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Improved Assay for Spinal Fluid Glutamine, and Values for Children with Reye's Syndrome

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We describe a simple assay for spinal fluid glutamine, in which glutaminase is used to deaminate glutamine and the liberated ammonia is then measured with phenol hypochlorite. The concentration of glutamine in spinal fluid is increased in coma of hepatic origin. Spinal fluid glutamine was increased above normal at the time of admission in 20 of 27 cases of Reye's syndrome, of which hepatic dysfunction is apparently a feature. There was no correlation between the initial concentration of glutamine in spinal fluid and the ultimate outcome in these patients.

Additional Keyphrases: *diagnostic aid • blood ammonia*

Several reports describe increased glutamine concentration in the spinal fluid of patients in hepatic coma (*1–4*). Current evidence suggests that the encephalopathy of Reye's syndrome is related to hepatic dysfunction. Thus, one might expect that glutamine concentrations in spinal fluid would be abnormally high in Reye's syndrome, as has in fact been reported in three of four cases (*3*).

Most investigators estimate spinal fluid glutamine by the method of Whitehead and Whittaker (*5*), in which glutamine is de-aminated with H_2SO_4 and the liberated ammonia determined with either Nessler's reagent or phenol hypochlorite (*1–4*). Glutaminase is used to de-aminate glutamine in several methods described for determinations in plasma or urine (*6, 7*). These methods involve separating the liberated ammonia by distillation or with cation-exchange resin before the ammonia is measured.

We describe here an assay for spinal fluid glutamine in which the ammonia liberated by glutaminase is measured directly, and present data from 27 cases of Reye's syndrome.[3]

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[3] *Ed. note:* In mid-February and mid-May 1974, the wire services reported an "outbreak" (>250 cases) of this noncontagious children's disease in the U. S., where B-type influenza was prevalent. Such seasonal upsurges are not unusual, and are evidently a nonspecific reaction to viral infection.

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Materials and Methods

Patients

Spinal fluid samples were obtained on admission from patients with Reye's syndrome admitted to Khon Kaen Provincial Hospital, Khon Kaen, Thailand. The diagnosis of Reye's syndrome was based on previously published criteria (*8*). Samples for use as controls were obtained from children with an acute encephalopathy other than Reye's syndrome undergoing diagnostic lumbar puncture at either Khon Kaen Provincial Hospital or Colorado General Hospital, Denver, Colo.

Materials

Glutaminase (EC 3.5.1.2; Grade IV; Sigma Chemical Co., St. Louis, Mo. 63178) was dissolved, in a concentration of 50 or 100 μg/ml (175 to 350 U/liter), in sodium acetate buffer (50 mmol/liter, pH 4.9). The activity of the enzyme was not measured except frequently to check that the de-amination reaction was complete in the time allowed. Phenol color reagent was either obtained from Sigma or made by dissolving 50 g of phenol and 0.25 g of sodium nitroprusside in 1 liter of water. Alkaline hypochlorite reagent was either obtained from Sigma or prepared by dissolving 25 g of NaOH and 2.1 g of sodium hypochlorite in 1 liter of water. Glutamine standard (Sigma) was prepared by dissolving 50 mg of glutamine in 100 ml of water. The supplier claimed 99%+ purity for the standard. The standard, after de-amination, gave 98% of the absorbancy of an equivalent ammonium sulfate standard. All reagents were stored at 4 °C.

Readings were made with 1-cm cuvettes, in a Model DU 2 spectophotometer (Beckman Instruments, Fullerton, Calif. 92634).

Method

Add 20 μl of water, standard, or spinal fluid to 0.2 ml of glutaminase, mix, and incubate for 30 min at 37 °C. Add 1.0 ml of phenol reagent followed immediately by 1.0 ml of alkaline hypochlorite, with thorough mixing. After a further 30 min of incubation at 37 °C, read the absorbance at 640 nm.

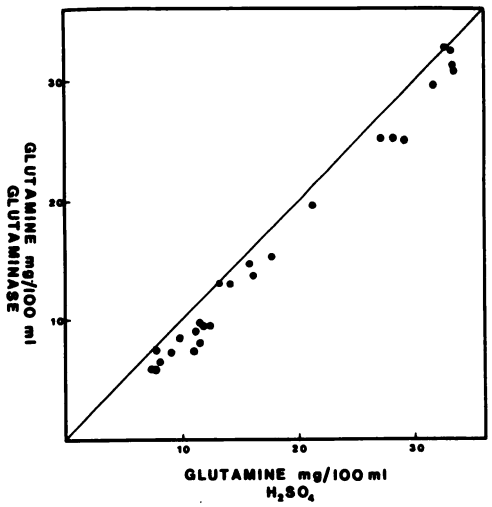

Fig. 1. Comparison of apparent glutamine in spinal fluid with H_2SO_4 and glutaminase methods
The higher values were obtained from patients with Reye's syndrome

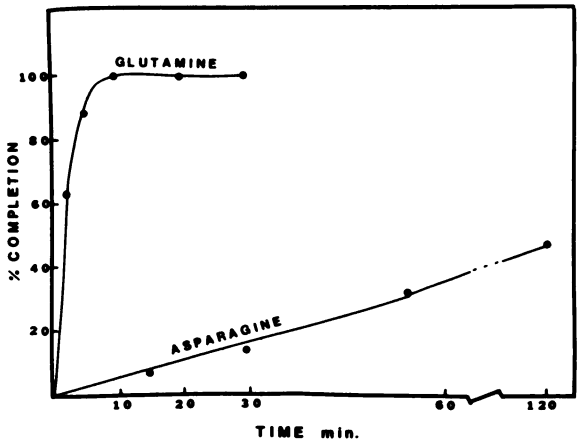

Fig. 2. Rate of deamination of glutamine and asparagine
Enzyme concentration of 0.1 mg/ml. Glutamine and asparagine concentration 50 mg/100 ml

Before the above assay was developed, and later for comparison of the present method with a previous method, some spinal fluids were assayed by the method of Whitehead and Whittaker (5) as modified by Hourani et al. (2).

Results

Assay

The standard curve for the assay is linear to at least 40 mg of glutamine per deciliter. In Figure 1, the results of the H_2SO_4 and glutaminase methods are compared; the latter gives consistently lower results, and this difference is particularly significant at low glutamine concentrations. In 12 recovery experiments, apparent recovery of glutamine added to spinal fluid averaged 99.8% (range 91–109%). The following compounds gave no color with the above method: urea (100 mg/100 ml), alanine, aspartate, arginine, glutamate, D-glutamine, glycine, γ-amino butyrate, histidine, hydroxyproline, isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, or valine (all 50 mg/100 ml), and cystine or uric acid (10 mg/100 ml). Asparagine is de-aminated by glutaminase, but much more slowly than is glutamine (Figure 2). The possible contribution of asparagine was checked in two samples from patients with Reye's syndrome. The apparent glutamine concentration after a 30-minute incubation with enzyme was 22.4 and 33.8 mg/100 ml, and after a 120-minute enzyme incubation 21.5 and 34.6 mg/100 ml, respectively. In addition, any preformed ammonia present will result in apparent glutamine. The error from this source can be corrected for by omitting the enzyme from a control sample of spinal fluid. The apparent glutamine from this source averaged 0.6 mg/100 ml (range, 0.2–1.4 mg/100 ml) for four spinal fluids (Table 1), and this correction is not made for the results reported here.

Table 1. Preformed Ammonia and Stability of Glutamine in 4 Samples of Spinal Fluid

Fresh		2 days at room temp.		7 days at −20°C	
Preformed NH_4^+[a]	Glutamine	Preformed NH_4^+	Glutamine	Preformed NH_4^+	Glutamine
mg/100 ml					
0.3	8.4	1.2	7.4	0.2	8.1
0.2	7.8	0.5	7.7	0.2	7.6
0.4	6.1	0.3	6.3	0.2	5.4
1.4[b]	36.1	3.3	33.1	0.9	34.9

[a] Expressed as apparent glutamine.
[b] Specimen from patient with Reye's syndrome.

Glutamine is relatively stable in spinal fluid. The values obtained for four spinal fluids left at room temperature for two days averaged 95% (range, 88–103%) of the value obtained for fresh spinal fluid. The apparent recovery after seven days at −20 °C was 95% (range, 89–97%) (Table 1).

During the first part of this work we used an enzyme concentration of 50 μg/ml; however, at this concentration the de-amination reaction is not complete within 30 min if enzyme is used that has been stored for longer than two weeks at 4 °C. At an enzyme concentration of 0.1 mg/ml, the enzyme is sufficiently stable for at least two months when stored at −20 °C.

Patients

Spinal fluid glutamine concentrations in patients with Reye's syndrome and in controls are given in Figure 3. If only levels measured by the method reported here are considered, the controls averaged 8.7 mg/100 ml (range, 5.8–14.1 mg/100 ml). Of the 27 patients with Reye's syndrome, 20 had a spinal fluid glutamine concentration greater than 15 mg/100 ml. There was no apparent relationship between the ini-

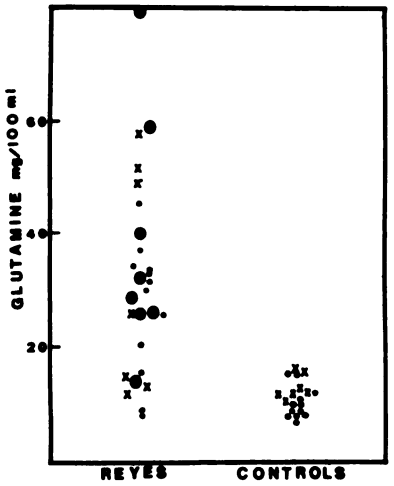

Fig. 3. Spinal fluid glutamine in cases of Reye's syndrome and in controls

X = H_2SO_4 method; ●, glutaminase method; ⊕ or ⊙, survivor. All survivors in this study recovered completely

tial concentration of glutamine in spinal fluid and the final outcome.

Discussion

The present method for determining spinal fluid glutamine offers apparent advantages in ease, specificity, and volume required. The only interfering compounds found should cause no problem in the clinical application of the assay. If a more specific assay for glutamine is desired, it is easy to measure the contribution of preformed ammonia. We have not encountered any bloody spinal fluid samples, and so do not know if blood interferes with this method.

Although we use 30 min for both incubations, less time is required. The de-amination reaction is complete within 15 min at an enzyme concentration of 0.1 mg/ml, and the color development with phenol hypochlorite is complete within 15 min at 37 °C. Decreasing the time for the first incubation will also decrease any contribution from asparagine.

Several investigators have shown that spinal fluid glutamine is increased in patients who are comatose secondary to acute hepatic necrosis or chronic liver disease (*1–4*). In such patients there has generally been a better correlation between values for spinal fluid glutamine and the clinical course than between values for blood ammonia and the clinical course (*2, 3*). Plasma glutamine concentrations are usually not elevated in cases of coma secondary to chronic liver disease (*9*); therefore we did not measure plasma glutamine as part of this study.

The finding that patients with Reye's syndrome also have elevated spinal fluid glutamine is consistent with other evidence of hepatic dysfunction in this disorder. In our subjects, there was no apparent correlation between the initial spinal fluid glutamine and the ultimate outcome.

We believe the measurement of spinal fluid glutamine to be a useful diagnostic test in Reye's syndrome. Values for spinal fluid glutamine were abnormally high in 74% of our patients, a percentage comparable to that found for other tests (blood sugar, SGPT, prothrombin time, and blood ammonia) used as aids in the diagnosis of Reye's syndrome (*8*).

Presumably, increased blood ammonia and spinal fluid glutamine both reflect hepatic dysfunction. It has previously been demonstrated that survivors of Reye's syndrome usually have a normal blood ammonia value on admission (*8*); however, this is not the case with spinal fluid glutamine. Thus it seems likely that some patients with Reye's syndrome have a normal blood ammonia and an elevated spinal fluid glutamine, and vice versa. Unfortunately we have ammonia values for only a few of these patients. Therefore, for the present, it would seem preferable to measure both blood ammonia and spinal fluid glutamine.

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