

Toxic levels of ammonia in human brain abscess

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OBJECTIVE Brain abscesses could lead to cerebral symptoms through tissue destruction, edema, changes in brain architecture, and increased intracranial pressure. However, the possibility that the pus itself could contribute to symptoms has received little attention. Brain abscesses are areas of tissue destruction, proteolysis, and formation of free amino acids, which are energy substrates for bacteria and possible sources of ammonia. Ammonia is neurotoxic, may cause brain edema, and could contribute to the symptoms of brain abscesses.

METHODS The authors analyzed the extracellular phase of pus from 14 patients with brain abscesses with respect to ammonia and amino acids. For comparison, CSF from 10 patients undergoing external ventricular drainage was included. The ammonia-forming ability of *Streptococcus intermedius* and *Staphylococcus aureus*, two common microbial isolates in brain abscesses, was studied in vitro.

RESULTS In brain abscesses ammonia was 15.5 mmol/L (median value; range 1.7–69.2 mmol/L). In CSF ammonia was 29 μ mol/L (range 17–55 μ mol/L; difference from value in pus: $p < 0.001$). The total concentration of amino acids in brain abscesses was 1.12–16 times higher than the ammonia concentration ($p = 0.011$). The median glucose value in pus was 0 mmol/L (range 0–2.1 mmol/L), lactate was 21 mmol/L (range 3.3–26.5 mmol/L), and pH was 6.8 (range 6.2–7.3). In vitro, *S. intermedius* and *S. aureus* formed ammonia at 6–7 mmol/L in 24 hours when incubated with 20 proteinogenic amino acids plus γ -aminobutyric acid (GABA), taurine, and glutathione at 1 mmol/L.

CONCLUSIONS Intracerebral abscesses contain toxic levels of ammonia. At the concentrations found in pus, ammonia could contribute to the brain edema and the symptoms of brain abscesses.

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KEY WORDS brain abscess; ammonia; cerebrospinal fluid; amino acids; lactate; infection

A BRAIN abscess could lead to cerebral symptoms by causing tissue destruction and edema as well as changes in brain architecture and intracranial pressure, but the possibility that the pus itself could contribute to symptoms has received little attention. However, brain abscesses have been shown to contain amino acids at high concentrations.^{9,15,19} Amino acids are energy substrates for bacteria,²⁶ and bacterial metabolism of amino acids entails formation of ammonia;⁶ therefore, brain abscesses could contain ammonia. Ammonia is neurotoxic; its deleterious effects on the brain, as can be seen in hyperammonemic states, include vasogenic edema formation,³⁵ astro-

cytic swelling and alterations in neurotransmission,^{14,22,33} impaired astrocytic potassium handling,³⁴ and a host of biochemical effects¹⁸ that could contribute to the various symptoms of hyperammonemic disorders.²⁰

To see if brain abscesses contain ammonia we determined the extracellular concentration of ammonia in pus from patients with brain abscesses and, for comparison, in CSF from patients with external cerebroventricular drains. The ability of *Streptococcus intermedius* and *Staphylococcus aureus*, two common microbial causes of brain abscesses in humans,^{4,11} to produce ammonia from amino acids was investigated in vitro.

ABBREVIATIONS GABA = γ -aminobutyric acid; GSH = glutathione, reduced form; HPLC = high-performance liquid chromatography; OD₆₀₀ = optical density at 600 nm.

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Methods

Patients and Samples

The study was approved by the Regional Ethics Committee for Medical Research in the southern and eastern part of Norway. Patients were recruited from 2011 to 2014. Informed, written consent was obtained from all participants or (in the case of children) their parents, in accordance with the Declaration of Helsinki. Pus from 14 patients with brain abscesses was collected at surgery. Nine of these patients were recently described with respect to amino acid levels in pus, clinical symptoms, and microbiological findings.⁹ The CSF used for the comparison was from 10 patients undergoing drainage of the cerebral ventricles through an external ventricular drain as part of the treatment for increased intracranial pressure. Of these patients, who were recently described with respect to amino acid levels in CSF⁹ (permission to reuse data granted by Elsevier), 6 had suffered subarachnoid hemorrhage, 3 had brain tumors, and 1 had congenital hydrocephalus. Intracranial pressure was normal at the time of sampling, and CSF was clear and colorless. Samples were aspirated into polypropylene syringes, cooled on ice, and rapidly centrifuged at 3000g for 10 minutes at 4°C. Supernatants were removed and stored at -70°C until analysis, which in most cases took place within 4 hours. Prior to surgery, patients underwent MRI or CT scans of the brain. Volumes of abscesses were calculated from the MR or CT images, using the ellipsoid formula.^{9,27} Pus samples from all patients with brain abscess underwent culture and analysis by polymerase chain reaction for microbiological diagnosis, and pus smears were investigated by light microscopy after May-Grünwald-Giemsa staining.²³

In Vitro Formation of Ammonia From Amino Acids

The *S. intermedius* and *S. aureus* used in the study were from The Culture Collection, University of Göteborg, Sweden (Catalog Nos. CCUG 43515 and CCUG 46923, respectively). The bacteria were grown on blood agar for 16 hours and suspended at approximately 10 µl bacteria/200 µl phosphate-buffered saline. A solution was made of all 20 proteinogenic amino acids plus γ -aminobutyric acid (GABA), taurine, and reduced glutathione (GSH) at 1 mmol/L in (mmol/L) NaCl 125, NaHCO₃ 25, KCl 2.8, MgCl₂ 1, CaCl₂ 1, and NaH₂PO₄ 0.3; pH was 7.3. The GSH was included, because it is present in the brain at approximately 1 mmol/L.⁴⁰ In some experiments a single amino acid was omitted from the amino acid solution to study its importance for ammonia formation. Glucose was not added, and to mimic anaerobic conditions, solutions were not saturated with oxygen. A similar solution, but with pH 6.5 to mimic the acid conditions in pus, was made, but results were similar to those obtained with a solution with pH 7.3, so only the latter are given here. The amino acid concentrations were chosen on the basis of a previous study documenting millimolar concentrations of amino acids in brain abscesses.⁹ Bacterial suspensions were diluted in the amino acid solution at 10 µl/ml and adjusted to an optical density at 600 nm (OD₆₀₀) of 0.28 for both streptococci and staphylococci. At 0, 1, 4, and 24 hours, samples were taken for analysis of ammonia and amino acids.

Biochemical Analyses

Ammonia was analyzed by spectrophotometry, using a Vitros DT60 II spectrophotometer (Ortho-Clinical Diagnostics). The method was linear in the range 10–500 µmol/L; $r = 0.999$; $p = 4 \times 10^{-8}$, Pearson's test. Samples with ammonia concentrations above the detection limit were diluted with distilled water, as recommended by the manufacturer. Three pus samples were analyzed for ammonia by both indirect potentiometry with an ammonium-selective electrode (Modular P800, Roche) and by reflectance spectrophotometry (Vitros DT60 II spectrophotometer). The values obtained (24–36 mmol ammonia/L) with reflectance spectrophotometry were $99\% \pm 9\%$ (mean \pm SD) of the values obtained by indirect potentiometry. To see if amino acids could produce false ammonia measurements, ammonia was measured in 20 mmol/L solutions of all 20 proteinogenic amino acids, GABA, taurine, hypotaurine, and GSH. In none of the solutions was there an ammonia signal detected by reflectance spectrophotometry; this was true also for a solution of urea at 100 mmol/L.

Amino acids were analyzed by high-performance liquid chromatography (HPLC) and fluorescence detection after precolumn derivatization with *o*-phthalaldehyde, using an 1100 series HPLC unit (Agilent), as described previously.⁹

Supernatants of brain abscess pus were analyzed by reflectance spectrophotometry for glucose and lactate as described previously,¹⁷ and pH was measured with a pH meter (inoLab pH 7110, WTW GmbH) that was calibrated before each reading.

Data Presentation and Statistics

Values for amino acids in abscess pus were not normally distributed according to the Kolmogorov-Smirnov normality test. Therefore, all data are presented as median values with full range (minimum and maximum values). For the same reason groups were compared statistically, using the Kruskal-Wallis and Dunn tests for multiple comparisons, or the Wilcoxon matched-pairs signed-rank test, as appropriate. In vitro data on ammonia formation from amino acids were analyzed by 1-way ANOVA with the Dunnett test for multiple comparisons or a Newman-Keuls all pairwise comparison, as appropriate. A p value < 0.05 was considered significant.

Results

Focal and General Brain Abscess Symptoms—Microbiology

All 14 patients with brain abscess had cerebral symptoms of varying severity (Table 1). Most symptoms were focal, reflecting the localization of the abscess. Eight patients had seizures, of whom 7 had focal seizures; in 1 patient only generalized seizures were observed. Five patients were disoriented. None of the patients were comatose. All patients had pronounced perilesional vasogenic edema, as could be seen on CT or MRI scans of the brain.

Light microscopy detected an abundance of polymorphonuclear leukocytes in all brain abscess samples, confirming the presence of pus. In pus from 8 patients, microbiological analysis identified *S. intermedius*; in 3 patients

TABLE 1. Individual data in 14 patients with brain abscess*

Age (yrs), Sex	Symptoms	Localization	Microbiology Findings	Ammonia (mmol/L)	Amino Acids (mmol/L)	Vol (cm ³)
58, F	Disorientation, rt-sided paresis	Lt frontal lobe	<i>S. intermedius</i>	69.2	94.2	5.4
72, M	Seizures & paresis lt leg	Rt frontal lobe	<i>A. aphrophilus</i> , <i>P. micra</i> , <i>P. acnes</i> , <i>F. nucleatum</i>	38.8	58.8	12
62, F	Headache, disorientation	Lt cerebellar	<i>S. intermedius</i>	36.4	65.2	8.2
55, F	Seizures, headache	Rt parietal lobe	<i>S. intermedius</i>	33.9	70.0	19
21, M	Disorientation, fever	Rt frontal lobe	<i>S. intermedius</i>	28.1	52.2	18
59, M	Disorientation, seizures	Rt parietal lobe	<i>H. parainfluenzae</i> , <i>Prevotella</i> sp., <i>F. nucleatum</i>	23.9	26.7	3.1
3, F	Seizures, malaise	Lt frontal lobe	<i>S. intermedius</i>	18.2	77.1	45
61, M	Hemiparesis, seizures, expressive aphasia	Lt frontal lobe	<i>S. anginosus</i>	12.7	99.3	35
79, M	Ataxia, dysarthria	Rt cerebellar	<i>S. anginosus</i>	8.3	24.7	14
3, F	Seizures, malaise	Rt hemisphere†	<i>S. intermedius</i>	8.2	71.2	0.8
42, F	Headache, malaise	Rt parietal lobe	<i>S. anginosus</i>	6.8	37.7	2.0
45, F	Headache, fever, rt-sided facial paresis	Lt frontal lobe	Unknown bacterium	4.5	18.7	3.1
41, F	Seizures, malaise	Rt parietal lobe	<i>S. intermedius</i>	2.4	32.9	8.6
84, M	Hemianopia, disorientation, seizures	Rt occipital lobe	<i>S. intermedius</i>	1.7	27.7	32

* Data include age and sex, symptoms prior to surgery, abscess localization (cerebral lobe or cerebellar hemisphere), bacteria in pus identified by culture and/or polymerase chain reaction, ammonia level, total concentration of amino acids in extracellular phase of pus, and abscess volume. Data reprinted with permission from Dahlberg et al.: High extracellular concentration of excitatory amino acids glutamate and aspartate in human brain abscess. *Neurochem Int* 69:41–47, copyright 2014, Elsevier.

† This patient had four abscesses in her right cerebral hemisphere, of which one in the frontal lobe was analyzed biochemically.

Streptococcus anginosus was found (Table 1). One patient had a mixed bacterial flora of *Aggregatibacter aphrophilus*, *Parvimonas micra*, *Propionibacterium acnes*, and *Fusobacterium nucleatum*; another had *Haemophilus parainfluenzae*, *Prevotella* species, and *F. nucleatum*. In 1 patient microbiological identification was not achieved.

Ammonia Levels in Brain Abscesses and CSF

The median extracellular concentration of ammonia in brain abscesses was 15.5 mmol/L (range 1.7–69.2 mmol/L; Fig. 1a). The total concentration of amino acids, which may serve as energy substrates for bacteria²⁶ and therefore may be a source of ammonia, was 1.12–16 times higher (difference between ammonia and amino acid levels: $p = 0.011$, Wilcoxon test). The level of ammonia correlated with the sum of amino acids ($r = 0.56$; $p = 0.046$; Spearman test). The median level of individual amino acids varied from 0.43 mmol/L (arginine) to 33.5 mmol/L (alanine). The two arginine metabolites, citrulline and ornithine, were present in pus at median values of 2.1 mmol/L and 0.54 mmol/L, respectively. The levels of amino acids were similar to recently published values.⁹

The median glucose concentration in pus was 0 mmol/L (range 0–2.1 mmol/L); in 8 patients glucose could not be detected. Lactate concentration in pus was 21 mmol/L (range 3.3–26.5 mmol/L), illustrating mainly anaerobic conditions in the brain abscesses. The pH level was 6.8 (range 6.2–7.3), in agreement with previous measurements.¹⁰ There was no correlation between these parameters (glucose, lactate, or pH) on the one hand and ammonia levels on the other ($p > 0.35$).

The time from symptom onset to surgical evacuation of

the abscesses ranged from 4 to 56 days (median 10 days) and did not correlate with ammonia concentration in pus ($r = 0.35$; $p = 0.24$). Abscess volume ranged from 0.8 to 45 cm³ (median value 11.7 cm³; Table 1) and did not correlate with pus level of ammonia ($r = -0.19$; $p = 0.54$). Seizures were produced by both large and small abscesses and were seen at various concentrations of ammonia.

In CSF the concentration of ammonia was 3 orders of magnitude below that in brain abscesses, with a median value of 29 μ mol/L (difference from abscess values, $p < 0.001$; Fig. 1b), which agrees with previously reported normal values for CSF²¹ and which is similar to normal serum values.¹² The total level of amino acids was 554 μ mol/L (median value; range 360–870 μ mol/L), which was significantly lower than abscess values ($p < 0.001$), with median values ranging from 0 (citrulline was not detectable) to 415 μ mol/L (glutamine). Apart from glutamine, amino acids had median values below 30 μ mol/L.

Ammonia Formation From Amino Acids by *S. intermedius* and *S. aureus* In Vitro

S. intermedius, a common cause of brain abscesses,^{4,11} was found in 8 pus samples (Table 1). In vitro, *S. intermedius* formed ammonia from amino acids. Within 1 hour of incubation in a medium that contained all 20 proteinogenic amino acids plus GABA, taurine, and GSH at 1 mmol/L, ammonia had reached a concentration of approximately 0.75 mmol/L (Fig. 2a). The concentration of arginine was reduced by 60% ($p = 0.10^{-6}$). Arginine metabolites citrulline and ornithine were present at approximately 0.5 and 0.1 mmol/L, respectively. The concentration of other amino acids was not significantly changed. At 4 hours, ammo-

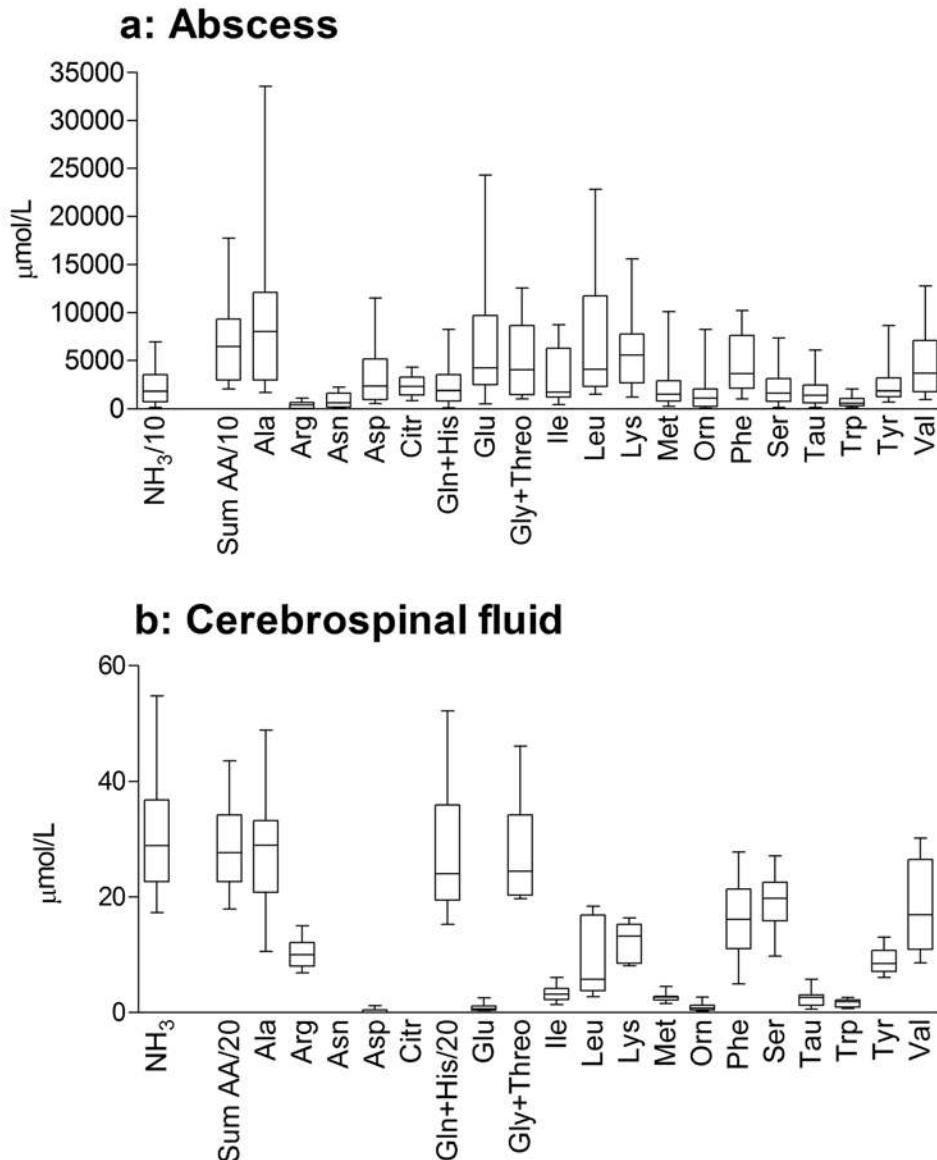


FIG. 1. Acid levels in the extracellular phase of pus from brain abscesses and CSF. **a:** Pus was from 14 patients with brain abscesses. **b:** CSF was from 10 patients undergoing external cerebroventricular drainage. The plots show the median value, with the box representing the two middle quartiles and the whiskers denoting minimum and maximum values. Values for ammonia (NH_3) and total level of amino acids (Sum AA) are divided by 10 in the upper panel; in the lower panel the total level of amino acids is divided by 20. Amino acids were analyzed by HPLC. The HPLC procedure does not resolve glutamine and histidine or glycine and threonine, hence "Gln+His" and "Gly+Threo." Ala = alanine; Arg = arginine; Asn = asparagine; Asp = aspartate; Citr = citrulline; Gln = glutamine; Glu = glutamate; Gly = glycine; His = histidine; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; Orn = ornithine; Phe = phenylalanine; Ser = serine; Tau = taurine; Threo = threonine; Trp = tryptophan; Tyr = tyrosine; Val = valine. Reprinted with permission from Dahlberg et al.: High extracellular concentration of excitatory amino acids glutamate and aspartate in human brain abscess. *Neurochem Int* 69:41–47, copyright 2014, Elsevier.

nia had increased to approximately 1.5 mmol/L. At this time point arginine could not be detected, but citrulline was present at approximately 0.5 mmol/L and ornithine at approximately 0.3 mmol/L. The concentration of other amino acids was not significantly changed. Omission of arginine from the amino acid solution abolished ammonia production at 4 hours of incubation.

At 24 hours, ammonia was approximately 6 mmol/L in amino acid solutions incubated with *S. intermedius* (Fig. 2a). At this time point arginine, aspartate, aspara-

gine, and serine were undetectable. The levels of GSH and glutamate were reduced by $35\% \pm 8\%$ and $56\% \pm 6\%$, respectively, and the levels of isoleucine, leucine, valine, tryptophan, and tyrosine were reduced by 14%–24% ($p < 0.01$; individual data not shown). The levels of the other measured amino acids were not significantly changed. Solutions without bacteria had unchanged concentrations of amino acids after 24 hours of incubation. After 24 hours the OD_{600} of *S. intermedius* had increased by $96\% \pm 5\%$ ($p = 10^{-9}$), reflecting bacterial growth, and when the incu-

bation media were transferred to blood agar and cultured overnight, there was massive growth of *S. intermedius*.

We wished to know whether different bacteria might produce ammonia through different pathways, so we analyzed ammonia formation by *S. aureus*, a common cause of brain abscesses.⁴ After 1 hour of incubation in the amino acid-containing medium, *S. aureus* had produced ammonia at approximately 0.2 mmol/L (Fig. 2b). The levels of alanine, arginine, and threonine tended to decrease by approximately 10% but without reaching statistical significance. At 4 hours of incubation, the concentration of ammonia was approximately 1.3 mmol/L. At this time point the levels of several amino acids had decreased significantly: alanine by 13%, glutamine by 20%, arginine by 22%, and threonine by 25%. Omission of arginine from the amino acid solution reduced ammonia levels by $29\% \pm 7\%$ at 4 hours of incubation ($p = 0.03$; $N = 4$), whereas omission of threonine led to a $41\% \pm 3\%$ reduction ($p = 0.00017$; $N = 4$). Omission of alanine or glutamine did not change ammonia formation significantly at 4 hours.

At 24 hours of incubation with *S. aureus*, the mean level of ammonia was approximately 7.5 mmol/L. At this time point arginine, alanine, threonine, and glycine were undetectable. Asparagine was reduced by $90\% \pm 2\%$ and glutamate by $70\% \pm 2\%$. The concentrations of isoleucine, leucine, lysine, tyrosine, and GSH were reduced by 15%–26% ($p < 0.003$; individual data not shown). Unlike the situation for *S. intermedius*, the level of aspartate was reduced only by $31\% \pm 9\%$, whereas methionine had increased by $45\% \pm 3\%$ ($p < 10^{-5}$). Ornithine was present at approximately 0.2 mmol/L, and citrulline could not be detected (Fig. 2b). After 24 hours, OD₆₀₀ had increased by $312\% \pm 7\%$ ($p = 10^{-14}$), and when the incubation media were transferred to blood agar and cultured overnight, there was massive growth of *S. aureus*.

Discussion

Ammonia Is High in Brain Abscesses and May Contribute to Cerebral Symptoms

The present study demonstrates very high concentrations of ammonia in brain abscesses in human patients; the median value was 3 orders of magnitude higher than the median value in CSF. The latter value was similar to normal values for CSF and serum.^{12,21} To our knowledge, this is the first report that abscesses anywhere in the body may contain ammonia at high concentrations.

A brain abscess is surrounded by a capsule composed of reactive astrocytes and fibrous tissue.³ The capsule is not a diffusion barrier, however,^{25,39} so ammonia would be able to diffuse from the abscess cavity into the surrounding brain tissue. Here, ammonia may contribute to edema formation^{14,33,35} and interfere with neurotransmission and astrocytic potassium handling, as is illustrated in hyperammonemic states.^{14,22,34} Serum ammonia in patients with coma due to hepatic encephalopathy was 0.35 mmol/L or below.^{2,32} The patients in the present study had pus ammonia levels well above that value. Therefore it is probable that ammonia in pus contributes to the neurological symptoms in patients with brain abscess.

Some factors may influence the degree to which am-

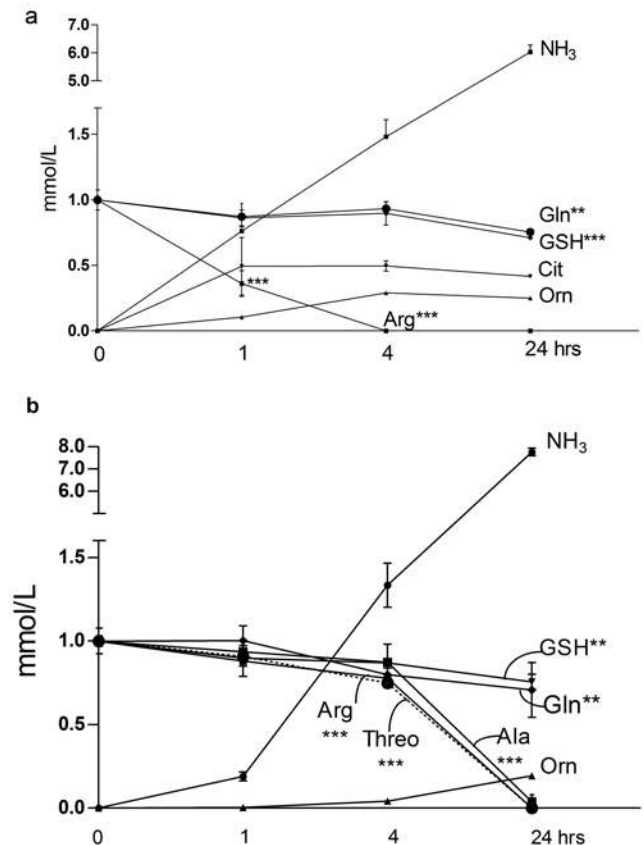


FIG. 2. Graphs showing formation of ammonia from amino acids in vitro by *S. intermedius* and *S. aureus*. The *S. intermedius* or *S. aureus* were incubated in Krebs buffer with all 20 proteinogenic amino acids plus GABA, taurine, and reduced GSH at 1 mmol/L. The concentrations of ammonia and amino acids (including citrulline and ornithine, which were formed from arginine) were measured at 0, 1, 4, and 24 hours. Only amino acids that were significantly reduced at 4 hours are shown (except citrulline and ornithine, which increased). **a:** Formation of ammonia by *S. intermedius*. At 1 and 4 hours, arginine was the only amino acid that was significantly reduced, and arginine metabolites citrulline and ornithine were formed together with ammonia. Omission of arginine from the incubation medium abolished ammonia formation at 4 hours. At 24 hours arginine, asparagine, aspartate, and serine were absent, and levels of isoleucine, leucine, valine, tryptophan, and tyrosine were reduced by 14%–24% (not shown); these amino acids were not consumed at 1 or 4 hours. **b:** Formation of ammonia by *S. aureus*. The level of several amino acids was reduced after 4 hours of incubation. Omission of threonine from the incubation medium reduced ammonia formation by 41% at 4 hours, whereas omission of arginine from the incubation medium reduced ammonia formation by 29% (not shown). Omission of alanine or glutamine did not cause a significant decrease in ammonia formation at 4 hours. At 24 hours even glycine was absent, asparagine was reduced by 90%, and glutamate by 70% (not shown). Data are expressed as mean \pm SD values; $N = 4$ per value. Most SD values have been omitted for legibility. Asterisks denote values that were different from value at 0 hour, as follows: ** $p < 0.01$ and *** $p < 0.001$ (1-way ANOVA with Dunnett test for multiple comparisons). See legend to Fig. 1 for definitions of abbreviations.

monia in pus may cause neuronal dysfunction in the brain tissue surrounding the abscess. First, the volume of the abscess, together with the ammonia concentration, will determine the amount of ammonia that is available for diffusion into the surrounding brain tissue. Second, the pH of

the pus will influence the diffusibility of ammonia from the abscess cavity. Ammonia as NH_3 is highly diffusible across phospholipid membranes,^{1,24} but with a pKa of 9.2, more than 99% of the ammonia is NH_4^+ at pH 7, and less than 1% is NH_3 . At lower pH, which may occur in a brain abscess, an even greater fraction of the ammonia is protonated and has a restricted diffusibility compared with unprotonated ammonia.

It is a limitation of the present study that we do not have data on the ammonia level in the brain tissue surrounding the abscesses and that the effect of the high concentration of ammonia in pus remains a matter of speculation. Experimental studies with microdialysis of abscesses and the surrounding brain tissue may be required to address this issue. Another limitation of this study is the small number of patients, which precludes statistical evaluation of the pathogenic role of ammonia versus other factors that accompany a brain abscess, such as tissue destruction and inflammation, changes in brain architecture, and increased intracranial pressure.

Possible Sources of Ammonia in a Brain Abscess

Amino acids were probably precursors of ammonia in the brain abscesses. High concentrations of amino acids that were precursors of ammonia in vitro in this study, including arginine, threonine, alanine, and glutamine, were recently reported in pus from human brain abscesses.⁹ DNA, RNA, and other nitrogen-containing compounds could be additional important sources of ammonia.⁷ Urease-positive bacteria may cause formation of ammonia from serum urea, but of the bacteria identified in the present study, only some strains of *H. parainfluenzae* have urease activity.³¹ Urease is absent in *S. intermedius* and *S. anginosus*,³⁸ and in *A. aphrophilus*,³⁰ *F. nucleatum*,¹⁶ *P. micra*,²⁹ *P. acnes*,²⁸ and *Prevotella* species.³⁶

Several ammonia-forming pathways may be active in a brain abscess, including ammonia formation from glutamine by phosphate-activated glutaminase, an enzyme that may originate from invading leukocytes⁵ or from damaged neurons.¹³ In the present study, *S. intermedius* and *S. aureus* produced ammonia at high concentrations in vitro, but seemed to do so along somewhat different pathways. Within the first 4 hours of incubation, *S. intermedius* primarily used arginine as a source of ammonia. This finding probably reflected an active arginine deiminase pathway, which leads to formation of ammonia, citrulline, ornithine, and adenosine 5'-triphosphate (ATP).⁸ Formation of citrulline from arginine entails the stoichiometric formation of 1 molecule of ammonia, whereas formation of ornithine entails the stoichiometric formation of 2 molecules of ammonia. In contrast to *S. intermedius*, *S. aureus* metabolized several amino acids (arginine, threonine, alanine, glutamine) within the first 4 hours of incubation, meaning that more ammonia-forming pathways were active in *S. aureus*. A preference of *S. aureus* for arginine and threonine under anaerobic conditions was recently demonstrated in vitro.³⁷ Both *S. intermedius* and *S. aureus* presumably metabolize different amino acids according to their availability, but the presence of citrulline and ornithine at a high concentration in pus in this study suggested that arginine is an important source of ammonia in brain abscesses.

Conclusions

Ammonia can reach toxic levels in brain abscesses in human patients, and ammonia may contribute to the brain pathology (edema) and symptoms caused by the abscess. Brain abscesses should probably be counted among disease states that can entail a toxic effect of ammonia on the brain, along with liver failure, portocaval shunting, infections with urease-producing bacteria, and inborn errors of urea metabolism.

Addendum

Since the submission of this manuscript the authors have completed analysis of pus from 10 patients with subdural empyemas. In these 10 patients the median ammonia concentration was 318 $\mu\text{mol/L}$ (range 46–1038 $\mu\text{mol/L}$), in agreement with a much lower level of amino acids in this type of pus, as previously reported.⁹

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Author Contributions

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