

## AMMONIUM UPTAKE BY THE EXTREMITIES AND BRAIN IN HEPATIC COMA<sup>1, 2</sup>

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Certain compounds containing or liberating ammonium may induce the syndrome of impending hepatic coma in susceptible patients with hepatic cirrhosis or Eck fistula (1-4). This syndrome is often but not always associated with increased ammonium concentrations in the blood (5, 6).

Although the origin and sources of elevated blood ammonium concentrations are not entirely clear, several factors contributing to them have been investigated. Decreased ammonium removal by the liver, resulting from either inadequate urea synthesis or from portal-systemic shunting of blood (7-10), is an important factor since increased arterial blood ammonium concentrations rarely occur if the liver and its circulation are normal. Elevated blood ammonium concentrations from an increased ammonium load are also observed in patients with liver disease. An increased load may result from the oral or intravenous administration of ammonium salts, amino acids and urea (1, 2, 11-13), from protein given orally (3, 14), and from gastrointestinal bleeding (15).

Previous investigations of patients with hepatic decompensation have demonstrated that the extremities and brain, which contain systems active in ammonium metabolism, are important sites for the removal of ammonium from the circulating blood (16-18). The roles of these peripheral sites in ammonium metabolism were evaluated in the present study also. In some instances, the extremities and brain of patients with severe hepatic

disease were found to remove significant amounts of ammonium from a given volume of blood. However, in other instances they failed to do so, and thereby contributed indirectly to the increased blood ammonium concentrations observed.

### METHODS

Three groups of patients were studied. The first consisted of 24 hospitalized patients without liver disease, the second of 27 patients with hepatic cirrhosis, and the third of 23 patients with hepatic cirrhosis and either impending hepatic<sup>4</sup> coma or coma. The last group is referred to subsequently as the "coma" group. Patients in the "coma" group were divided into one group without an added nitrogen load and a second group of patients with an added nitrogen load in the form of blood in their gastrointestinal tracts (evidence of upper gastrointestinal hemorrhage or massive epistaxis and blood swallowing). Pertinent clinical and laboratory data on all patients are presented in Tables I, II and III.

Patients were observed on the wards of Cleveland City Hospital, Crile Veterans Administration Hospital, and Boston City Hospital.<sup>5</sup> Confusion, coma and "flapping" tremor were graded from first to fourth degree; first degree indicated a mild, and fourth degree a serious, disturbance for each condition as previously described (20). Estimated daily protein intakes ranged from 30 to 100 grams for patients not in the "coma" group. Protein was withheld from confused or comatose patients for at least 24 and usually more than 48 hours before blood sampling.

Control was instituted over most factors known to increase blood ammonium ( $\text{NH}_4\text{-N}$ ) concentrations in patients with hepatic disease. No patient received ammonium salts within 72 hours of blood sampling. Blood samples were obtained only when patients had not received nitrogenous foods for the preceding 12 hours (13). Active gastrointestinal bleeding and epistaxis (15) were absent in all patients except some in the "coma" group. Because urea given intravenously may cause prompt increases in blood  $\text{NH}_4\text{-N}$  concentrations (13), patients

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<sup>2</sup> A report of this investigation was presented at the Twenty-Ninth Annual Meeting of the Central Society for Clinical Research, Chicago, Ill., November 9, 1956 (J. Lab. clin. Med. 1956, 48, 954).

<sup>3</sup> Russell M. Wilder Fellow of the National Vitamin Foundation.

<sup>4</sup> Characterized by mental confusion and a "flapping" tremor (19).

<sup>5</sup> Five patients in the "coma" group were studied at Boston City Hospital. Six patients in the cirrhosis group and one in the "coma" group were observed at Crile Veterans Administration Hospital in Cleveland.

TABLE I  
*Clinical and laboratory data in 24 patients without liver disease*

Patient	Age and sex	Diagnosis	Serum total bilirubin	Serum BSP	B.U.N.	Blood NH <sub>4</sub> -N (micrograms per 100 ml.)				
						mg./100 ml.	%	mg./100 ml.	Arm vein	Artery
1	68 M	Remote C.V.A.*	0.2	0	10	48	50	43	+ 2	+ 7
2	67 M	Remote C.V.A.*	0.5		22	47	26	52	-21	-26
3	42 M	Myotonia dystrophica	0.2	2	12	27	46	43	+19	+ 3
4†	65 M	Lues, ?pneumonia	0.6	7	17	55	55	60	0	- 5
5	75 M	Senility	0.1	0	21	73	86	78	+13	+ 8
6	65 M	Peptic ulcer	1.8	2	17	65	54		-11	
7	44 M	Peptic ulcer	0.3	1	12	46	49		+ 3	
8	70 F	Hypertension	0.7	7	22		61	49		+12
9	82 M	Senility	0.2	13	16		40	52		-12
10	74 M	Emphysema	0.3	9	8	46	47	60	+ 1	-13
11†	62 M	?Bronchogenic carcinoma	0.7	2	18	35	28	43	- 7	-15
12	36 F	?Cushing's disease	0.5		10	35	34	50	- 1	-16
13	63 M	Hypertension, fibrosarcoma	0.9	4	21	44	40	44	- 4	- 4
14†	26 M	Pneumonia	0.6	0	11	40	41	43	+ 1	- 2
15	42 M	Peptic ulcer	0.1	9	13	37	45	48	+ 8	- 3
16	52 M	Hypertension	0.5	8	14	43	38	41	- 5	- 3
17	41 M	Hypertension		4	23	32	49	43	+17	+ 6
18‡	42 F	Subsided pyelonephritis		3	20	22	21	23	- 1	- 2
19	28 F	Undiagnosed fever	0.6	8	21	23	22	27	- 1	- 5
20	67 M	?Pulmonary infarct	0.7	7	24	49	43	35	- 6	+ 8
21§	32 M	Lung abscess	0.1	1	10	25	47	44	+22	+ 3
22	42 F	Subsided pyelonephritis			10	22	34	30	+12	+ 4
23	35 M	Peptic ulcer		3	14	57	51	54	- 6	- 3
24	45 M	Emphysema	0.3	2	18	60	57	62	- 3	- 5

\* Cerebral vascular accident.

† Penicillin discontinued for at least 72 hours in Patients 4, 11 and 14.

‡ Gantrisin® discontinued for three days in Patient 18.

§ Patient 21 receiving penicillin.

|| Patient 22 receiving Panmycin® and nitrofurantoin.

in the normal and cirrhosis groups were selected so that none had elevated blood urea nitrogen concentrations. Patients having clinical evidence of shock or a systolic blood pressure below 90 mm. Hg were not included because animal experiments indicate that shock may produce increased blood NH<sub>4</sub>-N concentrations (21). When steroids, acetazolamide (Diamox®), analgesics or sedatives were given it is so indicated in the tables because some of these drugs have been used in the treatment of impending hepatic coma or have been implicated in its production. The patients studied received neither glutamic acid nor arginine which may lower blood NH<sub>4</sub>-N concentrations (20, 22-25). Neomycin and tetracycline antibiotics also may lower blood NH<sub>4</sub>-N concentrations in some patients with hepatic disease (26). Accordingly, antibiotics were used only as mentioned in Tables I, II and III.

Ammonium metabolism in extremities and brain was evaluated by analyzing NH<sub>4</sub>-N in blood samples obtained within three minutes of each other from a vein located in an extremity, an artery, and a superior jugular bulb. The uptake or release of NH<sub>4</sub>-N per given volume of blood circulating through the extremity and brain was obtained by calculating the arteriovenous and arteriojugular bulb blood NH<sub>4</sub>-N concentration differences.

Extremity venous blood was obtained from a forearm vein except as indicated for some patients in the coma group (Table III). The jugular vein was punctured, either in its superior bulb or just below this site, by directing a two inch number 20 gauge lumbar puncture needle with the bevel upwards superiorly and posteriorly from a site just under the ear in the space anterior to the mastoid process and posterior to the mandibular ramus.

Ammonium-nitrogen<sup>6</sup> in blood samples was determined by a modified Conway microdiffusion technique (27) with diffusion times of either 10 or 16½ minutes. In all but a few instances the values for NH<sub>4</sub>-N concentration represent the average of triplicate determinations for a given sample. Concentrations were calculated as recommended by Conway and were expressed as micrograms of NH<sub>4</sub>-N per 100 ml. of blood. The percentage of nitrogen recovered from a standard ammonium sulfate solution was used to estimate the per cent recovery of volatile base from blood. A modification was introduced

<sup>6</sup> Whether ammonium normally is present in circulating blood or whether it is formed only after blood is shed has not been established. Volatile base, released from blood upon addition of alkali, is assumed to be nearly all ammonium.

at Cleveland, *i.e.*, the rim of the Conway dish was greased rather than the entire underside of the cover glass. This permitted a greater recovery from an ammonium sulfate standard.

The ranges of blood NH<sub>4</sub>-N concentrations obtained in patients with and without liver disease were comparable in Cleveland and Boston. The reproducibility of NH<sub>4</sub>-N concentrations in two different blood samples drawn during the day from the same site in a given patient ranged from 0 to 23 micrograms per 100 ml. (mean, 6; S.D., plus or minus 5.2). This range of reproducibility was not significantly different in patients without liver disease compared to those with cirrhosis ( $p > 0.7$ ). Reproducibility in confused or comatose patients with cirrhosis was not tested.

Bromsulphalein concentration was determined in the serum 45 minutes after injection of 5 mg. of this dye

per kilogram of body weight (28). Serum bilirubin concentrations were performed by the standard technique (29). The methods of either Myers (30) or of Owings and Mandel (31) were employed for the determination of blood urea nitrogen.

The statistical evaluations presented were derived by the methods described in Snedecor's textbook (32).<sup>7</sup>

## RESULTS

### Blood ammonium-nitrogen concentrations

The NH<sub>4</sub>-N concentrations in blood obtained from different sites in the patient groups studied

<sup>7</sup> Dr. George F. Badger kindly reviewed the application of these statistical methods.

TABLE II  
Clinical and laboratory data in 27 patients with hepatic cirrhosis

Patient	Age and sex	Biopsy or autopsy	Physical examination*	Serum total bilirubin	Blood NH <sub>4</sub> -N (micrograms per 100 ml.)						
					mg./100 ml.	%	mg./100 ml.	Arm vein	Artery	Jugular bulb	A-V diff. extremities
1	33 F	No	Angiomata, ascites, edema	1.0	>20	8	47	70	76	+23	- 6
2	68 M	No	Ascites, edema	1.5	32	15	23	35	53	+12	-18
3	55 M	Yes		1.1	35	11	21	30	30	+9	0
4	49 M	Yes	Angiomata, edema	1.4	>20	8	19	29	47	+10	-18
5	68 M	Yes	Angiomata, ascites, edema	1.3	>20	17	53	89	75	+36	+14
6	40 M	Yes	Angiomata, ascites, edema	19.7		12	39	65	76	+26	-11
7	54 F	No	Ascites, edema	2.8	30	5	35	59	58	+24	+ 1
8†	39 F	Yes		6.1	27	13	41	66	65	+25	+ 1
9†	41 M	Yes	Ascites	0.4	7	16	23	39	40	+16	- 1
10	48 M	No	Angiomata	6.2	52	9	43	92	95	+49	- 3
11	62 M	Yes	Ascites, edema	1.2	21	17	31	54	53	+23	+ 1
12	68 M	Yes	Ascites, edema	1.3	58	14	64	104	88	+40	+16
13	57 F	No	Ascites, edema	4.9		6	35	87		+52	
14	45 F	No		0.9	16	6	35	60		+25	
15	44 F	No	Splenomegaly	1.2	13	9	78	87	86	+ 9	+ 1
16	58 M	Yes	Angiomata, ascites, splenomegaly	2.0	38	13	59	67	68	+ 8	- 1
17	63 M	Yes	Angiomata	5.6	23	8	36	37	60	+ 1	-23
18‡	72 M	Yes		0.8	9	19	45	49	48	+ 4	+ 1
19	58 F	Yes	Angiomata, ascites	0.3	23	11	78	91	78	+13	+13
20	49 M	Yes	Angiomata, ascites, edema	11.0	53	12	80	86		+ 6	
21	42 F	No	Angiomata, ascites	1.8	22	10	82	93	85	+11	+ 8
22	76 M	Yes	Angiomata, splenomegaly	0.9	34	20	77	79	78	+ 2	+ 1
23	71 M	No	Edema	1.4	23	15	80	94	98	+14	- 4
24	58 M	Yes	Angiomata, splenomegaly	0.9	27	13	83	106	120	+23	-14
25	56 M	No	Angiomata, ascites, edema	2.5		20	84	89		+ 4	
26§	67 M	Yes	Ascites, edema	0.9	27	15	69	128	96		+32
27	51 F	Yes	Splenomegaly, edema	1.8	51	8	86	86	84		+ 2

\* All patients had hepatomegaly except Patients 7 and 11.

† Penicillin was discontinued 72 hours prior to blood sampling in Patients 8 and 9.

‡ Patient 18, with miliary tuberculosis, had been receiving streptomycin, isoniazid and *p*-aminosalicylic acid for months.

§ Patient 26, with pulmonary tuberculosis, was receiving streptomycin. The NH<sub>4</sub>-N uptake in the extremities was not calculated for Patient 26 because the venous blood sample was not drawn simultaneously with those from the artery and jugular bulb.

|| Metastatic carcinoma with inferior vena caval obstruction in addition to cirrhosis.

TABLE III  
Clinical and laboratory data in 23 patients with cirrhosis and hepatic coma \*

Group	Patient	Age and sex	Diagnosis	Biopsy or autopsy	Physical findings†	Mental state	Tremor‡	Comment	Serum total bilirubin			Blood NH <sub>4</sub> -N (micrograms per 100 ml.)				
									mg./100 ml.	%	mg./100 ml.	NPN or B.U.N.	Antecubital (A) or femoral (F) vein	Artery	Jugular bulb	A-V diff. extremities
Without hemorrhage																
1	57 M	Cirrhosis	Yes	Icterus, angiomata, ascites	2° I.C.	0	No G.I. bleeding		39	23 NPN		139	251		-112	
2	62 M	Cirrhosis	Yes	Angiomata, ascites	1° I.C.	1+	11 days after paracentesis; 3 days after Diamox®		15	42 NPN		153	182	+ 64	- 29	
3	65 F	Cirrhosis	Yes	Ascites, edema	3° I.C.	2+	Spontaneous coma		28 NPN							
4	47 F	Cirrhosis	Yes	Angiomata, ascites	3° I.C.	1+	Had phenobarbital and compound F		42							
5	38 M	Cirrhosis, myocardial infarction	No	Angiomata, ascites	1° I.C.	1+	9 days after paracentesis		1.6	>40	17	(F) 145	226	+ 81	- 20	
6	52 M	Cirrhosis	No	Ascites	2° I.C.	3+	Had paraaldehyde, ACTH and reserpine		14			(A) 167	164	- 3	- 22	
7	69 M	Cirrhosis, carcinoma of pancreas	Yes		1° I.C.		4 days after operation		11.9	21		(F) 31	29	- 2		
8	57 M	Cirrhosis	Yes	Angiomata, ascites, edema	1° C.	0	Had Thorazine® and paregoric		64	21		(A) 266	271	+ 5	- 107	
9	53 M	Cirrhosis, hypertension, cardiac failure	No	Edema	3° I.C.	3+	Received Diamox®		34			(A) 28	38	+ 10	- 9	
10	49 M	Cirrhosis	Yes	Splenomegaly, ascites, edema	3° C.	0	Shock 24 hours previously		3.2	>20	36	(F) 265	297	+ 32	+ 59	
11	65 M	Cirrhosis	Yes	Angiomata, ascites, edema	1° I.C.	1+	150 Gm. protein diet previous 4 days		4.3	20	(F) 228	207	235	- 21	- 28	
12	61 M	Cirrhosis	No	Angiomata, ascites	1° I.C.	2+	Had paraaldehyde 48 hours after Diamox®		5.6		9	(A) 118	119	+ 1	- 26	
13	38 M	Cardiac cirrhosis	No	Ascites, edema	1° I.C.	3+	No further Diamox®		1.0	>20	39	(A) 112	121	+ 9	- 23	
14	51 F	Cor pulmonale	Yes	Splenomegaly, edema	2° I.C.	2+	No G.I. bleeding for 24 hours		1.8	51	20	(A) 155	156	+ 1	+ 23	
13					1° I.C.	1+	G.I. bleeding		41			(F) 51	112	+ 61	+ 10	
14					1° I.C.	3+	Epistaxis					(A) 142	185	+ 43	+ 44	
15					2° I.C.	2+	G.I. bleeding		1.2	24	36 NPN	(A) 62	197	+ 135	+ 40	
16	63 M	Cirrhosis	Yes	Angiomata, splenomegaly, ascites, edema	1° I.C.	±	G.I. bleeding		52	45 NPN	(F) 71	135	+ 64			
					1° I.C.	0			45 NPN	28 NPN	40 NPN	(A) 70	116	+ 46		
					2° C.		Epistaxis			203	203	276		- 73		
17	64 M	Cirrhosis, peptic ulcer	Yes	Angiomata, edema	2° I.C.	0	G.I. bleeding		4.4			(A) 120	185	+ 65	+ 8	
18	78 M	Cirrhosis	No	Angiomata, ascites, edema	3° I.C.	2+	G.I. bleeding		2.0			(A) 78	127	+ 49	+ 40	
					1° I.C.	1+			37			(F) 150	227	+ 77	+ 32	
					1° I.C.	0			37			(A) 109	152	+ 47	+ 21	
					3° I.C.	1+	G.I. bleeding		3.2			(F) 83	214	+ 131	+ 89	
					3° I.C.	3+			43			(A) 404	625	+ 221	+ 148	
					1° I.C.	1+			43			(A) 109	175	+ 66	+ 23	
19	61 M	Cirrhosis	No	Angiomata, splenomegaly, ascites	2° C.	1+	G.I. bleeding		3.5							
20	42 M	Cirrhosis	No	Angiomata, ascites	2° C.	2+	G.I. bleeding		5.1			(F) 112	205	+ 93	+ 33	
21	54 M	Cirrhosis	No	Angiomata, ascites, edema	3° I.C.	3+	G.I. bleeding		7.4	72	22	(A) 211	337	+ 126	+ 71	
22	64 M	Cirrhosis, hepatoma	Yes	Angiomata, ascites, edema	1° I.C.	1+	G.I. bleeding		4.9	70		(F) 87	168	+ 115	+ 53	
23	47 M	Cirrhosis	Yes	Angiomata, ascites, edema	1° I.C.	2+	G.I. bleeding		6.2			(F) 154	242	+ 185	+ 57	

\* Includes patients with either impending hepatic coma or coma. Patient 8 was the only one receiving an antibiotic (penicillin).

† The liver was palpable except in Patients 2, 3, 5, 11, 15, 20, 21 and 23.

‡ I.C., impending hepatic coma; C, hepatic coma. Both were graded from first to fourth degree (22).

|| Grading: tremor graded from 1 to 4+ (22).

||| Blood NH<sub>4</sub>-N determinations are presented separately when they were done on different days in the same patient.

¶ Expired during hospitalization.

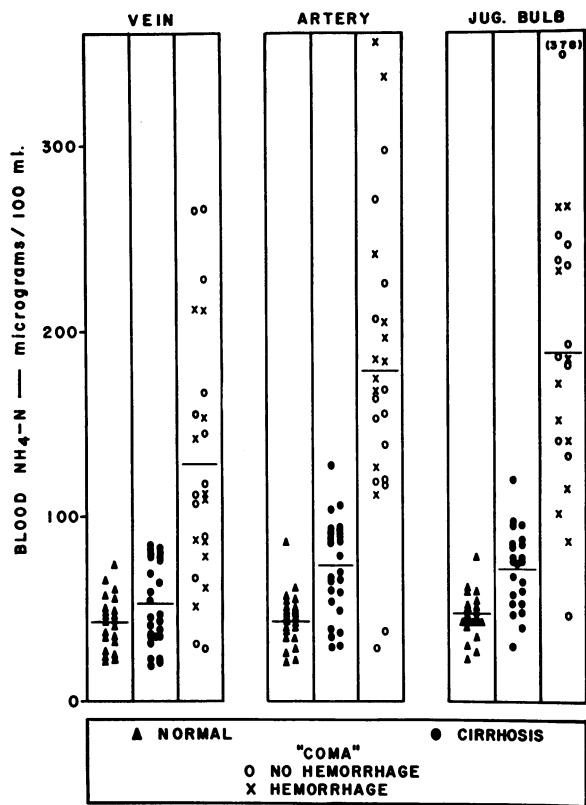


FIG. 1. BLOOD AMMONIUM CONCENTRATIONS

The plotted values represent the concentrations shown in Tables I, II and III. The horizontal line for each group of plotted values indicates the mean concentration for the group.

are charted in Figure 1. Ammonium-nitrogen concentrations in antecubital venous blood of the alert patients with cirrhosis were not significantly higher than those of the group without liver disease ( $p = 0.06$ ). However,  $\text{NH}_4\text{-N}$  concentrations measured in arterial or jugular bulb venous blood were significantly higher in the alert patients with cirrhosis than in those without liver disease ( $p < 0.01$ ).

Ammonium-nitrogen concentrations in blood drawn from each site were highest in the "coma" group ( $p < 0.01$ ) but the mean concentrations obtained in patients with and without hemorrhage were not significantly different from each other. Arterial and jugular bulb blood  $\text{NH}_4\text{-N}$  values overlapped less between cirrhosis and "coma" groups than did values found in venous blood obtained from the extremities. Although both arterial and jugular bulb blood  $\text{NH}_4\text{-N}$  concentrations correlated better with the above clinical classification than did those in extremity venous blood, concentrations in blood from all three sites correlated poorly with the depth of impending hepatic coma or coma (Table III).

#### Arteriovenous ammonium-nitrogen differences for the extremities

Arteriovenous blood  $\text{NH}_4\text{-N}$  concentration differences were calculated for individual patients

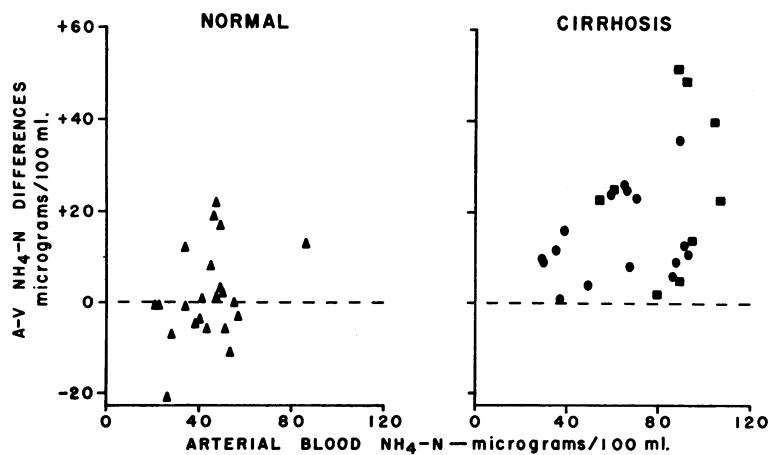


FIG. 2. EXTREMITY A-V AMMONIUM DIFFERENCES

The plotted values are derived from the data in Tables I and II. A positive arteriovenous difference indicates an uptake of  $\text{NH}_4\text{-N}$ ; a negative difference indicates a release.

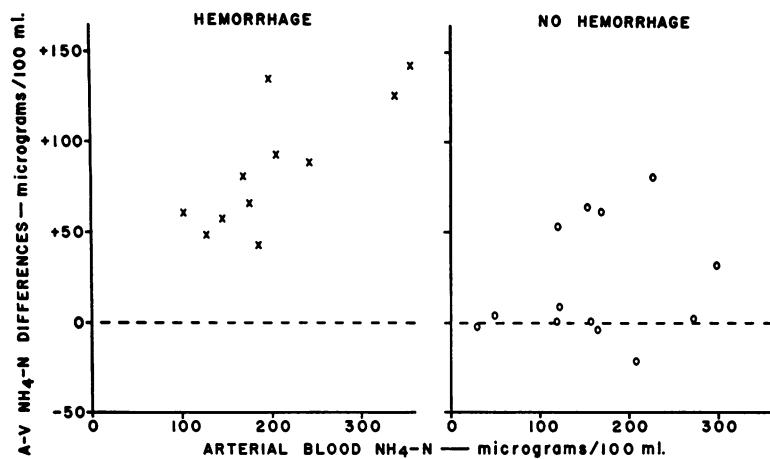


FIG. 3. EXTREMITY A-V AMMONIUM DIFFERENCES IN HEPATIC COMA

The plotted values are derived from data in Table III. Blood NH<sub>4</sub>-N concentrations and arteriovenous differences determined on separate days in an individual were averaged. A positive arteriovenous difference indicates an uptake of NH<sub>4</sub>-N; a negative difference indicates a release.

(Tables I, II and III). Each arteriovenous NH<sub>4</sub>-N difference for the extremities was plotted as a function of its arterial blood NH<sub>4</sub>-N concentration. In patients without liver disease, individual arteriovenous NH<sub>4</sub>-N differences were within a range of plus 22 to minus 21 micrograms per 100 ml. of blood. There was neither a significant average uptake nor release of NH<sub>4</sub>-N per given volume of blood circulating through the extremities. Moreover, there was no correlation between individual arteriovenous blood NH<sub>4</sub>-N concentration differences and their respective arterial blood NH<sub>4</sub>-N concentrations (Table I, Figure 2).

In contrast to the group without liver disease, alert patients with cirrhosis demonstrated positive arteriovenous NH<sub>4</sub>-N differences (uptakes) in their extremities (Table II and Figure 2). When arteriovenous NH<sub>4</sub>-N differences obtained for the upper extremities in alert patients with cirrhosis were plotted as functions of their respective arterial NH<sub>4</sub>-N values the resulting correlation was not significant (Figure 2) ( $r = 0.373$ ;  $p = 0.07$ ).

In the 11 "coma" patients with an added nitrogen load in the form of blood in their gastrointestinal tracts the extremities removed more NH<sub>4</sub>-N from the blood with increasing arterial NH<sub>4</sub>-N concentrations as expressed by the regression  $\hat{Y} = 0.343X + 15.5$  (Figure 3) ( $r = 0.77$ ;  $p < 0.01$ ). In contrast, only 4 of 11 patients

in the "coma" group without evidence of an added nitrogen load removed ammonium efficiently from a given volume of blood at increased arterial NH<sub>4</sub>-N concentrations (Figure 3). Thus, at a given arterial blood NH<sub>4</sub>-N concentration there was a greater removal of NH<sub>4</sub>-N from a given volume of blood circulating through the extremities of the group of confused or comatose patients with blood in their gastrointestinal tracts than in the group without hemorrhage.

#### Arteriovenous ammonium-nitrogen differences for the brain

Arteriovenous NH<sub>4</sub>-N differences for brain, as for the extremities, were plotted as functions of

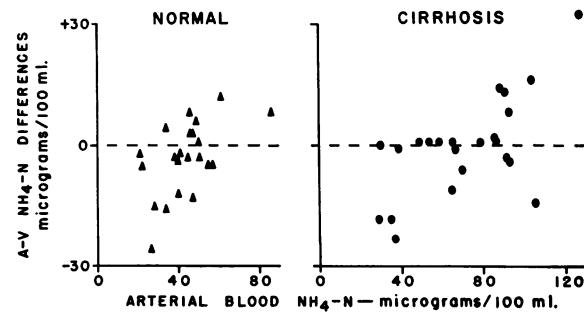


FIG. 4. BRAIN A-V AMMONIUM DIFFERENCES

The values plotted are derived from data in Tables I and II. A positive arteriovenous difference indicates an uptake of NH<sub>4</sub>-N; a negative difference indicates a release.

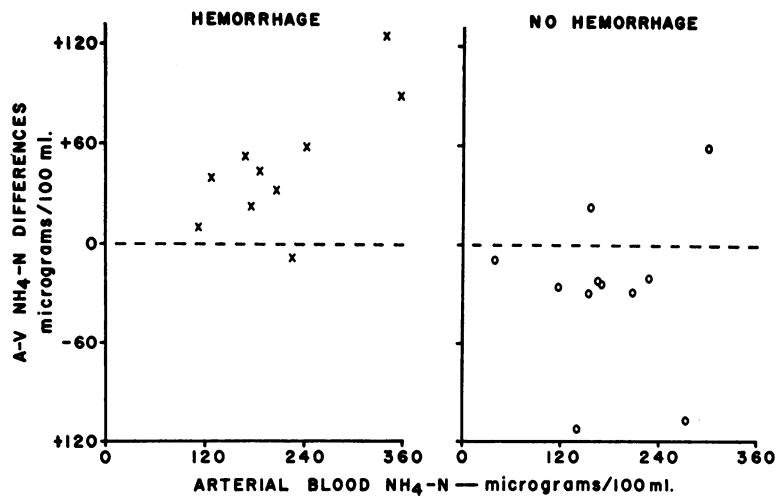


FIG. 5. BRAIN A-V AMMONIUM DIFFERENCES IN HEPATIC COMA

The plotted values are derived from the data in Table III. An average difference is plotted when these differences were determined for the same patient on separate days. A positive arteriovenous difference indicates an uptake of  $\text{NH}_4\text{-N}$ ; a negative difference indicates a release.

their corresponding arterial blood  $\text{NH}_4\text{-N}$  concentrations (Figures 4 and 5).

Arteriovenous differences across the brains of patients without liver disease or of alert patients with cirrhosis were neither consistently positive nor negative, although there was some correlation between this variable and arterial  $\text{NH}_4\text{-N}$  concentrations in both groups (Figure 4). In the "coma" group with blood in their gastrointestinal tracts, 9 of the 10 patients showed increasing removal of  $\text{NH}_4\text{-N}$  from a given volume of blood circulating through the brain with increasing arterial  $\text{NH}_4\text{-N}$  concentrations ( $r = 0.74$ ;  $p = 0.01$ ) as expressed by the regression  $\hat{Y} = 0.355X$  minus 28.8 (derived from all values shown in Figure 5). The uptake of  $\text{NH}_4\text{-N}$  from a given volume of blood circulating through the brain which occurred in this "coma" group was significant ( $p < 0.01$ ).

A significantly greater removal of  $\text{NH}_4\text{-N}$  per given volume of blood circulating through the brain occurred in those patients with hemorrhage than in those without ( $p < 0.01$ ). Most of the patients belonging to the latter group displayed a release of  $\text{NH}_4\text{-N}$  into blood circulating through their brains (Figure 5). However, although the average arteriojugular bulb blood  $\text{NH}_4\text{-N}$  difference for this group indicated an  $\text{NH}_4\text{-N}$  release into the blood, this was not statistically significant ( $p = 0.1$ ).

#### DISCUSSION

The removal or release of ammonium from a given volume of blood circulating through the extremities or the brain was obtained by calculating the arteriovenous concentration differences for patients without liver disease, for alert patients with cirrhosis, and for patients with hepatic coma. Although, as discussed below, the interpretation of arteriovenous blood ammonium differences is beset with some difficulties, the  $\text{NH}_4\text{-N}$  levels and arteriovenous  $\text{NH}_4\text{-N}$  differences in these peripheral sites differed in the groups of patients with cirrhosis when compared with values obtained for patients without hepatic disease. In the patients with liver disease the findings for the extremities differed in some respects from those for the brain.

Arteriovenous  $\text{NH}_4\text{-N}$  differences across the extremities ranged from plus 1 to plus 52 mg. per 100 ml. of blood ( $\text{NH}_4\text{-N}$  removed) for alert patients with cirrhosis, but were distributed within the range of minus 21 to plus 22 mg. per 100 ml. of blood for patients without liver disease (Figure 2). Arteriovenous  $\text{NH}_4\text{-N}$  differences across the brains were not significantly different in the control subjects compared to the values for the alert patients with cirrhosis (Figure 4). Thus there were differences in  $\text{NH}_4\text{-N}$  removal for: 1) the extremities of alert patients with cirrhosis

compared to those of the control subjects; and 2) the extremities as contrasted to the brains of the alert patients with cirrhosis.

The alert patients with cirrhosis had arterial  $\text{NH}_4\text{-N}$  concentrations that were elevated as compared to the values obtained for control subjects (Figure 1). The elevations of arterial blood  $\text{NH}_4\text{-N}$  concentrations in patients with cirrhosis were apparently sufficient to stimulate an enhanced removal of  $\text{NH}_4\text{-N}$  by the extremities but not by the brain. Further support for this hypothesis was obtained by comparing the arteriovenous  $\text{NH}_4\text{-N}$  differences for brain with those for the extremities in the group of patients in coma with hemorrhage (Figures 3, 5). Positive arteriovenous  $\text{NH}_4\text{-N}$  differences (uptakes) for both extremities and brain correlated with the arterial  $\text{NH}_4\text{-N}$  concentrations in these patients, but the uptakes of  $\text{NH}_4\text{-N}$  occurred at lower arterial concentrations in extremities than they did in brain. In addition, individuals in the coma group who demonstrated positive arteriovenous  $\text{NH}_4\text{-N}$  differences for extremities did not always have positive differences for brain (Table III). The explanation for these differences in the metabolism of ammonium in extremities compared to that in brain of the patients with cirrhosis studied is not known. However, ammonium disposal is known to differ in these sites (17), and other dissimilarities might exist as well, *e.g.*, in ammonium transport mechanisms.

The data obtained in comatose patients with hemorrhage suggest that both extremities and brain removed ammonium from a given volume of blood with about equal efficiencies once removal was initiated, since the regression of arteriovenous  $\text{NH}_4\text{-N}$  differences upon the arterial concentrations for these two sites was similar (0.343 for extremities and 0.335 for brain). This is at variance with the finding of Bessman, Fazekas, and Bessman (16), that at a given arterial  $\text{NH}_4\text{-N}$  concentration the extremities of patients with hepatic coma had twice as large an arteriovenous  $\text{NH}_4\text{-N}$  difference as the brain (17).

As predicted by Bessman and Bessman (17), arterial and jugular bulb blood  $\text{NH}_4\text{-N}$  concentrations correlated better with the classification of patients into normal, cirrhosis and "coma" groups than did the concentrations in antecubital and femoral venous blood (Figure 1). However,

even when arterial and jugular bulb venous blood was analyzed, there was considerable overlap of concentrations between different patient groups and a poor correlation between blood  $\text{NH}_4\text{-N}$  concentrations and coma depth in patients belonging to the "coma" group. Actually, an excellent correlation between coma depth and blood  $\text{NH}_4\text{-N}$  concentration might not be expected because of the many factors which may affect both variables.

Patients in the coma group were classified clinically according to the presence or absence of associated gastrointestinal bleeding. This classification was made because experience suggests that the prognosis of hepatic coma is better for patients with coma precipitated by a nitrogen load than for patients in whom mental symptoms occur without an associated precipitating factor. Whether or not arteriovenous  $\text{NH}_4\text{-N}$  differences increased with increasing arterial  $\text{NH}_4\text{-N}$  concentrations varied according to this clinical classification. Arteriovenous blood  $\text{NH}_4\text{-N}$  differences for extremities and brain usually increased with increasing arterial  $\text{NH}_4\text{-N}$  concentrations in confused or comatose patients with cirrhosis who had blood in their gastrointestinal tracts, *e.g.*, an added nitrogen load. In contrast,  $\text{NH}_4\text{-N}$  was not removed efficiently from a given volume of blood circulating through the extremities and brains in most of the patients in the "coma" group who did not have gastrointestinal bleeding. In fact, the brains of some of the latter patients released  $\text{NH}_4\text{-N}$  into the circulating blood. The difference in prognosis for the patients with, as contrasted to those without, an added nitrogen load might be related to these observed differences in  $\text{NH}_4\text{-N}$  metabolism.

The presence or absence of an added ammonium load (*e.g.*, gastrointestinal bleeding) probably did not *per se* account for the variations in arteriovenous  $\text{NH}_4\text{-N}$  differences because high arteriovenous differences were noted for the extremities of some alert patients in the cirrhosis group and for the extremities and brains of others in the "coma" group in whom no evidence of an extra ammonium load was detected. Furthermore, if an increased ammonium load to the systemic circulation were to initiate  $\text{NH}_4\text{-N}$  removal in the extremities only by increasing the arterial  $\text{NH}_4\text{-N}$  concentration, one would expect large arteriovenous  $\text{NH}_4\text{-N}$  differences across the extremities and brains of all patients having markedly ele-

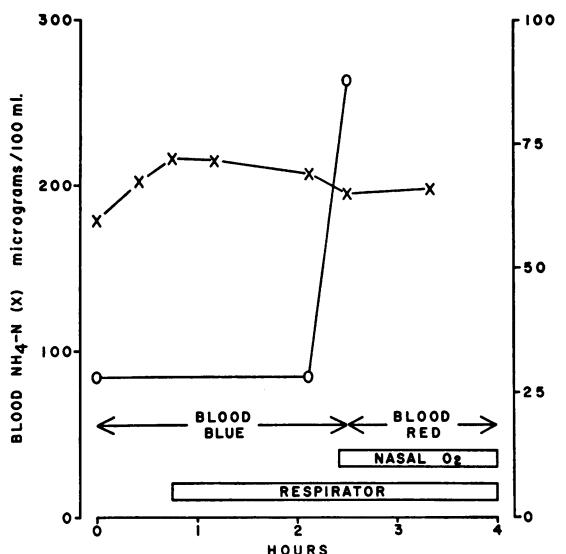


FIG. 6. EFFECT OF ARTERIAL BLOOD O<sub>2</sub> SATURATION ON BLOOD AMMONIUM CONCENTRATION

vated arterial NH<sub>4</sub>-N concentration. This did not always occur (Figures 3, 5). The observed variations in peripheral arteriovenous NH<sub>4</sub>-N differences might be explained more plausibly by an alteration in the ability of the peripheral intracellular systems either to metabolize ammonium or to initiate ammonium removal at elevated arterial NH<sub>4</sub>-N concentrations. The negative arteriovenous NH<sub>4</sub>-N difference across the brains of certain individuals in the coma group might only reflect an inability of their brains to cope with ammonium which is normally produced (33). For example, in animals ammonium concentrations in the brain may increase if fluoroacetate, an agent effective in blocking the tricarboxylic acid cycle, is administered (34). These possibilities require further elucidation.

Aside from difficulties inherent in measuring blood NH<sub>4</sub>-N,<sup>8</sup> interpretation of arteriovenous NH<sub>4</sub>-N differences is difficult for several reasons. An arteriovenous difference for a given tissue only determines whether or not there is an overall ammonium removal or release in that tissue; it provides no information concerning the ammonium transport mechanisms involved. The composition of the blood analyzed (arterial as con-

trasted to venous) might affect the amount of ammonium recovered during analysis. For instance, some investigators suggest that the ammonium concentration in blood is proportional to its oxygen content (35, 36). However, a patient with cor pulmonale studied in this laboratory did not demonstrate significant fluctuations in arterial blood NH<sub>4</sub>-N concentrations when his arterial oxygen saturation was increased *in vivo* from 28 to 88 per cent (Figure 6). When the quantity of blood circulating through the tissue is not known, arteriovenous differences can provide only semiquantitative estimates of tissue uptake or release. Abnormalities of blood flow do occur in patients with cirrhosis of the liver (37-40), but it seems unlikely that variations of blood flow through the extremities and brain solely accounted for the variations in arteriovenous NH<sub>4</sub>-N differences noted in the present study. The magnitude of changes in arteriovenous differences observed is probably too great to be explained by changes in blood flow alone. The incidence of clinical findings often associated with altered blood flow through peripheral tissues, *i.e.*, hypotension, palmar erythema, and spider angioma, was similar for individuals having high or low NH<sub>4</sub>-N uptakes per given volume of circulating blood. In addition, preliminary studies in this laboratory indicate that the rate of cerebral blood flow, found to be decreased in patients with hepatic coma (39, 40), does not correlate with the arteriovenous NH<sub>4</sub>-N difference across the brain. In accord with the above reservations, data concerning arteriovenous blood NH<sub>4</sub>-N differences for peripheral sites have been discussed only in reference to the results obtained for the control group of hospitalized patients without liver disease.

Considering the present findings and assuming that abnormal ammonium metabolism is important in hepatic coma, one might explain this disorder in several ways. The removal of ammonium occurring in the brains of patients with an added nitrogen load suggests that hepatic coma might result because the brain had to metabolize increased quantities of ammonium presented to it by the arterial blood. This process of ammonium disposition might deprive the brain of reactants that are required for other essential metabolic processes. The release of ammonium from the brains of some patients in coma not associated with an

<sup>8</sup> Since ammonium ion is not measured directly by methods devised to date, it is possible that volatile base released from other substances present in blood is also being measured.

added nitrogen load suggests that the confusion or coma associated with hepatic disease might ensue because certain metabolic functions of the brain concerned in ammonium disposal were affected adversely by an abnormally functioning liver. These mechanisms operating simultaneously in a patient with hepatic coma would make the identification and evaluation of each difficult.

#### SUMMARY

Ammonium nitrogen was determined in blood obtained practically simultaneously from an extremity vein, an artery and a superior jugular bulb in fasting patients without liver disease, with cirrhosis, and with cirrhosis and either impending hepatic coma or coma.

Arterial and jugular bulb blood ammonium concentrations were least in the group without liver disease, higher in the group with cirrhosis, and highest in the group with impending hepatic coma or coma. Ammonium concentrations in blood obtained from these sites correlated better with the above clinical classification than did those found in extremity venous blood. However, the correlation was not good enough to permit prediction of the clinical state in individual cases.

As judged by arteriovenous blood  $\text{NH}_4\text{-N}$  differences alone, there was no significant uptake or release of  $\text{NH}_4\text{-N}$  across the extremities and brains of patients without hepatic disease. In patients with liver disease, sufficient increases in arterial blood ammonium concentrations were usually associated with a removal of ammonium from a given volume of blood circulating through these peripheral sites. The extremities of some patients in both the cirrhosis and "coma" groups removed ammonium from a given volume of blood in proportion to the arterial blood ammonium concentrations. A similar removal of ammonium also occurred in the brains of some patients in the "coma" group. This was roughly proportional to the arterial blood ammonium concentration.

Ammonium was not always removed efficiently from a given volume of blood in the extremities and in the brain. Some patients in the cirrhosis group and some in the "coma" group demonstrated a poor ammonium removal for the extremities. Furthermore, not only did the brains of some patients in the latter group remove ammonium poorly but others showed significant re-

leases. In alert patients with cirrhosis the extremities removed ammonium from a given volume of circulating blood when the brain did not.

Confused or comatose patients with hepatic cirrhosis were classified into two groups according to the presence or absence of an added nitrogen load, *i.e.*, blood in the gastrointestinal tract. Although there was overlap between individual values, ammonium removal from given volumes of blood circulating through the extremities and brain was significantly greater in the group with an added nitrogen load than in the group without hemorrhage.

The status of peripheral ammonium removal may be important in the genesis and outcome of hepatic coma.

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#### REFERENCES

1. Gabuzda, G. J., Jr., Phillips, G. B., and Davidson, C. S. Reversible toxic manifestations in patients with cirrhosis of the liver given cation-exchange resins. *New Engl. J. Med.* 1952, **246**, 124.
2. Phillips, G. B., Schwartz, R., Gabuzda, G. J., Jr., and Davidson, C. S. The syndrome of impending hepatic coma in patients with cirrhosis of the liver given certain nitrogenous substances. *New Engl. J. Med.* 1952, **247**, 239.
3. Schwartz, R., Phillips, G. B., Seegmiller, J. E., Gabuzda, G. J., Jr., and Davidson, C. S. Dietary protein in the genesis of hepatic coma. *New Engl. J. Med.* 1954, **251**, 685.
4. McDermott, W. V., Jr., and Adams, R. D. Episodic stupor associated with an Eck fistula in the human with particular reference to the metabolism of ammonia. *J. clin. Invest.* 1954, **33**, 1.
5. Traeger, H. S., Gabuzda, G. J., Jr., Ballou, A. N., and Davidson, C. S. Blood "ammonia" concentration in liver disease, and liver coma. *Metabolism* 1954, **3**, 99.
6. Phear, E. A., Sherlock, S., and Summerskill, W. H. J. Blood-ammonium levels in liver disease and "hepatic coma." *Lancet* 1955, **1**, 836.

7. Bollman, J. L., and Mann, F. C. Studies on the physiology of the liver. XVIII. The effect of removal of the liver on the formation of ammonia. *Amer. J. Physiol.* 1930, **92**, 92.
8. Krebs, H. A. Urea synthesis in *The Enzymes*, J. B. Sumner and K. Myrback, Eds. New York, Academic Press Inc., 1952.
9. Roger, S., and Stahl, J. Correlation entre l'épreuve d'ammoniémie provoquée et l'histopathologie hépatique. *C. R. Soc. Biol. (Paris)* 1952, **146**, 1786.
10. White, L. P., Phear, E. A., Summerskill, W. H. J., and Sherlock, S. Ammonium tolerance in liver disease: Observations based on catheterization of the hepatic veins. *J. clin. Invest.* 1955, **34**, 158.
11. Kirk, E. Amino acid and ammonia metabolism in liver diseases. *Acta med. scand.* 1936, Suppl. **77**, 8.
12. Webster, L. T., Jr., and Davidson, C. S. Cirrhosis of the liver. Impending hepatic coma and increased blood ammonium concentrations during protein hydrolysate infusion. *J. Lab. clin. Med.* 1957, **50**, 1.
13. Webster, L. T., Jr., and Davidson, C. S. Sources of blood ammonium after feeding protein to patients with hepatic cirrhosis (abstract). *J. clin. Invest.* 1956, **35**, 742.
14. Fuld, H. Über die diagnostische verwerbarkeit von ammoniakbestimmungen im blut. *Klin. Wschr.* 1933, **12**, 1364.
15. Young, P. C., Burnside, C. R., Knowles, H. C., Jr., and Schiff, L. The effects of intragastric administration of whole blood on the concentration of blood ammonia in patients with liver disease. *J. Lab. clin. Med.* 1957, **50**, 11.
16. Bessman, S. P., Fazekas, J. F., and Bessman, A. N. Uptake of ammonia by the brain in hepatic coma. *Proc. Soc. exp. Biol. (N. Y.)* 1954, **85**, 66.
17. Bessman, S. P., and Bessman, A. N. The cerebral and peripheral uptake of ammonia in liver disease with an hypothesis for the mechanism of hepatic coma. *J. clin. Invest.* 1955, **34**, 622.
18. Bessman, S. P., and Bradley, J. E. Uptake of ammonia by muscle. Its implications in ammoniagenic coma. *New Engl. J. Med.* 1955, **253**, 1143.
19. Adams, R. D., and Foley, J. M. Neurological changes in more common types of severe liver disease. *Trans. Amer. neurol. Ass.* 1949, **74**, 217.
20. Webster, L. T., Jr., and Davidson, C. S. The effect of sodium glutamate on hepatic coma. *J. clin. Invest.* 1956, **35**, 191.
21. Nelson, R. M., and Seligson, D. Studies of blood ammonia in normal and shock states. *Surgery* 1953, **34**, 1.
22. Singh, I. D., Barclay, J. A., and Cooke, W. T. Blood-ammonia levels in relation to hepatic coma and the administration of glutamic acid. *Lancet* 1954, **1**, 1004.
23. McDermott, W. V., Jr., Wareham, J., and Riddell, A. G. Treatment of "hepatic coma" with 1-glutamic acid. *New Engl. J. Med.* 1955, **253**, 1093.
24. Bessman, S. P. The reduction of blood ammonia levels by certain amino acids (abstract). *J. clin. Invest.* 1956, **35**, 690.
25. Najarian, J. S., and Harper, H. A. A clinical study of the effect of arginine on blood ammonia. *Amer. J. Med.* 1956, **21**, 832.
26. Fisher, C. J., and Faloon, W. W. Episodic stupor following portacaval shunt. Observations on etiology and therapy. *New Engl. J. Med.* 1956, **255**, 589.
27. Conway, E. J. *Microdiffusion Analysis and Volumetric Error*, 3rd ed. New York, D. Van Nostrand Company, Inc., 1950.
28. Gaebler, O. H. Determination of bromsulphalein in normal, turbid, hemolyzed or icteric serums. *Amer. J. clin. Path.* 1945, **15**, 452.
29. Ducci, H., and Watson, C. J. The quantitative determination of the serum bilirubin with special reference to the prompt-reacting and the chloroform-soluble types. *J. Lab. clin. Med.* 1945, **30**, 293.
30. Myers, V. C. *Practical Chemical Analysis of Blood*, 2nd ed. St. Louis, C. V. Mosby Company, 1924.
31. Owings, R. H., and Mandel, E. E. Studies in non-protein nitrogen. I. A convenient method for measuring urea in blood. *Proc. Soc. exp. Biol. (N. Y.)* 1951, **78**, 363.
32. Snedecor, G. W. *Statistical Methods*, 4th ed. Ames, Iowa, The Iowa State College Press, 1946.
33. Weil-Malherbe, H. Significance of glutamic acid for the metabolism of nervous tissue. *Physiol. Rev.* 1950, **30**, 549.
34. Benitez, D., Pscheidt, G. R., and Stone, W. E. Formation of ammonium ion in the cerebrum in fluoracetate poisoning. *Amer. J. Physiol.* 1954, **176**, 488.
35. Iber, F. L., and Chalmers, T. C. Biochemical observations on the use of 1-glutamic acid in the treatment of hepatic coma. *J. clin. Invest.* 1957, **36**, 706.
36. Fisher, C. J., Faloon, W. W., Auchincloss, J. H., Eich, R., and Gilbert, R. Alteration in blood ammonia with changing oxygen concentrations (abstract). *Gastroenterology* 1957, **33**, 269.
37. Kowalski, H. J., Abelmann, W. H., and McNeely, W. F. The cardiac output in patients with cirrhosis of the liver and tense ascites with observations on the effect of paracentesis. *J. clin. Invest.* 1954, **33**, 768.
38. Abelmann, W. H., and Hutcheson, J. M., Jr. Peripheral blood flow in patients with cirrhosis of the liver at rest and during exercise. *Clin. Res. Proc.* 1956, **4**, 147.
39. Wechsler, R. L., Crum, W., and Roth, J. L. A. The blood flow and O<sub>2</sub> consumption of the human brain in hepatic coma (abstract). *Clin. Res. Proc.* 1954, **2**, 74.
40. Fazekas, J. F., Ticktin, H. E., Ehrmantraut, W. R., and Alman, R. W. Cerebral metabolism in hepatic insufficiency. *Amer. J. Med.* 1956, **21**, 843.