℃ vs. 4℃. Based on 4 different K_2SO_4-solutions.

9 Figures

Figure 1:
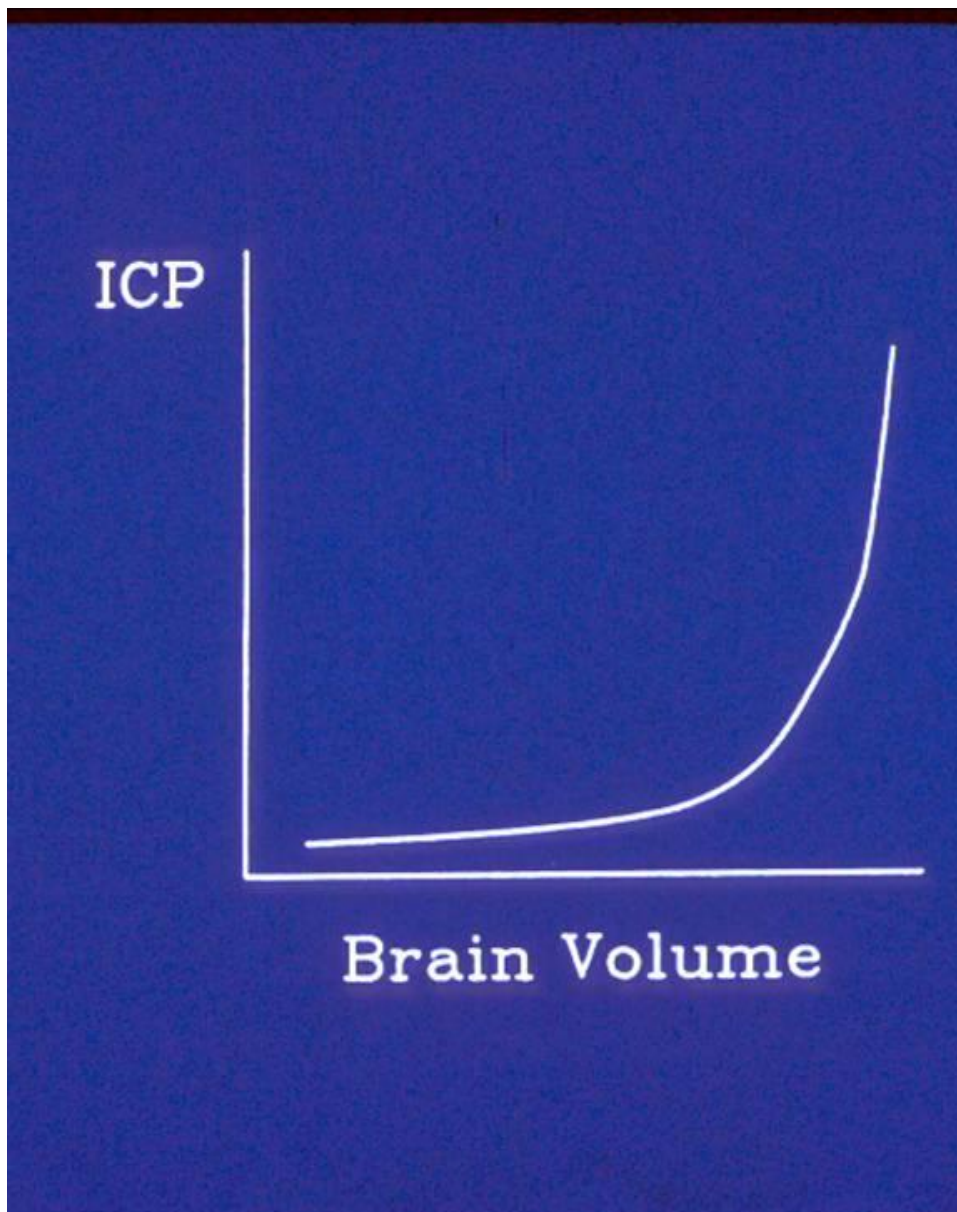
Relationship between brain volume and ICP is not linear. (16)

Figure 2:

r = 0.610
r^2 = 0.372
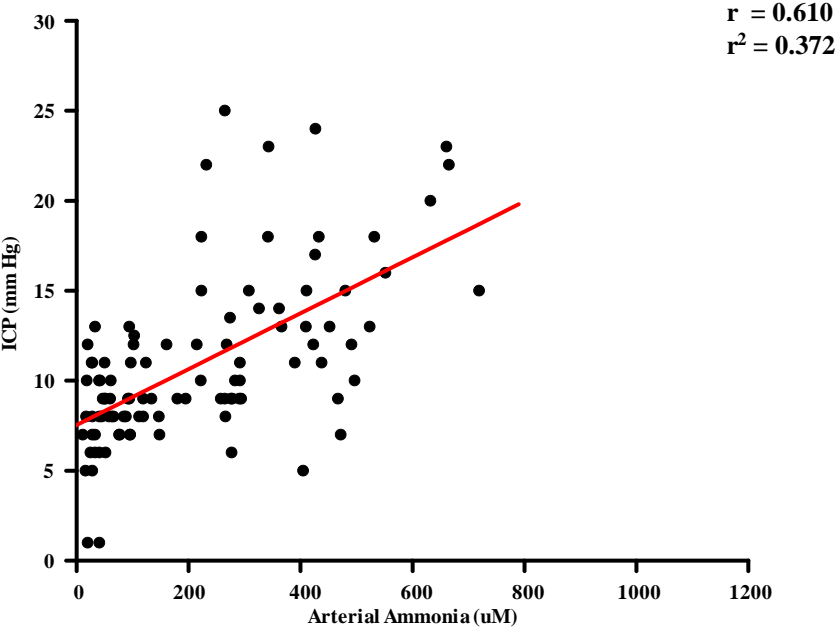
Correlation between ICP and arterial ammonia (p<0.001) (17).

Figure 3:
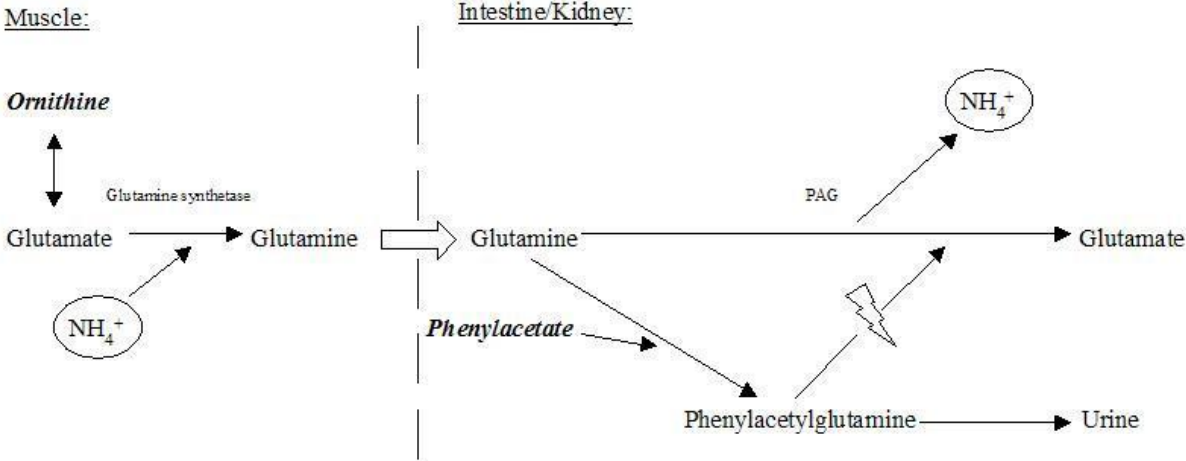
Schematic diagram demonstrating the mechanism of L-ornithine phenylacetate.
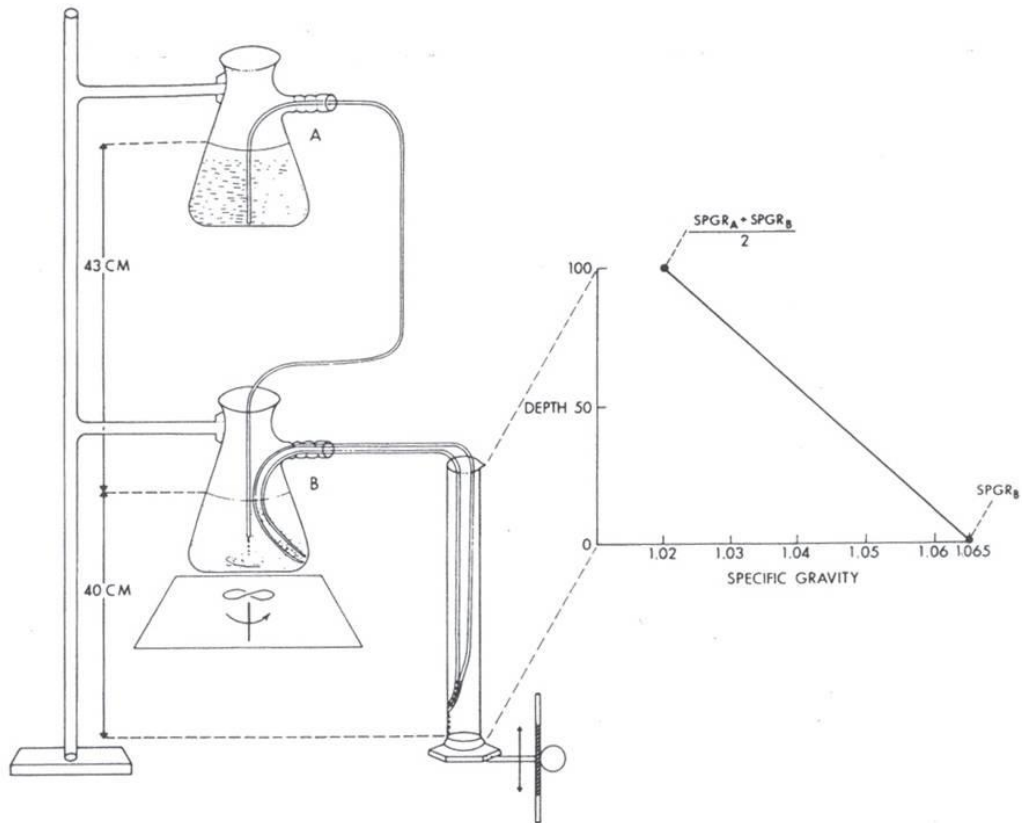
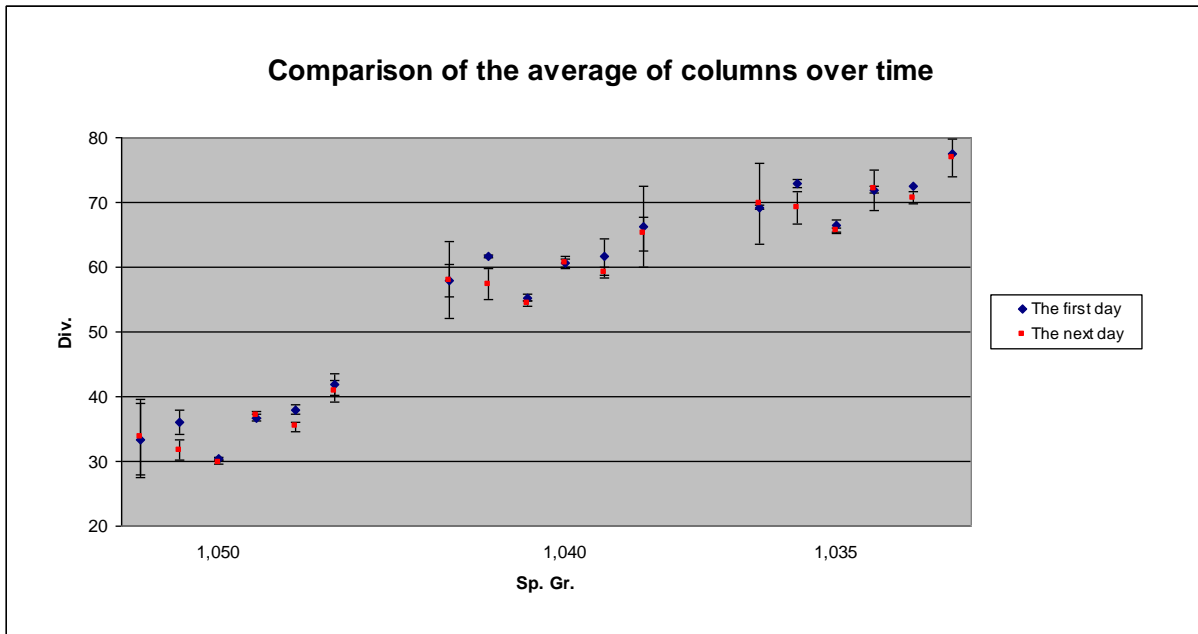The set up system for the gravimetric technique for measurement of cerebral oedema (15)

Figure 5:
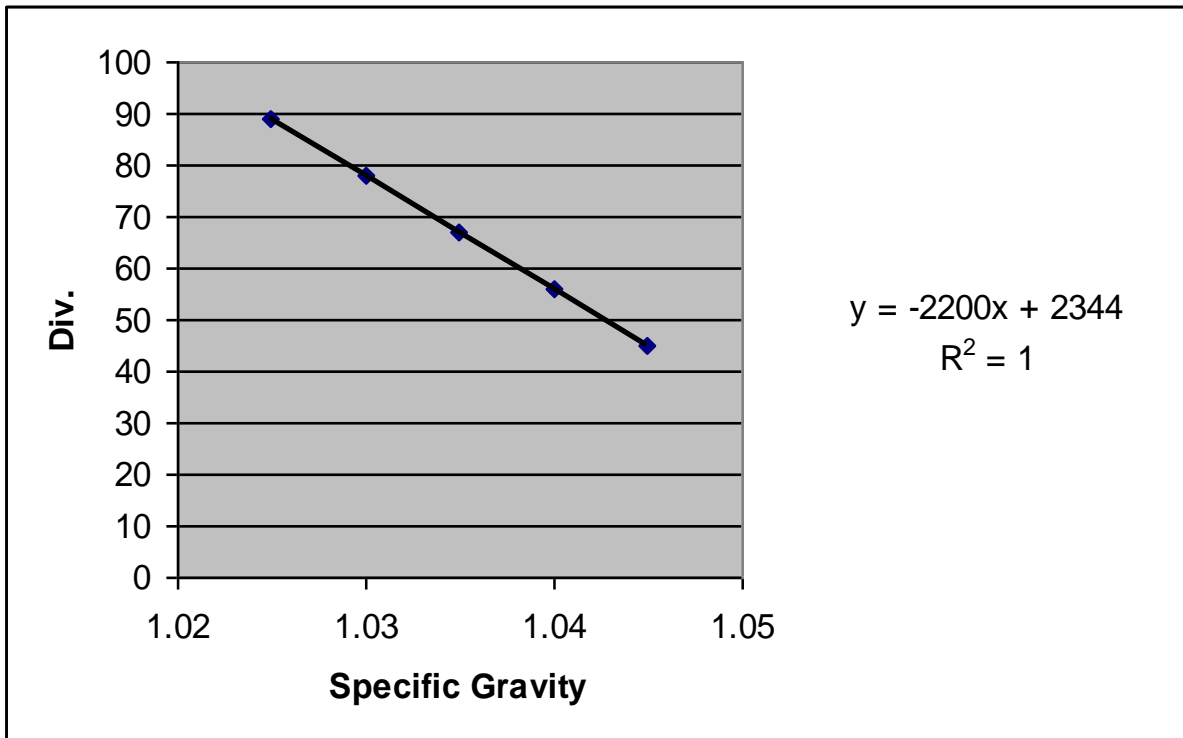
Theoretical relationship between specific gravity and graduate division using K_2SO_4 as standards (15).
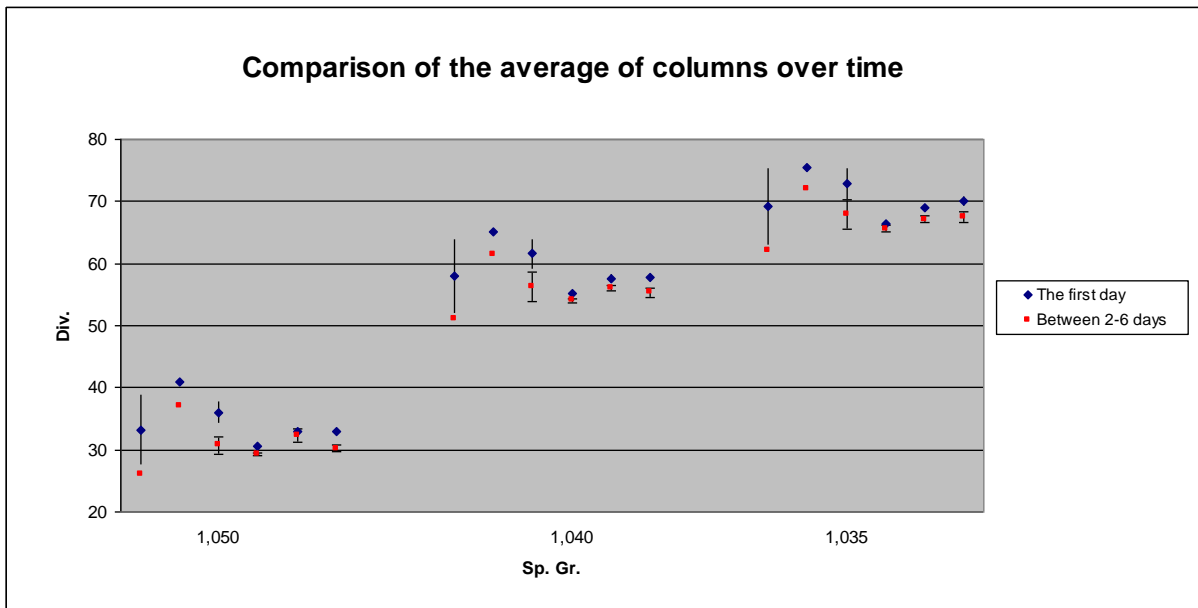
Figure 6:
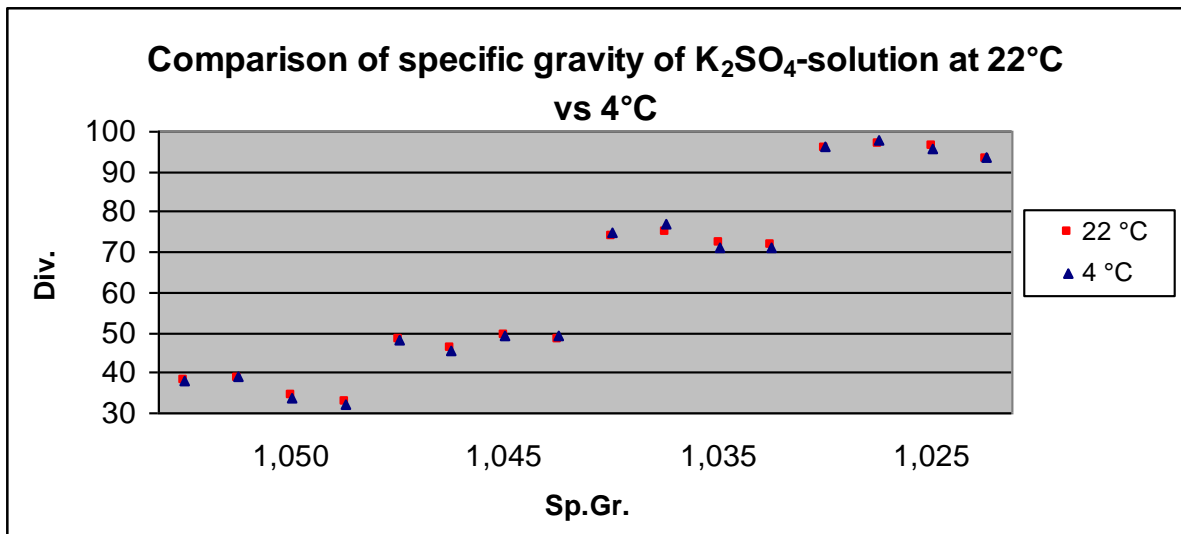
Comparison of specific gravity of K_2SO_4-solutions at 22°C vs. 4°C. The measurements are done in 4 different columns.
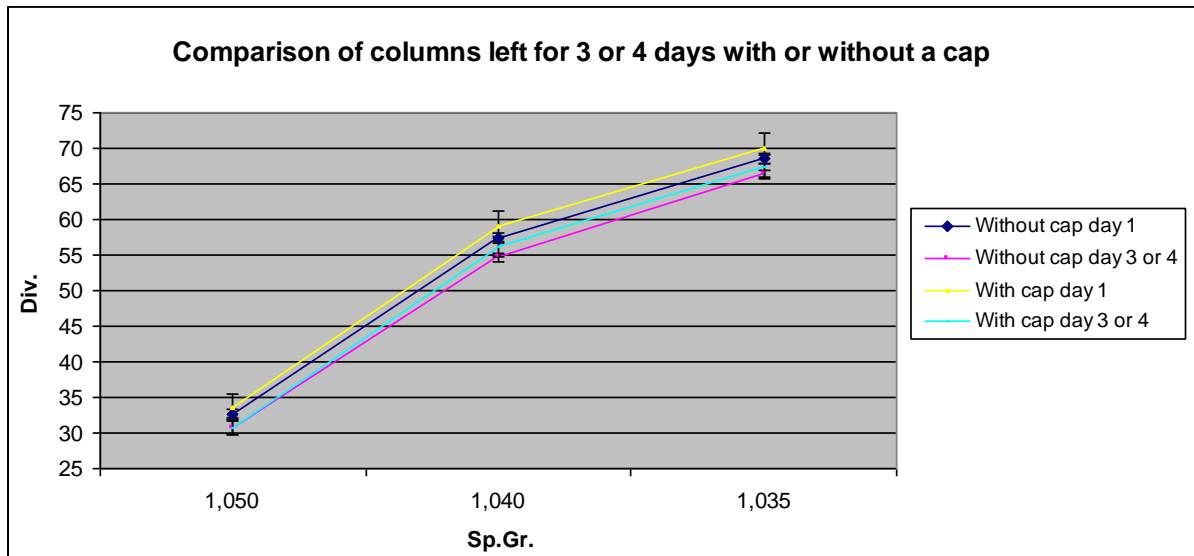
Figure 7:

Comparison of K_2SO_4-solution in columns left for one day. The measurements are done in 6 different columns and for 3 different K_2SO_4-solutions (Sp.Gr. of 1.050, 1.040, 1.035).

Figure 8:

Comparison of the average of columns over time

Comparison of K$_2$SO$_4$–solution in columns left for between 2-6 days. The measurements are done in 6 different columns and for 3 different K$_2$SO$_4$-solutions (Sp.Gr. of 1.050, 1.040, 1.035).

Figure 9:

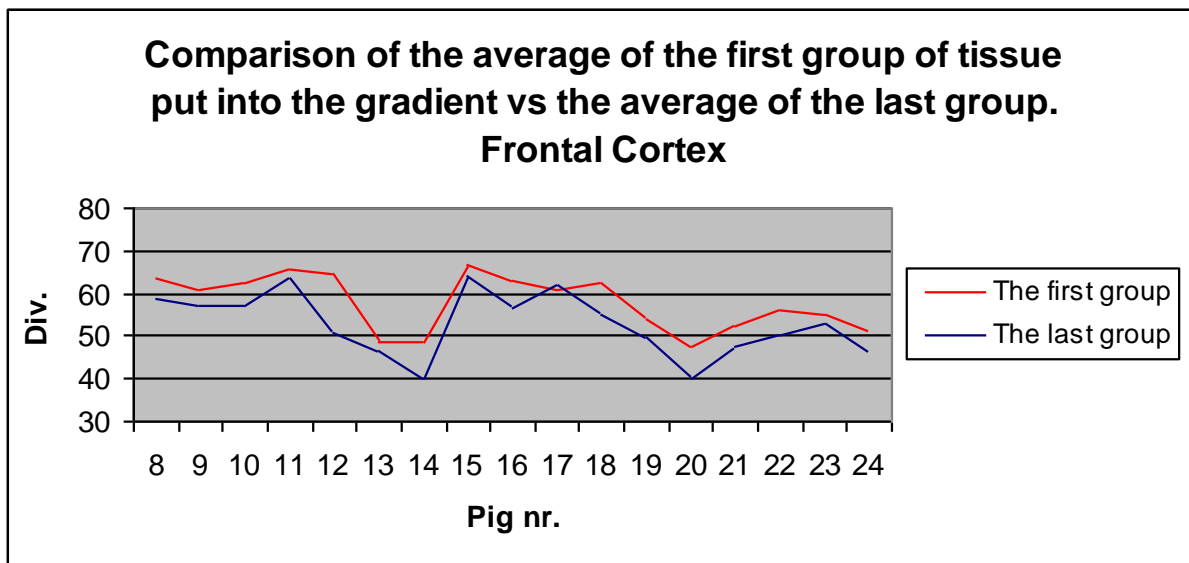**Comparison of columns left for 3 or 4 days with or without a cap**

Comparison of K_2SO_4–solutions in columns left for three or four days with or without a cap. After one day the differences are even smaller.

Figure 10:

**Comparison of original and reduced gradient (Brainstem)**
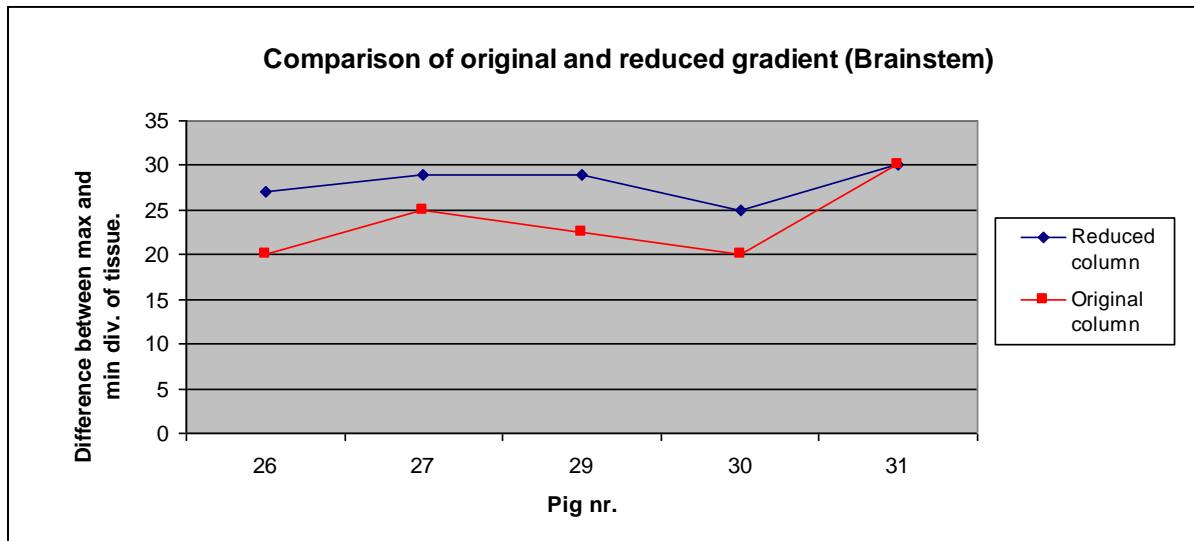
The sensitivity of the columns made with a reduced gradient (Sp.Gr. 1,032-1,05) vs the original gradient (Sp.Gr. 1,020-1,065). Comparison of the difference between the highest and lowest tissue in the two columns/gradients. 1 ml in the reduced columns is equal to 0.4 ml in the original columns because of the reduced gradient. This ratio of 0.4 was used to calculate and compare the two gradients. Time of measurement was 2 minutes for both gradients.

Figure 11:

Comparison of the average of the first group of tissue put into the gradient vs the average of the last group.
Frontal Cortex

Frontal cortex. Comparison of the average of the first group of tissue dropped into the column and the average of the second (last) group of tissue. We immersed 5 pieces the first time, waited 2 minutes for equilibration, then the last 3 pieces was immersed. Sometimes a piece of tissue went into a K_2SO_4-bubble and was taken out of the results.
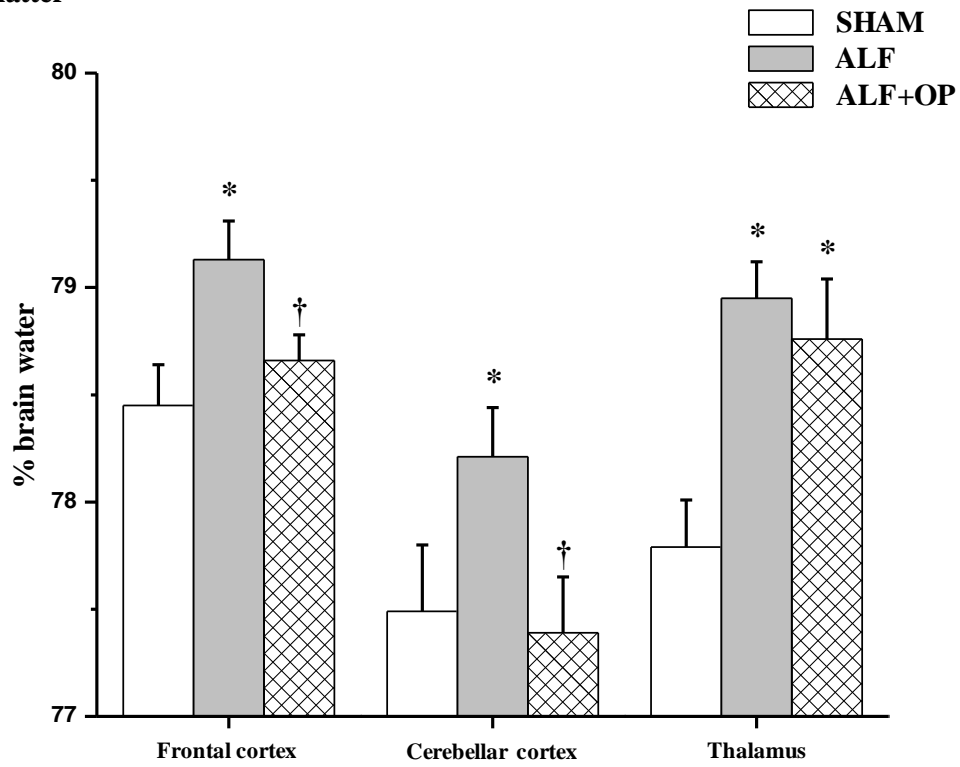
Figure 12:

Comparison of the average of the first group of tissue put into the gradient vs the average of the last group. Frontal white matter
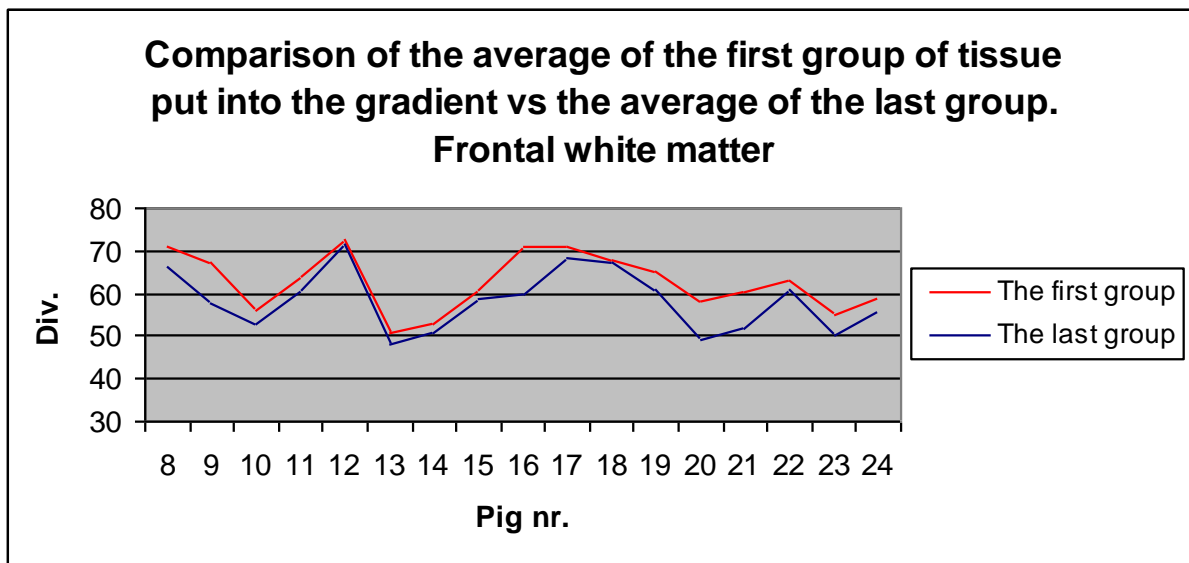
Frontal white matter. Comparison of the average of the first group of tissue dropped into the column and the average of the second (last) group of tissue. We immersed 5 pieces the first time, waited 2 minutes for equilibration, then the last 3 pieces was immersed. Sometimes a piece of tissue went into a K_2SO_4-bubble and was taken out of the results.
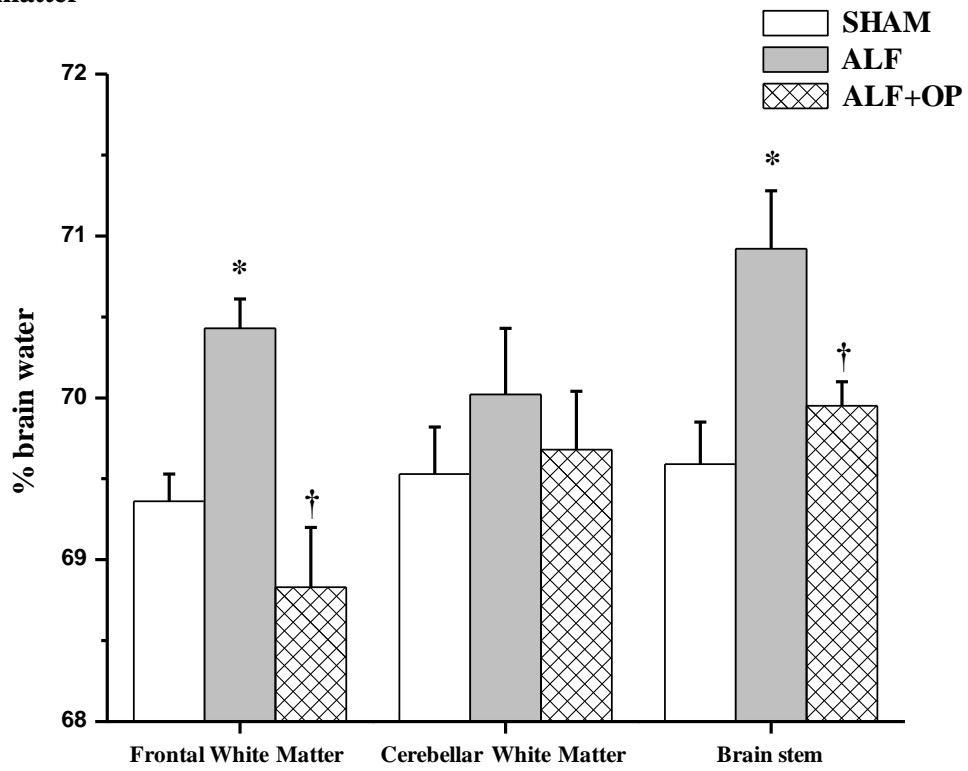
Figure 13:

Grey matter

* = p < 0.05 vs SHAM; † = p < 0.05 vs ALF

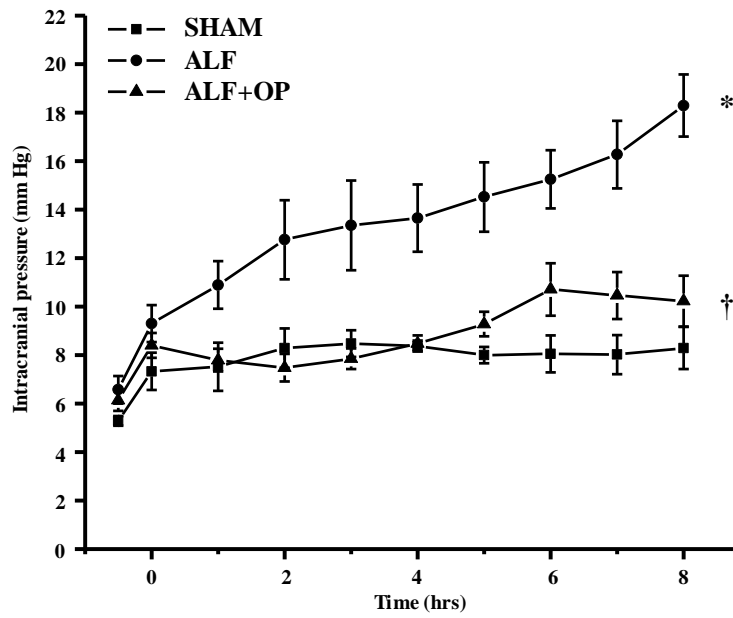Percent brain water in different parts of grey matter in the brain.

Figure 14:

White matter

* = p < 0.05 vs SHAM; † = p < 0.05 vs ALF

Percent brain water in different parts of white matter in the brain.

Figure 15:

ICP levels in pigs with ALF, ALF+OP, and sham operated controls (* = ALF vs sham, $P_{GT} <$ 0.001; † = ALF vs ALF+OP, $P_{GT} = 0.001$ (17).

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