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Research Article

THE METABOLISM OF AMMONIA AND α-KETO-ACIDS IN LIVER DISEASE AND HEPATIC COMA[1]

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A causal relationship between ammonia intoxication and hepatic coma is suggested by the reproduction of the syndrome of impending hepatic coma in sensitive patients with liver disease by substances from which ammonia can be derived (1) and is supported by demonstration of deranged ammonia metabolism in liver disease (2). The frequent finding of elevated peripheral vein ammonia concentrations in hepatic coma (3) has encouraged incrimination of ammonia in the genesis of the syndrome (4, 5). Conversely, reservations have been expressed that such results may signify no more than impaired nitrogen metabolism secondary to liver disease (6, 7). More recently Bessman and Bessman (8) have questioned the validity of observations based on peripheral vein blood alone by demonstrating significant arterio-venous ammonia difference in hepatic coma. Moreover, a close relationship has been reported between arterial concentrations and neurological status in a patient with this complication (9).

The purposes of this paper are: 1) Further assessment of the significance of arterial concentrations and A-V ammonia differences in hepatic coma, directing particular attention to the effect of eliminating from the diet nitrogenous substances from which ammonia may be derived; and 2) Investigation of a possible relationship between disordered ammonia metabolism and the elevated blood pyruvate and α-ketoglutarate concentrations reported in liver disease (10, 11). Arterial concentration and tissue metabolism of ammonia have been compared with the neuropsychiatric state and with the protein intake in uncomplicated liver disease and hepatic coma. Simultaneous estimations of blood pyruvate and α-ketoglutarate were performed. The relationship of the keto-acids to ammonia metabolism was further studied in a smaller group of patients with liver disease by measuring blood concentrations of these substances in response to the administration of ammonium chloride.

MATERIAL AND METHODS

Patients. Twenty-seven patients (16 male and 11 female) in impending hepatic coma or coma were studied. Ages were distributed between 29 and 70 years. Twenty patients had cirrhosis associated with chronic alcoholism. The etiology of cirrhosis was uncertain in three patients. One patient had hemochromatosis and another Wilson's disease. One patient had an hepatic lymphoma and the remaining patient had carbon tetrachloride poisoning. Liver failure, evident from progressive jaundice and severe ascites, was judged the basis of hepatic coma in 16 patients. Other factors precipitating coma included major gastrointestinal hemorrhage (5 patients), acute pyogenic infections (3 patients), intolerance of nitrogenous substances with deterioration of liver function (2 patients with portacaval anastomoses), paracentesis abdominis (1 patient) and a major surgical operation (1 patient).

Eleven patients with cirrhosis in the absence of hepatic coma were also studied (10 chronic alcoholics and 1 in whom the etiology of liver disease was uncertain). A group of 18 control subjects without evidence of hepatic, renal or metabolic disorder was recruited from hospital patients and staff.

The diagnosis of liver disease was made on clinical and biochemical grounds; histological confirmation from biopsy or autopsy specimens was available in 27 patients, including 18 of the 20 patients who died in hepatic coma.

Neuropsychiatric assessment. Patients in coma (including impending hepatic coma) were examined daily or more often, assessment being based on the clinical syndrome reported by Adams and Foley (12) and assisted by EEG records when necessary. The charac-

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TABLE I

Cerebral (arterial-jugular bulb difference) and peripheral (arterial-peripheral vein difference) uptake and release of ammonia during terminal two days of hepatic coma *

	Patient and diagnosis	Serum bilirubin (*mg./100 ml.*)	Ascites (0–+++)	Arterial NH_3N (*μg./100 ml. blood*)	A-V difference Ammonia "uptake" (+) or "release" (−) Cerebral (*μg./100 ml.*)	Peripheral (*μg./100 ml.*)
GA	Cirrhosis of the alcoholic	26.0	+++	275	+15	+61
SU	Cirrhosis of the alcoholic, massive gastrointestinal hemorrhage	18.4	+++	60	+12	+16
ST	Cirrhosis of the alcoholic, massive gastrointestinal hemorrhage	2.9	+++	250	+66	+83
BC	Cirrhosis of the alcoholic, lobar pneumonia	6.4	0	271	+4	+1
MC	Cirrhosis of the alcoholic	11.0	+++	171	−75	−97
GE	Wilson's Disease	1.9	+++	229	−90	+42
BE	Cirrhosis of the alcoholic, massive gastrointestinal hemorrhage	23.6	++	354		+4
EA	Cirrhosis of the alcoholic	22.0	+++	262		−1
MG	Cirrhosis of the alcoholic	19.0	++	200		−11
MS	Hepatic lymphoma, gastrointestinal hemorrhage	0.5	0	114		−4
SP	Carbon tetrachloride poisoning	38.0	+	52		−11
MK	Cirrhosis of the alcoholic	33.1	+++	160		+63

* Although in coma, the patients were not moribund.

teristic fluctuation and frequent disparity between psychiatric and objective neurological findings permitted an accurate distinction only between impending coma and coma for the purpose of this report, stuporous patients responding only to strong stimuli being placed in the latter category.

Protein intake. Protein intake, during or immediately prior to hepatic coma arising in hospitalized patients, was assessed from the ward diet or calculated from the restricted protein diet, usually a low-sodium milk product,[4] on which patients were maintained. Progressive or advanced hepatic coma was treated with exclusion of all nitrogenous substances (protein, drugs, etc.) from the diet and with broad spectrum antibiotics by mouth (7). Chlortetracyclene in doses of 2 to 4 gm. daily was administered for the duration of the neurological syndrome. The possibility of gastrointestinal bleeding was checked by inspection of feces, examination of stools for occult blood and, in fatal cases, at autopsy.

Biochemical methods. Blood ammonia was determined by a modification (13) of Conway's method (14); blood pyruvate and α-ketoglutarate were estimated using the method of Seligson and Shapiro (15). Blood was drawn without stasis into specially cleaned syringes and was introduced into the Conway units or flasks at the bedside. The accuracy of these methods in our hands, calculated from the standard deviation from mean recoveries, was ± 3 per cent for ammonia, using standards and blanks, ± 6 per cent for α-ketoglutarate and ± 9 per cent for pyruvate. The recoveries for keto-acids were carried out using human blood. "Uptake" of ammonia or keto-acids by brain or peripheral tissues was assumed when the differences between concentrations in arterial and appropriate venous blood (A-V difference) were positive and, conversely, release is represented by negative differences.

Administration of ammonium chloride. Alterations in blood ammonia, pyruvate and α-ketoglutarate concentrations following the administration of ammonium chloride were measured in 5 patients with liver disease, whose clinical and biochemical findings are given in Table III, and in 4 control subjects. Three other control subjects received ammonium chloride prior to determination of arterial and peripheral vein ammonia concentrations. Ammonium chloride was given by mouth (as non-enteric coated capsules) or intravenously (2 per cent solution in water or sodium chloride infused over 45 minutes) in 3.0 or 4.0-gm. doses. Individuals with severely impaired liver function, extensive portal-systemic collateral venous systems or previous evidence of neuropsychiatric sensitivity to nitrogenous substances received the smaller amount by mouth in view of their susceptibility to hepatic coma induced by ammonium salts (1). Control subjects were given ammonium chloride intravenously as use of the oral route would have prevented most of the ammonia from reaching the peripheral tissues owing to its removal from portal blood by a healthy liver (2). Blood specimens (arterial, peripheral venous, or both) were taken immediately prior to administration of the salt and at varied 30-minute intervals up to four hours for determination of blood ammonia, pyruvate and α-ketoglutarate concentrations. Values are reported at 0, 30 to 60, 90 to 120 and 180 to 240 minutes, representing actual readings or mean levels where more than one estimation was made in the relevant period.

[4] Lonalac®, Mead Johnson and Company, Evansville, Indiana.

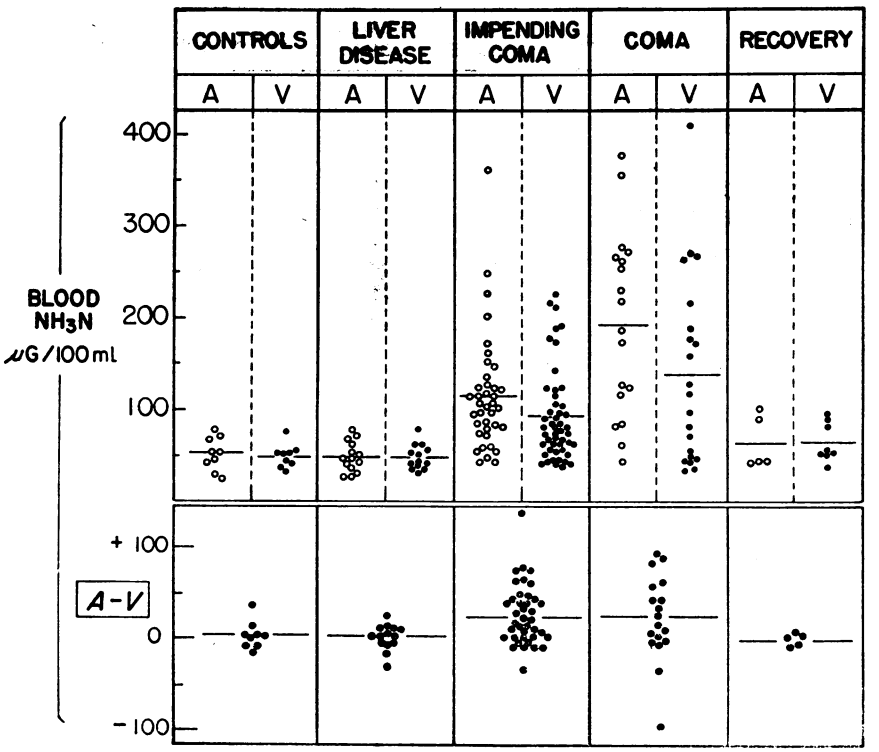

FIG. 1. ARTERIAL (A) AND PERIPHERAL VEIN (V) AMMONIA CONCENTRATIONS WITH A-V DIFFERENCES IN CONTROL SUBJECTS AND PATIENTS WITH LIVER DISEASE WITH AND WITHOUT HEPATIC COMA

RESULTS

Blood ammonia and keto-acid concentrations in hepatic coma

Comparisons were made between the arterial and peripheral vein ammonia concentrations and A-V difference of ammonia in the control group and in patients with liver disease and hepatic coma (Figure 1). Control subjects and patients with liver disease without coma had similar fasting values of ammonia in arterial and venous blood (upper limit of normal 75 μg. per 100 ml.) and A-V differences in both groups indicated that a small and variable uptake or release of ammonia by peripheral tissues occurred in the fasting state. During impending hepatic coma the mean arterial concentration (113 μg. per 100 ml.) was elevated, but a quarter of the readings remained within the normal range. The mean concentration was lower in the peripheral vein (92 μg. per 100 ml.), a half of the values being within normal limits. These findings were associated with a greater positive A-V difference of ammonia in peripheral tissues in the majority of cases. In patients who had progressed to coma, higher mean values of arterial and peripheral vein ammonia were found (193 and 139 μg. per 100 ml., respectively) with a smaller proportion of readings (about 10 per cent arterial and 25 per cent venous) still remaining in the normal range. The A-V difference was still predominantly positive but relative equilibrium was not uncommon and tissue release of ammonia, occasionally of a high degree, was sometimes observed. Values obtained from patients in the phase of recovery, but still exhibiting residual neuropsychiatric disorder, showed a return to normal blood ammonia concentrations and tissue equilibrium of ammonia.

Blood α-ketoglutarate and pyruvate values were determined simultaneously with ammonia (Figure 2). Mean concentrations of both substances in fasting patients with liver disease (α-ketoglutarate 20.7, pyruvate 112 μM per liter) were above mean control values (α-ketoglutarate 11.5, pyru-

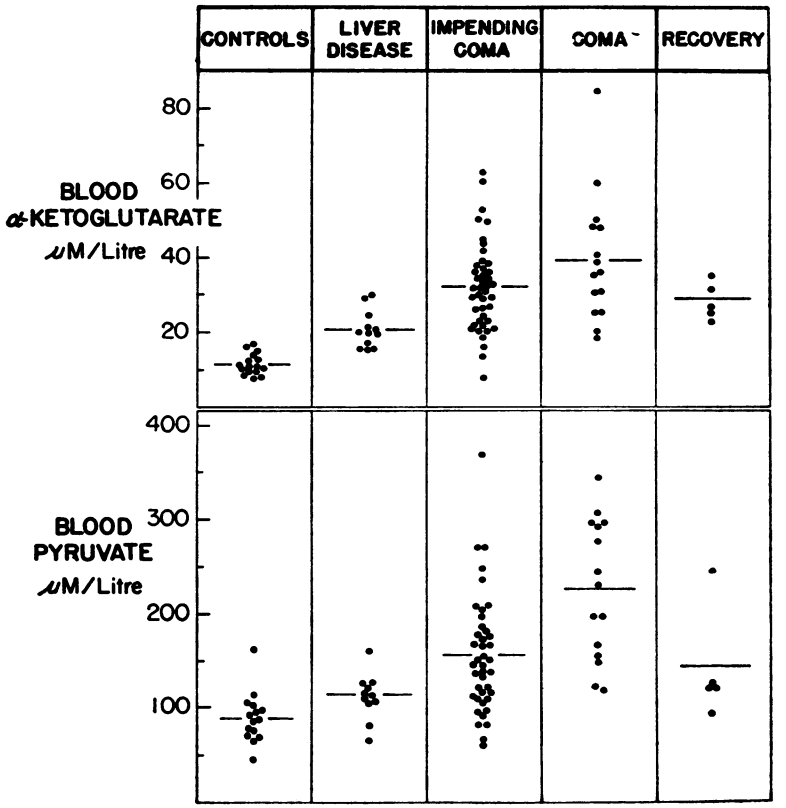

FIG. 2. BLOOD α-KETOGLUTARATE AND PYRUVATE CONCENTRATIONS IN CONTROL SUBJECTS AND PATIENTS WITH LIVER DISEASE WITH AND WITHOUT HEPATIC COMA

vate 89 μM per liter), but there was a considerable overlap in pyruvate values. Progressive increase in mean values in impending coma (α-ketoglutarate 32.3, pyruvate 154 μM per liter) and coma (α-ketoglutarate 39.7, pyruvate 225 μM per liter) was observed, but the scatter was wide. A third of the α-ketoglutarate and half of the pyruvate values during impending coma and coma were below the upper limits found in patients with uncomplicated liver disease.

The influence of nitrogenous material in the intestines on blood ammonia concentration in hepatic coma

Blood ammonia concentrations during hepatic coma were studied in relation to nitrogenous material in the intestine (dietary protein, gastrointestinal bleeding, drugs such as ammonium chloride, etc.) (Figure 3). The highest arterial ammonia concentrations occurred when coma was precipitated by gastrointestinal hemorrhage or by intolerance to nitrogenous substances and the mean levels in patients on conventional home or ward diets exceeded that found when coma was associated with low protein feeding. Only one value in each group was within the normal range. During treatment by total protein withdrawal and antibiotics, two-thirds of arterial ammonia readings remained high during the first 48 hours, but there was a striking decline towards normal in all patients during the second to fifth-day period, although only three values fell within the normal range. In the sixth to tenth-day period, the majority of arterial ammonia concentrations remained at or near normal, but a rise occurred in some patients and a further elevation in the mean value was observed in patients who survived on this regimen for more than 10 days, in the absence of protein feeding, gastrointestinal hemorrhage or uremia. Those who recovered and the fatal cases

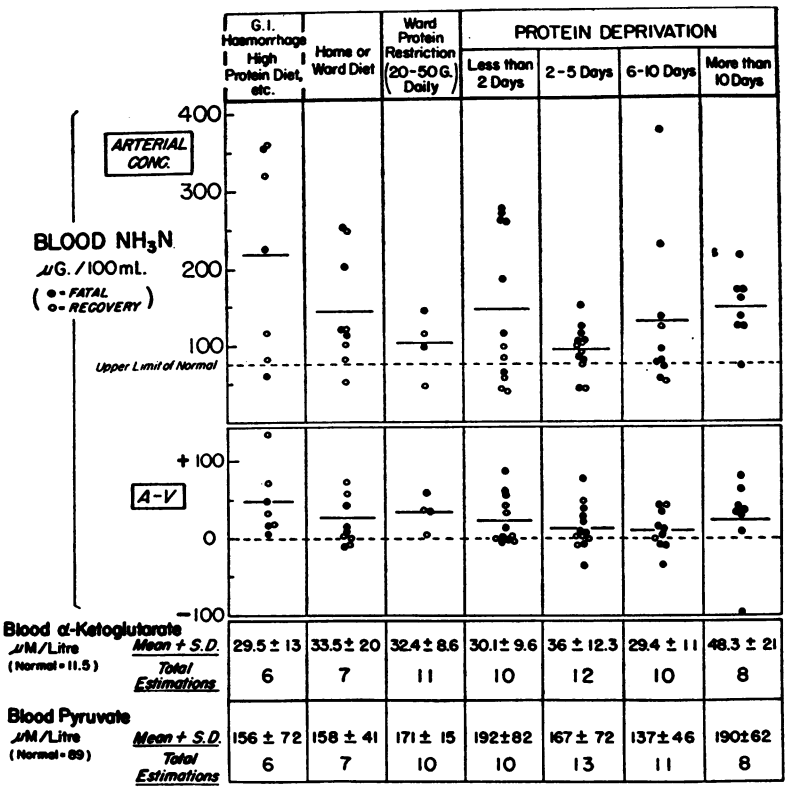

FIG. 3. ARTERIAL AMMONIA CONCENTRATIONS, A-V AMMONIA DIFFERENCES AND BLOOD α-KETOGLUTARATE AND PYRUVATE CONCENTRATIONS DURING HEPATIC COMA IN RELATION TO NITROGENOUS MATERIAL IN THE GASTROINTESTINAL TRACT

had arterial ammonia concentrations which were comparable in the early stages, but in patients who survived they returned towards normal more rapidly after protein withdrawal.

It was not possible to demonstrate a similar relationship between blood pyruvate or α-ketoglutarate concentrations and intake of nitrogenous material (Figure 3). Mean values for both substances were high at all stages, particularly in patients who survived more than 10 days, but the scatter was wide.

Uptake of ammonia by peripheral tissue and brain

Peripheral A-V ammonia difference in relation to arterial concentrations greater than 100 μg. per 100 ml. was compared in control subjects who had received ammonium chloride to patients with liver disease who also had received ammonium chloride or were in the early phase of impending hepatic coma (Figure 4). Although uptake of ammonia occurred in both groups, it was greater in control subjects in relation to arterial concentration and the impaired uptake in patients with liver disease was particularly striking at ammonia concentrations greater than 200 μg. per 100 ml. No difference was observed between patients with uncomplicated liver disease and those in impending hepatic coma.

Uptake of ammonia was the usual finding during the course of hepatic coma (Figures 1 and 3) and was related to arterial concentration. Tissue equilibrium or release of ammonia despite high arterial concentrations was not infrequent, however, and these findings were mainly limited to the terminal phase of coma. The arterial concentrations and A-V ammonia differences in 12 unconscious, but not moribund, patients who were studied during the ultimate 2 days of coma are reported in Table I. Cerebral and peripheral A-V differences of ammonia were determined

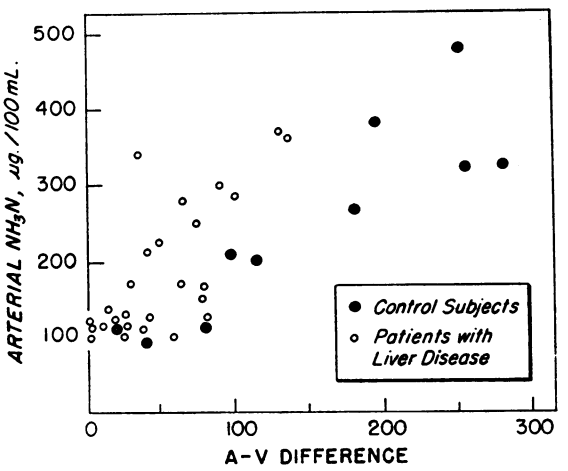

FIG. 4. PERIPHERAL TISSUE AMMONIA UPTAKE IN CONTROL SUBJECTS AND PATIENTS WITH LIVER DISEASE

simultaneously in 6 patients. Variable uptake of ammonia occurred at both sites in 3 patients (GA, SU, ST), two of whom had massive gastrointestinal bleeding, and a fourth (BC) showed neither uptake nor release at either site despite a high arterial concentration. Ammonia release of a high degree was taking place from both brain and peripheral tissues in the fifth patient (MC) and the remaining subject (GE) exhibited release of cerebral ammonia associated with peripheral uptake. Of the other 6 patients, in whom peripheral studies alone were performed, only one (MK) had a positive A-V difference, the remainder showing ammonia equilibrium, although arterial concentrations were high in all but one instance.

A-V differences of keto-acids

Peripheral A-V differences of pyruvate and α-ketoglutarate were determined on 14 occasions during impending coma, coma or ammonium chloride administration (*vide infra*). Pyruvate levels were higher in the vein in all but one instance, the mean A-V difference being − 15 μM per liter (S.D. ± 14). There was less evidence of peripheral tissue release of α-ketoglutarate. The mean A-V difference was − 2.1 μM per liter (S.D. ± 2.6), a small positive difference being found on three occasions.

Cerebrospinal fluid

Investigation of cerebrospinal fluid (Table II) showed relatively small amounts of ammonia and α-ketoglutarate in control subjects, although pyruvate concentrations were comparable to those in arterial blood. In hepatic coma very high ammonia values, comparable with but not clearly related to arterial concentrations, were found. With one exception, cerebrospinal fluid pyruvate values reflected and exceeded the arterial concentration in hepatic coma. Despite high arterial values, spinal fluid α-ketoglutarate concentrations remained relatively low, but a linear increase with arterial values occurred. High pyruvate and α-ketoglutarate values coincided in blood and cerebrospinal fluid but were unrelated to ammonia concentrations at either site.

Blood keto-acid concentrations in response to ammonium chloride administration

Control subjects demonstrated no constant alteration in mean blood α-ketoglutarate concentra-

TABLE II

Arterial and cerebrospinal fluid concentrations of ammonia, pyruvate and α-ketoglutarate in control subjects and in terminal hepatic coma

	Ammonia (NH_3N μg./100 ml.)		Pyruvate (μM/Liter)		α-Ketoglutarate (μM/Liter)	
	Arterial	C.S.F.	Arterial	C.S.F.	Arterial	C.S.F.
Controls (7 subjects)						
Mean ± S.D.	52	20 ± 6*	112	107 ± 25	11.5	1.1 ± 0.8
Hepatic coma						
Patient M. C.	171	408	295	275	85.5	6.5
R. Y.	122	161	190	218	36.5	3.9
B. E.	71	76	111	206	34.5	2.6
G. E.	229	92	92	123	27.7	2.0

* From Clarke, Parsons Smith, Sherlock, and Summerskill (29).

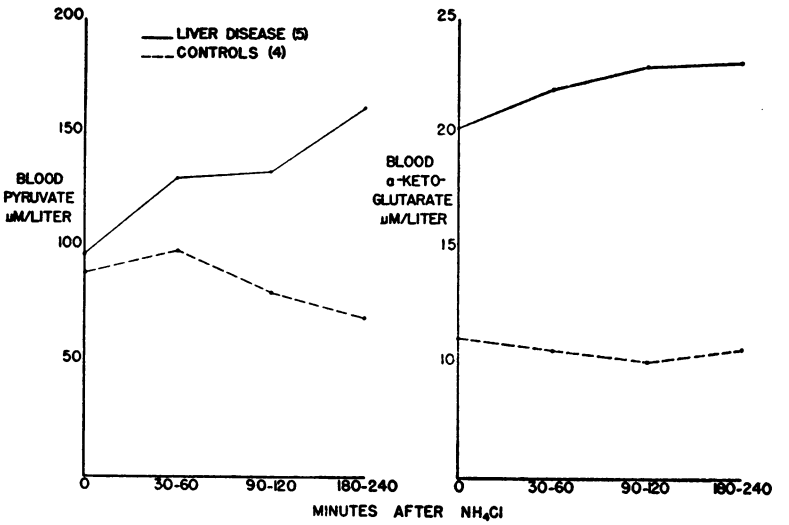

FIG. 5. MEAN BLOOD PYRUVATE AND α-KETOGLUTARATE CONCENTRATIONS FOLLOWING ADMINISTRATION OF AMMONIUM CHLORIDE TO CONTROL SUBJECTS AND PATIENTS WITH LIVER DISEASE

tions following the administration of 4 gm. ammonium chloride intravenously, but a late fall in mean pyruvate level was evident (Figure 5). Similarly, individual α-ketoglutarate values showed little variation from the fasting reading in this group, but pyruvate concentrations, after an early increase in 2 patients, declined as the test continued (Table III). All patients with liver disease exhibited a rise of pyruvate and α-ketoglutarate concentration both in mean values (Figure 5) and individual readings (Table III). The more striking elevations occurred in pyruvate levels which progressed steadily to a maximum at the 3 to 4-hour determination. One patient (B. A.), in whom sensitivity was anticipated owing to chronic nitrogen intolerance following portacaval anastomosis, showed a 95 per cent increase in pyruvate concentration after only 3.0 gm. of ammonium chloride by mouth, but other patients receiving the drug by the oral route (S.E., T.A.) exhibited smaller augmentations of blood pyruvate values than those receiving intravenous ammonium chloride (B.U., C.O.), despite comparable or worse liver function.

The mean elevation of blood α-ketoglutarate concentrations in patients with cirrhosis in response to ammonium chloride was less, but of a magnitude beyond the error of the method. It was a constant finding in every patient (Table III), and the greatest increase of α-ketoglutarate concentration occurred by 30 to 60 minutes, thus preceding the greatest alteration in pyruvate values.

Ammonia uptake by peripheral tissues following the administration of ammonium chloride was demonstrated by elevation of blood ammonia levels and positive A-V ammonia differences in both groups (Table III), coinciding in patients with liver disease with elevation of α-ketoglutarate and preceding the major rise in pyruvate levels. The return to fasting values occurred earlier in patients receiving the drug intravenously, but further assessment of individual tolerance was not considered relevant to the study.

The sequence of events is demonstrated in Figure 6. Following oral ammonium chloride, rapid elevation of blood ammonia levels was accompanied by a rise in peripheral tissue uptake of ammonia and α-ketoglutarate concentration. The steady rise of blood pyruvate values continued after blood levels of ammonia and α-ketoglutarate had declined and after the patient returned to his initial neuropsychiatric state.

DISCUSSION

Increased peripheral vein ammonia concentrations reported in patients with liver disease uncomplicated by hepatic coma (2, 3, 5, 7, 16) are

TABLE III

*Blood pyruvate, α-ketoglutarate and ammonia concentrations following * ammonium chloride administration (4.0 gm. intravenous infusion unless otherwise indicated) in control subjects and patients with liver disease*

Patient, Age, Sex	Serum bilirubin (mg.%)	Ascites	Impending coma	Blood pyruvate (μM/Liter) 0	30–60	90–120	180–240	Blood α-ketoglutarate (μM/Liter) 0	30–60	90–130	180–240		Blood ammonia† (NH_3N μg./100 ml.) 0	30	60	90	120	180
Control subjects																		
A. N., 29, F				86	62	55	53	10.5	8.2	8.8	9.9							
S. I., 46, M				66	134	80	81	8.1	10.7	8.6	9.7	A	27	388	118	44	34	
												A-V	−15	+196	+20	−18	−10	
F. L., 52, M				114	97	103	66	16.0	13.7	13.7	13.5							
C. R., 66, M				92	105	85	79	10.0	9.4	9.4	9.4							
			Mean	89	99	81	72	11.1	10.5	10.1	10.6							
Liver disease																		
B. U., 44, M	9.0	Transient	0	126	195	198	241	29.8	32.2	32.2	33.3	A	71		373		71	71
												A-V	−6		+129		−9	−5
T. A.,‡ 46, F	1.0	+++	0	81	95	113	135	17.9	19.0	21.4	23.2	A	43	279	302		167	
												A-V	−14	+66	+90		+80	
C. O., 61, M	1.0	0	0	67	78	71	112	14.4	15.6	17.9	18.1							
B. A.,§ 65, M	6.0	0	Recurrent	118	160	180	206	22.8	24.9	26.9	23.3							
S. E.,§ 70, M	3.0	0	Transient	92	123	110	110	16.9	19.8	17.5	18.4	A	89	320		360		284
												A-V	+2	+34		+136		+98
			Mean	97	130	134	161	20.3	22.2	23.2	23.3							

* Time in minutes.
† A—Arterial, A-V—Arterial-Peripheral Vein Difference.
‡ Ammonium chloride, 4.0 gm. by mouth.
§ Ammonium chloride, 3.0 gm. by mouth.

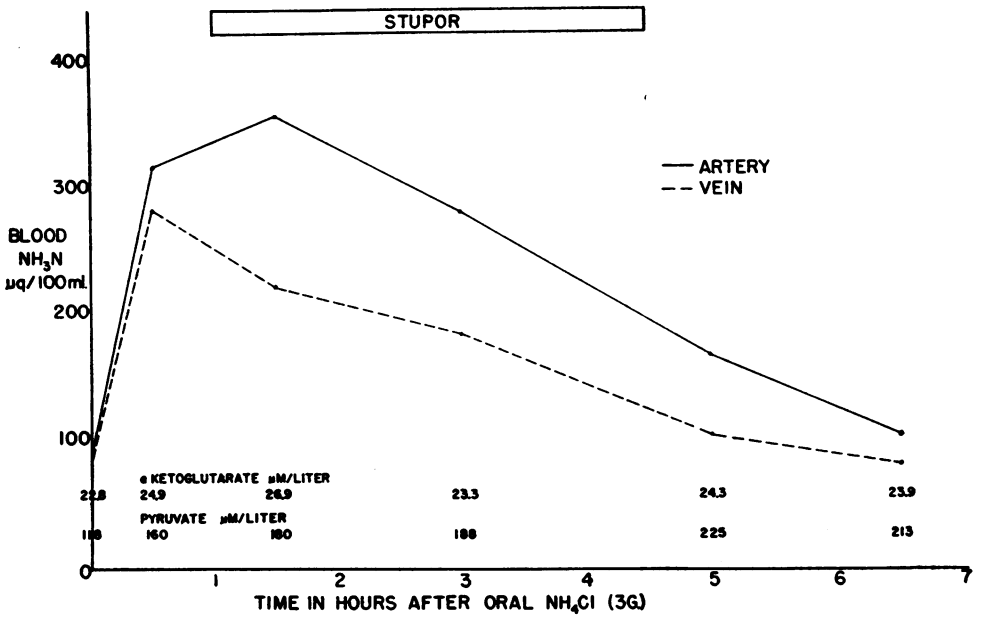

FIG. 6. PERIPHERAL TISSUE UPTAKE OF AMMONIA (A–V DIFFERENCE) AND BLOOD KETO-ACID CONCENTRATIONS DURING EPISODE OF IMPENDING HEPATIC COMA PRECIPITATED BY AMMONIUM CHLORIDE

variably affected by fasting (17, 18), but in our patients both arterial and venous values were normal under such circumstances. Impaired ammonia tolerance in liver disease (2), however, permits abnormal augmentations of blood ammonia following the ingestion of protein (19), blood (20), and other nitrogenous material (1). The highest ammonia concentrations in coma itself were also found by us to be related to these factors. During treatment with protein withdrawal and oral broad spectrum antibiotics, which presumably reduces the formation of ammonia and other toxic substances by suppression or change of intestinal flora (7, 21, 22), arterial ammonia concentrations fell towards, but seldom to, normal. Simultaneously, clinical improvement often occurred and the decline in arterial ammonia values was earlier and greater in patients who subsequently recovered.

The finding of rising arterial ammonia concentrations later in the course of fatal cases, despite continued treatment and the absence of gastrointestinal bleeding or uremia, could only sometimes be related to release of ammonia from peripheral tissues or brain. Possible additional sources of ammonia include the kidneys, which function abnormally in hepatic coma (23), and the failing liver, as high ammonia levels have been reported in renal and hepatic vein blood in hepatic coma (7, 16). The parent compound is unknown and extensive investigation of the phenomenon of ammonia release from mouse brain failed to identify its origin (24, 25). It is relevant, however, that Diamox® may liberate ammonia from the brain by direct inhibition of enzyme function, with elevation of arterial ammonia levels and the production of impending hepatic coma (18), and it also releases ammonia from the kidney into the renal vein (26).

The high incidence of elevated arterial ammonia concentrations in hepatic coma, with a terminal rise in fatal cases or a prompt fall on recovery, supports incrimination of ammonia intoxication in the genesis of the condition. However, the relationship between clinical status, arterial ammonia concentrations and ammonia uptake by brain and muscle reported by others (8, 9) is at variance with the frequently poor correlation in our patients. The occasional finding of normal values in coma also demands consideration.

Ammonia uptake by the brain, resulting in depletion of available α-ketoglutarate (27), has been

suggested as the basic disorder of cerebral metabolism in hepatic coma (8), but our findings suggest that removal of ammonia may be accomplished without injury to the organism and that clinical deterioration is more readily related to failure of this removal system. Thus, although uptake of ammonia at the periphery occurred in the earlier stages of coma, the efficiency of this process was impaired relative to normal capacity, usually to an extent that could not be explained by increased blood flow in hepatic disease. Under these circumstances, progressive deterioration in ammonia removal would result in negligible uptake, equilibrium or even release of ammonia by peripheral tissues and brain, despite high arterial concentration. Such findings were, in fact, characteristic of the late stages of coma in our patients.

An increased metabolic load is thrown on brain, muscle and other tissues by the abnormal and prolonged rises in blood ammonia which follow absorption of ammonia from the portal vein in some patients with liver disease (2). This may explain the impairment of peripheral ammonia uptake which was found even in the absence of hepatic coma, and stresses the importance of these adjuvant sites of ammonia removal. The pre-existing efficiency of these pathways may therefore determine the onset of coma in some instances, and minor overloading could account for the personality changes (28) and EEG abnormalities (29) sometimes observed in liver disease in the absence of objective neurological changes. The threshold of coma, depending on the magnitude of the insult and previous efficiency of cerebral and peripheral tissue ammonia removal, is therefore unlikely to be closely related to blood ammonia concentrations, which reflect ammonia derived from the gastrointestinal tract modified by endogenous uptake and release at various sites.

Evidence of derangement of intermediary metabolism by ammonia was obtained by the administration of ammonium chloride to patients with liver disease. The elevation of blood pyruvate and α-ketoglutarate in response to this procedure suggests that the high keto-acid values found in hepatic coma were also related to impaired ammonia metabolism, although imperfect metabolism by a failing liver (30) may be an additional factor. Biochemical interpretation of these changes is difficult as pyruvate and α-ketoglutarate may be involved simultaneously in more than one reaction.

The rise in pyruvate and α-ketoglutarate concentrations in response to ammonium chloride infusion in liver disease is compatible with a defect in intermediary metabolism (11). It is therefore relevant that Amatuzio, Shrifter, Stutzman, and Nesbitt (31) demonstrated accumulation of blood pyruvate in response to glucose infusion in hepatic coma, a finding which would be in accord with inhibition of final glucose oxidation by the high blood ammonia content in this condition. The delayed fall in pyruvate values found in control subjects receiving ammonium chloride could result from utilization of pyruvate in the tissues for transamination reactions.

Additional biochemical findings in hepatic coma compatible with deranged ammonia metabolism include high blood concentrations of glutamine (32), asparagine (33), and other amino acids, all of which may reflect increased amidation and transamination involved in disposal of ammonia. Precise details of the metabolic disorder still remain uncertain and our investigation failed to support or exclude the theory of cerebral α-ketoglutarate depletion (8). Augmentation rather than removal of peripheral blood α-ketoglutarate occurred in response to ammonium chloride administration in patients with liver disease and greatly increased amounts were available in the blood in hepatic coma. Nevertheless, the "blood-brain barrier" (34) may prevent replenishment of this keto-acid in the brain from peripheral blood (8). Examination of the spinal fluid revealed comparatively small quantities of α-ketoglutarate, which is compatible with the hypothesis that it passes from blood to spinal fluid with difficulty, but the higher concentrations present in hepatic coma fail to exclude its availability to the brain.

Certain practical points are emphasized by this investigation. It was confirmed that similar clinical and biochemical abnormalities occur in hepatic coma regardless of the etiology of the liver disease or nature of the precipitating factor (7). Blood ammonia estimations, particularly arterial concentrations, may be of diagnostic assistance in the fasting patient, but the close relationship postulated between arterial values, tissue uptake of ammonia and clinical status (9) was unreliable be-

cause blood ammonia concentrations reflect the amount of ammonia entering the circulation from the gastrointestinal tract and elsewhere, modified by release as well as uptake in various tissues, and the relationship between these factors is variable. The results of this investigation suggest the employment of antibiotics in treatment of hepatic coma (35). Glutamic acid also reduces blood ammonia values (36, 37) and its variable effect as a therapeutic agent may in part be due to relative impotence in the presence of a large influx of ammonia in the non-protein-deprived patient or the transient nature of its action in severe liver disease (38). On the other hand, its precursors, ammonia and α-ketoglutarate, were found in excess in peripheral blood in hepatic coma and elevated concentrations of glutamic acid itself have been reported in this condition (39). It appears from these considerations that assessment of any agent in the therapy of hepatic coma should be compared with the effect of protein deprivation and include estimations of arterial ammonia concentrations while also taking into account the importance of nitrogenous material in the gastrointestinal tract.

SUMMARY

1. In 27 patients studied in hepatic coma, blood ammonia concentrations were more frequently elevated in the artery than in the peripheral vein, but a good correlation with clinical status was evident at neither site. Fasting patients with uncomplicated liver disease had normal blood ammonia concentrations and the height of arterial values in hepatic coma was broadly related to the amount of nitrogenous material in the intestines. Values fell towards normal with protein withdrawal and broad spectrum antibiotic therapy, but a later elevation occurred in fatal cases, despite continuation of this regimen. The origin of this ammonia was uncertain and could only sometimes be attributed to release from brain or muscle.

2. Uptake of ammonia by peripheral tissues was impaired in liver disease relative to normal capacity. During the late stages of coma, poor uptake, equilibrium or release of ammonia by peripheral tissues or brain occurred, despite high arterial concentrations.

3. Elevation of blood pyruvate and α-ketoglutarate values paralleled the high blood ammonia concentration in hepatic coma. A rise in blood concentrations of these keto-acids followed the administration of ammonium chloride to patients with liver disease, but did not take place in control subjects. It is suggested that the high concentrations of keto-acids in hepatic coma represent a defect in intermediary metabolism due to impaired utilization of ammonia and faulty removal from the blood by a diseased liver. Concentrations of pyruvate in cerebro-spinal fluid were comparable to those in arterial blood, but relatively small amounts of α-ketoglutarate were found there, although values were above normal in hepatic coma.

4. The significance of blood ammonia concentrations in hepatic coma must take into account ammonia entering the system from the gastrointestinal tract, uptake or release of ammonia at various sites and the possibility of pre-existing defects in ammonia utilizing systems. Protein withdrawal with broad spectrum antibiotics effectively reduced blood ammonia values.

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