Advances in Hepatic Encephalopathy and Urea Cycle Diseases

5th International Symposium on Ammonia
Semmering, Austria, May 16–19, 1984

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Drug Dosage

The authors and the publisher have exerted every effort to ensure that drug selection and dosage set forth in this text are in accord with current recommendations and practice at the time of publication. However, in view of ongoing research, changes in government regulations, and the constant flow of information relating to drug therapy and drug reactions, the reader is urged to check the package insert for each drug for any change in indications and dosage and for added warnings and precautions. This is particularly important when the recommended agent is a new and/or infrequently employed drug.

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Preface

This volume contains the papers presented at the 5th International Symposium on Ammonia, held in Semmering, Austria, May 16–19, 1984 and covers a wide spectrum of research activities conducted to uncover the mistery of hepatic encephalopathy. The contributions include various topics such as metabolism, endocrinology, neurochemistry, electrophysiology, nutrition etc., reflecting the enormous increase in knowledge of many aspects of liver failure.

The possible metabolic derangements in liver failure are not a simple consequence of the breakdown of several functions of the liver but of a series of interactions of almost any organ in the body. Against this background it was not surprising that many different hypotheses of the pathogenesis of hepatic encephalopathy have been promoted and that still the right solution is lacking. This development in research was evident when Dr. *I. Szam* (Budapest, Hungary) the president of the 1st International Symposium on Ammonia, reviewed the past symposia and their highlights.

The position of ammonia as "frontrunner" and candidate toxin for the mediation of hepatic encephalopathy was strenghtened in this symposium. Important interactions between ammonia and the metabolism of branched chain amino acids (BCAA), the permeability of the blood brain barrier and the altered neurotransmission in hepatic encephalopathy have been presented and hence, ammonia could serve as an important link to other hypotheses. The liver plays an even more complex role in ammonia metabolism then recognized before. The landmark work to this topic by Dr. *D. Häussinger* (Freiburg, FRG) on „New Concepts in Hepatic Ammonia Metabolism and pH Regulation" was awarded with the *Friedrich Wewalka Award* 1984. Therapeutic strategies to lower blood ammonia concentration in urea cycle diseases have been considerably improved and may also provide a new approach to the treatment of hepatic encephalopathy.

The "false neurotransmitter" hypothesis of the pathogenesis of hepatic encephalopathy by *Fischer and Baldessarini* lead to trials to treat hepatic en-

cephalopathy with BCAA alone or with BCAA enriched amino acid solutions. After a period of great enthusiasm about the possible therapeutic values on intravenous BCAA, first controlled studies have tempered our hopes. Therefore, these proceedings start with the panel about the "Future Clinical Trials in Treatment of Acute Hepatic Encephalopathy" which was excellently chaired and edited by Dr. *N. Tygstrup* from Copenhagen. Undoubtly the highly qualified panelists and the competent discussants in the auditorium made this panel to one of the most exciting events of the symposium.

The new concepts of receptor mediated changes as causal pathogenic factor of HE included not only the "classical" neurotransmitters serotonin, dopamine and noradrenaline but also an altered amino acidergic neurotransmission was presented, stressing the role of the major inhibitory neurotransmitter of the mammalian brain, gamma-aminobutyric-acid (GABA), in the pathogenesis of hepatic encephalopathy. In addition the possible role of alterations of the blood brain barrier in liver failure was discussed in detail by well recognized experts in this field. However, it became clear that the different animal models used to study the effects of liver failure and the different methods used to detect and to quantitate possible changes yielded different and even contradictory results. Future studies of brain function in hepatic encephalopathy may clarify many issues and new facilities may result in a better understanding of events mediating hepatic encephalopathy.

We like to express our graditude to all participants of the symposium for contributing their efforts and for their cooperation. Our special thanks are due to Mrs. *Reingard Kleinberger* for her secretarial work and to Karger, Munich, especially *H. Rupprecht* and *W. Kunz* for providing the rapid publication of this volume. Furthermore we are extremly thankful to the numerous sponsors of this conference, without which we not have had the possibility to organize and publish this conference for the scientific community.

Vienna, October 1984

Gunter Kleinberger
Peter Ferenci
Peter Riederer
Heribert Thaler

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Past and Future of the Ammonia Symposia

I. Szám

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The word "ammonia" was originated in Egypt. Since the 18th–20th centuries b.C. pilgrims came from afar on their camels to the Ammon-church. Here camel excrement accumulated in abundance, was burned, and from the ashes "sal ammonium" (NH_4Cl) was obtained.

Hepatic encephalopathy was first described by *Hippocrates* (460–370) b.C. [10]. Studies on ammonia metabolism also have a long experimental and biochemical history, going back to the work of the *Pawlow* School on meat intoxication in dogs with Eck fistulas [9, 15]. In 1932 the ornithine cycle of urea synthesis was discovered [13]. Important milestones in research on ammonia metabolism include the work of *Burchi* [2], *van Caulert* [3, 4], *Mc Dermott and Adams* [14], *Bessman and Bessman* [1], *Summerskil et al.* [20], and *Stahl* [18, 19].

My research on ammonia metabolism began in a peculiar way. In 1953 I investigated the pathomechanism and therapy of acute pulmonary edema, elaborating an experimental model for producing pulmonary edema: In Wistar rats experimental pulmonary edema was produced by intraperitoneal injection of 40–50 mg/100 g body wight of ammonium chloride [7]. Between 1953–1972 we elucidated the details of the pathophysiology and therapy for this experimental NH_4Cl — intoxication [35, 37]. Histologic and electrophysiologic examinations showed that neurohistologic changes and bioelectric disturbances of the brain precede the pulmonary edema produced by ammonium chloride [8, 22, 23]. Ganglion cell alteration of anoxic-sclerotic type and intracellular edema and vacuolisation were found in the cortex and reticular formation of rats with experimental ammonium chloride poisoning [8]. In experimental ammonium chloride intoxication bradypnea, bradycardia [23, 35], hypercatecholaminemia [39], convulsions, and marked changes in cerebral bioelectric activity such as spikes, changes in amplitude and frequency were observed [22, 23]. These findings suggested that the noxious effect of the ammonium ion on the central nervous system was responsible for the symptoms of NH_4Cl intoxication.

In 1971 the ultrastructure of the lung was studied by electron microscope in various phase of ammonia intoxication. An edematous impregnation was proven in the endothelial cells of pulmonary capillaries and the respiratory epithelium [35]. Alveolar transudation was shown to develop only in a later phase. The experimental pulmonary edema induced by ammonium chloride develops by increasing the permeability of the respiratory membrane [35]. The symptoms of NH_4Cl poisoning were ascribed to the ammonium ion itself and not to acidosis; since the symptoms can also be produced by other ammonium salts which do not cause acidosis [35].

In another experimental study carried out on Wistar rats the ammonia concentration of brain tissue was found to markedly increase in the course of poisoning by hyperbaric oxygen [25, 28, 30].

After the pathophysiology of pulmonary edema due to ammonium was ascribed, the frequency of pulmonary edema in hepatic coma was investigated. Examining autopsy material from 4561 cases, we found that pulmonary edema occurs 2.5 times more frequently during hepatic coma than might be expected from control dissection materials. The higher frequency of pulmonary edema in hepatic coma can probably be traced back to endogenous ammonia intoxication [24, 35].

Subsequently we developed a new modified ion exchange method for determining plasma ammonia concentration. Plasma ammonia was isolated on a cation exchange resin (Varion-KS), then eluted into 4 M sodium chloride solution, and the ammonium content of the eluate was determined by means of the modified Berthold reaction [21, 27].

In 1968 we demonstrated hyperammonia in cor pulmonale decompensatum with right heart failure and established that endogenous ammonia intoxication can play an important role in the development of CNS symptoms in severe forms of cor pulmonale with encephalopathy [26, 29, 32, 38]. In cases of cor pulmonale, right heart failure and CNS symptoms there was a negative correlation between hyperammonia and arterial oxygen saturation, a positive correlation between hyperammonemia and $paCO_2$, a negative correlation between hyperammonemia and arterial blood-pH [42]. In a severe stage of pickwickian syndrome [34] and mucoviscidosis (cystic fibrosis of the pancreas), hyperammonemia was also demonstrated [17], a new simple quantitative writing test was described for psychometry in hepatic encephalopathy [40] and the role of respiration in the elimination of ammonia was elucidated [41].

Between 1964 and 1973 we investigated the effect of different amino acids in experimental NH_4Cl poisoning and in patients with hyperammonemia. The effect of arginine, arginine-malate, aspartic acids salts, ornithine-α-ketoglutarate was tested on 462 Wistar rats with experimental NH_4Cl intoxication. The results were compared with the findings in control animals poisoned with ammonium chloride without being pretreated. Significant protection was observed in the groups treated with arginine, arginine-malate, aspar-

Table I. International Ammonia Symposia, 1972–1984

Place	Date	President(s)	Number of			Costs of the organisation
			Participants	Countries	Papers	
I. Budapest	May 11–13, 1972	*D. Müting* *I. Szám*	57	6	30	132.000 HUF
II. Strasbourg	May 16–18, 1974	*J. Stahl*	70	7	31	30.000 FRF
III. Baden near Vienna	May 11–14, 1977	*F. Wewalka*	70	9	38	210.000 ATS
IV. Heidelberg-Mannheim	May 7–11, 1980	*E. Holm*	150	12	46	63.000 DM
V. Semmering Austria	May 16–19, 1984	*G. Kleinberger*	130	18	72	100.000 DM

tic acid salts, and ornithine-α-ketoglutarate. Clinical investigations were carried out in patients with liver diseases and hyperammonemia. After infusions of arginine-malate and ornithine-α-ketoglutarate plasma ammonia levels decreased [31, 33, 36, 37].

During a scientific meeting in Bad Kissingen, 5th February 1971, *D. Müting* and I proposed founding a Working Group and organizing a conference on ammonia metabolism. The Ist International Symposium took place in Budapest, 11–13 May 1972, presided over by *D. Müting* and *I. Szám*, and had 57 participants from six countries. The main topics of this first conference were the methods of ammonia determination, ammonia metabolism in liver diseases, ammonia metabolism in extrahepatic diseases, and the pharmacology of ammonia metabolism. An important event at this first meeting was the report of *H. A. Krebs* (Oxford) on the discovery of the ornithine cycle of urea synthesis [13]. The lively response to this first Symposium provided a good opportunity to demonstrate the aim of our Working Group, i.e., to discuss different aspects of ammonia metabolism and metabolic disorders. Although the symposium was successful, I did not dream that four ammonia symposia would follow.

The 2nd. International Symposium, held 16–18 May 1974 in Strasbourg, was chaired by *J. Stahl* and 70 participants arrived from seven countries. This second conference again focused on the pathophysiology, diagnosis, and treatment of hyperammonemia. Important results were reported and discussed extensively.

F. Wewalka presided over the 3rd Symposium held in the Weikersdorf Castle in Baden near Vienna, 11–14 May 1977 and to which 70 participants came from nine countries. *J. Stahl* gave the Keynote lecture entitled his honorary lecture, "How have ammonia studies contributed to the understanding of hepatic encephalopathy?" The methods of ammonia determination, the correlation of hyperammonemia with encephalopathy, the problems of hepatic coma were discussed anew.

The IVth International Symposium on Amino Acid and Ammonia Metabolism was held in Heidelberg (FRG), 8–11 May 1980 with 150 participants from 12 countries. This meeting continued the tradition of the three earlier conferences. The scientific organisation was in the hands of *E. Holm* as chairman. Important contributions on liver-brain relationship, deviation in amino acid metabolism, ammonemia in extrahepatic diseases such as malignant tumors, and therapy with amino acid mixtures were introduced into the program. The most important data of the previous four symposia are summarized in table I. The papers of the symposia have been published in four volumes [11, 12, 37, 43].

The 12 years following the first symposium showed that our initial idea had borne fruit. The symposia on ammonia have served many functions: they have provided a forum for presentation and discussion of new and important results, served as a meeting ground for old and new friends, and furthered the cross-fertilisation of ideas from papers and discussions in an informed atmosphere. The findings proved useful also for the pharmaceutical industry.

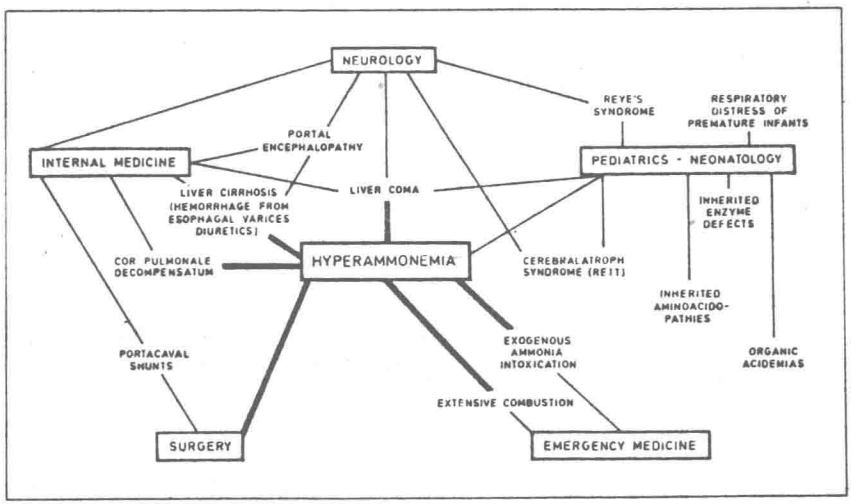

Fig. 1. Diseases associated with hyperammonemia

Table II. Inherited disorders with hyperammonia

I. Urea cycle defects

1. Ornithine transcarbamylase deficiency
2. Carbamyl phosphate synthetase I deficiency
3. Argininosuccinate synthetase deficiency (Citrullinemia)
4. Argininosuccinate lyase deficiency (Argininosuccinic aciduria)
5. Low arginase activity (Argininemia)
6. N-acetyglutamate synthetase deficiency

II. Dibasic aminoacidopathies

1. Ornithinemia
2. Periodic hyperlysinemia (L-lysin dehydrogenase defect)
3. Lisinuric protein intolerance

III. Organic acidemias

1. Propionic acidemia (Propionyl CoA-carboxylase defect)
2. Methylmalonic acidemia (Methylmalonyl CoA mutase or cobalamin metabolism defect)
3. Isovaleric acidemia (Isovaleryl CoA dehydrogenase defect)
4. Multiple carboxylase deficiency (Holocarboxylase synthetase or biotin transport defect)
5. Ethylmalonic acidemia (Multiple acyl CoA dehydrogenase defect)
6. Glutaric acidemia (Multiple acyl CoA dehydrogenase defect)
7. Methyl-3-hydroxybutiric acidemia (Acetoacetyl CoA thiolase, β-ketothiolase defect)

The most important diseases associated with hyperammonemia are summarized in figure 1. In recent years the inherited disorders with hyperammonemia have attracted more interest. Table II presents the inherited hyperammonemic syndromes [6].

Any overview of past accomplishments must also touch upon future prospects. Many participants of the Ammonia Symposia expressed their desire that the tradition be continued. It seems advisable to enlarge the topics to include interdisciplinary problems, but the techniques of ammonia determination, the inborn errors of ammonia metabolism, Reye's syndrome, the patho-

physiology of neurotransmission in hepatic encephalopathy, branched-chain amino acids and keto acids, the problems of parenteral nutrition, therapy for hepatic coma will in all probability remain fascinating topics for the future symposia as well. Together with my colleagues *E. Holm* and *G. Kleinberger*, we propose that the VIth Symposium should take place in May, 1987 in Maastricht, Holland, with *P. Soeters*, who has agreed to make the necessary arrangements, as chairman. We would like to request proposals for special topics.

As a fitting conclusion to my opening lecture, I should like to remember the deceased members of our Working Group who participated in the previous international workshops and are not among us today.

Béla Balás, Senior Physician in the Department of Clinical Chemistry of the Hungarian Institute of Rheumatism, Balneology and Physiotherapy died of myocardial infarction 8 February 1973 in Budapest at the age of 61. He was born in Nagybánya in 18 July, 1912. After graduating in medicine from the University of Budapest in 1936, he specialized in clinical chemistry and worked as a leading physician in different hospitals in Hungary. In 1951 *Balás* became the Medical Director of the Clinical Laboratories of the Hungarian Institute of Rheumatism, Balneology and Physiotherapy. He also founded the San Marco College for Assistants in Laboratory Medicine in Budapest. At the Ist Ammonia Symposium he lectured on the role of the kidneys in ammonia metabolism and also helped organize our first conference.

László Murányi, Associate Professor of Pediatrics at Szeged, died of a malignant lymphoma in Budapest on 21 February 1979, at the age of 51. He was born in Szentendre on 7 August 1927. He studied medicine at the University of Budapest, graduating in 1952. Between 1953 and 1963 he specialized in pediatrics, respiratory pathophysiology, infectious diseases, and clinical pharmacology. In 1975 he became Director of the Perinatal Intensive Care Center at the University Medical School in Szeged, Hungary. About one hundred excellent papers and chapters in books made him well-known to the scientific community. He gave valuable lectures at the Ist and 3rd Symposia on Ammonia Metabolism: in 1972, *Dr. Murányi* demonstrated the simultaneous changes in ammonia concentration of the blood and in acid-base metabolism during exchange transfusion of newborns with hyperbilirubinemia and in 1977 he reported on ammonia metabolism in the respiratory distress syndrome in newborns.

Friedrich Wewalka, Professor of Gastroenterology and Hepatology, was 60 years old at his death on 17 May 1980. He was born on 22 January 1920 in Vienna and graduated with a degree in medicine from the University of Vienna. After the war he specialized in hepatology and gastroenterology, becoming the head of the Plasma-Protein Laboratory of the Ist Medical Department of the University of Vienna. He recognized early the importance of endoscopic examinations, and achieved important results in epidemiologic