

## Ammonia Metabolism: Reye's Syndrome as a Model for Elevated Ammonia.

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### OBJECTIVES:

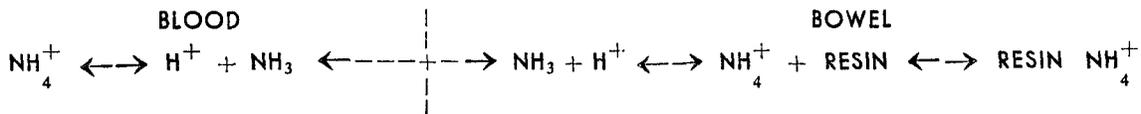
1. Determine if ammonia is elevated in Reye's syndrome in Thailand;
2. Develop ion exchange resins as treatment for elevated ammonia;
3. See if treatment of elevated ammonia will increase survival in Reye's syndrome.

BACKGROUND: Elevated ammonia has been reported to be associated with encephalopathy in several disease states. Ammonia has been reported elevated in Reye's Syndrome in the United States.

Blood ammonia will be measured in patients with encephalopathy and fatty degeneration of the viscera (EFDV) in order to determine: (1) if it could have etiologic significance in the encephalopathy; (2) if EFDV is similar to Reye's syndrome in this respect. Blood ammonia will also be measured in patients with Japanese B encephalitis to see if blood ammonia can be used to differentiate the two conditions.

It is anticipated that if the encephalopathy of EFDV is related to elevated ammonia that this would prove a useful model for study of treatments of elevated ammonia. EFDV would be a good model for the following reasons: (1) lack of other medical problems as in chronic hepatic failure; (2) probable reversible nature of lesion (vs acute fulminant hepatitis); (3) well defined end point (death); (4) large number of cases available over relatively short period.

Another phase of this study will look at the possibility of using an ionic exchange resin in the treatment of elevated ammonia. Ionic exchange resins have been shown to lower blood ammonia in laboratory animals. Decreasing stool pH has also been shown to lower blood ammonia. It is hoped to combine these effects by using an acidic ionic exchange resin enema that would work according to the following equation.



It is hoped that this might prove to be a simple, effective therapy for conditions characterized by elevated ammonia.

PROGRESS: (1) Ammonia Assay. The ammonia assay, although a modification of a previously reported principle<sup>(1)</sup>, is reported here in some detail because it offers certain advantages in the volume of blood required, speed and ease of analysis and accuracy of results:

Blood is drawn and immediately transferred to tubes (containing 50 $\mu$  dried heparin) on ice and mixed. 3.0 ml of blood is added to 3.0 ml  $\sim$  3.0 M ice cold perchloric acid, mixed and centrifuged in the cold. To 3.0 ml iced supernatant is added 1.5 ml  $\sim$  3.0 M KOH (Conc. of perchloric acid and KOH need not be exactly 3.0 M but should be adjusted so that neutralized extract is neutral to slightly acidic) 0.01 ml 0.2% methyl red is added and the solution titrated to an orange or "just" yellow color with the above solutions of perchloric acid and KOH (with practice it is possible to easily perform this titration, this involves a small dilution but if perchloric acid and KOH are properly prepared this is less than 1%). The

neutralized solution is centrifuged in the cold and the supernatant removed and allowed to come to room temperature. To cuvettes add 2.0 ml supernatant, 1.0 ml PO<sub>4</sub> buffer-NADH (prepared immediately before use by dissolving NADH in 0.5 M PO<sub>4</sub> buffer pH = 7.5 and adjusting to read .600 to .700 at 340 mμ in spectrophotometer) and 0.1 ml 0.25 M α Ketoglutarate. Mix and read Initial OD<sub>340</sub>. Add .05 ml glutamate dehydrogenase, mix, and let stand 30' at room temperature, read final OD<sub>340</sub>.

$$\text{NH}_4^+ \text{ in } \mu\text{m/ml} = \Delta\text{OD}_S - \Delta\text{OD}_B \times .76$$

$$\Delta\text{OD} = \text{Initial-final OD}_{340}$$

S = Sample  
B = Water Blank

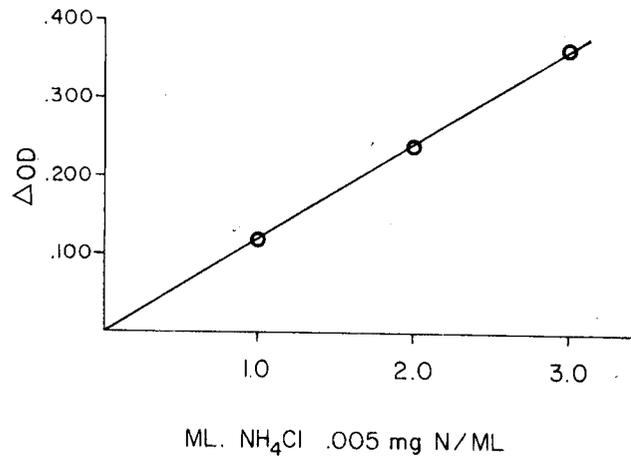


Figure 1. shows a representative standard curve.

Other experiments showed reproducibility is good (duplicates within 5-10%) and that recovery from blood of added ammonia is complete. The neutralized extract is stable up to 2 weeks following addition of 0.5 ml 0.5 M PO<sub>4</sub> buffer pH=7.5 to 2.0 ml extract and freezing and storing at -60°C.

Total time until extract is stable is about 30 min. total time for assay about 90-120 min. 10-15 samples can be run by one operator simultaneously.

2. Removal of NH<sub>4</sub><sup>+</sup> by ion exchange resin. Kayexalate is used as the resin. It is converted from its Na<sup>+</sup> form to a Na<sup>+</sup>-K<sup>+</sup> form by repeated washing with a solution containing 3,770 mg Na<sup>+</sup> (as NaCl) and 200 mg K<sup>+</sup> (as KCl)/100 ml and then repeated distilled H<sub>2</sub>O washing. (Washing is most easily performed using suction on a large funnel). The completeness of equilibration is then tested by mixing a quantity of resin with a solution containing 377 mg Na<sup>+</sup> and 20 mg K<sup>+</sup>/100 ml (i.e. approximate plasma concentration) and measuring the change in the concentration of Na<sup>+</sup> and K<sup>+</sup>.

The resin thus prepared will remove NH<sub>4</sub><sup>+</sup> in vitro but the removal is dependent on salt concentration (Table 1) as well as the amount of resin (Table 2).

Table 1. 68 mg resin added to 10 ml above solutions containing .001 mg N/ml as NH<sub>4</sub>Cl.

Na mg/100 ml	K mg/100 ml	% NH <sub>4</sub> <sup>+</sup> REMOVED
377	20	24
188	10	36
94	5	39
37.7	2	57
0	0	100

Table 2. Resin added to 10 ml solution containing 377 mg Na, 20 mg K/100 ml and .001 mg N/ml as NH<sub>4</sub>Cl.

mg RESIN	% NH <sub>4</sub> <sup>+</sup> REMOVED
34	4.4
68	17.1
136	28.2
272	42.7

Two attempts have been made to lower blood ammonia in monkeys using the resin by stomach tube and enema. These attempts were unsuccessful. However, several technical difficulties were encountered including low control blood pH (probably secondary to anesthesia), only mildly elevated ammonia, and poor distribution of resin (resin remained in stomach and rectum). Further attempts will be made to overcome these difficulties.

3. Blood ammonia in EFDV In Thailand. Measurement of levels of blood ammonia in patients with EFDV will be done in the near future.

REFERENCES:

(1) Clin. Chem. Acta. October 1969, p. 185