LITERATURE CITED

1. E. Ya. Stan, A. V. Popova, and M. P. Chernikov, Vopr. Pitan., No. 1, 18 (1979).
2. E. Ya. Stan and M. P. Chernikov, Byull. Éksp. Biol. Med., No. 8, 64 (1982).
3. E. Ya. Stan, S. D. Groisman, K. B. Vasil'shchikov, and M. P. Chernikov, Byull. Éksp. Biol. Med., No. 7, 10 (1983).
4. E. Ya. Stan, A. P. Ekimovskii, S. I. Aleinik, et al., Vopr. Med. Khim., (1987) (in press).
5. M. P. Chernikov, G. V. Nikol'skaya, E. Ya. Stan, et al., Vopr. Pitan., No. 2, 21 (1974).
6. G. K. Shlygin, L. S. Vasilevskaya, M. P. Chernikov, and G. V. Nikol'skaya, Byull. Éksp. Biol. Med., No. 2, 9 (1971).
7. V. Brantl, H. Teschemacher, A. Henschen, and F. Lottspieck, Z. Physiol. Chem., 360, 1211 (1979).
8. T. Mitsuma, T. Nogimori, and M. Chays, Exp. Clin. Endocrinol., 84, 324 (1984).
9. J. Nedvidkova, E. Kasafirek, A. Dlabac, and V. Felt, Exp. Clin. Endocrinol., 85, 249 (1985).
10. K. G. Reymann, A. N. Chepkova, K. Sculzeck, and T. Ott, Biomed. Biochim. Acta, 44, 749 (1985).
11. R. Schick, A. Holland, A. Fuente, et al., Acta Endocrinol. (Copenhagen), Suppl. 253, 80 (1983).
12. V. Schusdziarra, R. Schick, A. Fuente, et al., Endocrinology, 112, 885 (1983).
13. V. Schusdziarra, R. Schick, A. Fuente, et al., Endocrinology, 112, 1948 (1983).
14. C. Zioudrou, R. A. Streaty, and W. A. Klee, J. Biol. Chem., 254, 2446 (1979).
15. C. A. Zittle and J. H. Custer, J. Dairy Sci., 46, 1183 (1963).

FREE SERUM AMINO ACIDS IN RATS WITH HEPATIC FAILURE DUE TO SMALL INTESTINAL FISTULAS

V. I. Yukhtin, R. O. Labotkin, A. R. Kushelev, and M. I. Savina

UDC 616.36-008.64-092.9-02:616.341-007.253/-07:616.153.466-074

KEY WORDS: free amino acids; small intestinal fistulas; hepatic failure.

Clinical observations on patients with hepatic failure (HF) of varied etiology [12, 13] and experimental investigations [7, 11] have revealed uniform changes in the free amino acid (AA) spectrum of the blood (Fig. 1). An increase in the total AA content with a considerable rise in the level of aromatic AA and a fall in the content of branched-chain AA has now begun to be used as a diagnostic test for HF [14, 15]. The metabolic basis for the development of changes in the ratio between AA and their participation in the development of hepatic coma have been partly elucidated [10, 12]. It is noteworthy that all pathological processes studied were the result of primary damage to hepatocytes (hepatotoxic poisons, viral and nonviral hepatitis) or of a slowly developing dystrophic process (cirrhosis of the liver, etc.). It will be noted that the catabolic reactions in these processes are of moderate intensity [1]. Previously, however, the writers showed that HF against a background of high catabolic activity appears after exhaustion of the compensatory–adaptive reactions of metabolism [4, 5]. The biochemical and morphological symptoms of HF of this pathogenesis have the characteristic features of developing HF and of active reorganization of metabolism in order to maintain gluconeogenesis. About 50-60% of free AA and of AA obtained in the course of proteolysis is utilized for gluconeogenesis and ketogenesis by the body when there are functioning small-intestinal fistulas [2]. This degree of AA utilization after total exhaustion of the energy reserves of the body, as takes place in the presence of small intestinal fistulas, must have some effect on the AA spectrum in HF, developing as a result of functioning of small intestinal fistulas. Despite the acute necessity of determining the AA spectrum in the presence of

Department of General Surgery of the Pediatric Faculty, and Department of Biochemistry of the Interlaboratory Complex, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 10, pp. 426-428, October, 1987. Original article submitted October 17, 1986.

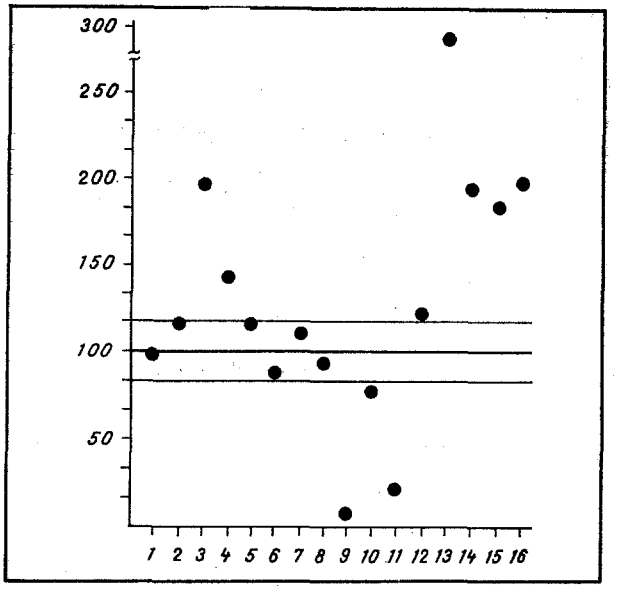

Fig. 1. Percentage deviation of content of free AA from normal HF of varied etiology (after [11, 12]). Here and in Fig. 2: 1-16 denotes: Ala, Lys, Gly, Glu, Ser, Pro, Thy, Arg, Val, Leu, Ile, His, Asp, Tyr, Phe, and Met, respectively.

such complex pathology as small intestinal fistulas, no data on this problem could be found in the literature.

The aim of this investigation was to study the free AA spectrum in the stage of HF developing as a result of functioning of total small-intestinal fistulas.

EXPERIMENTAL METHOD

Experiments were carried out on 60 noninbred albino rats weighing 200-300 g. Under multicomponent anesthesia (diazepam 1 ml/kg, ketamine 50 mg/kg, and fentanyl 2.5 mg/kg, intramuscularly) using a technique described by the writers previously [4], total single high small intestinal fistulas (HIF) were produced in 20 rats and medium-high small-intestinal fistulas (MIF) also were formed in 20 rats. A group of 20 rats subjected to no manipulations served as the control. Animals with fistulas and control animals were kept in pairs in cages under standard conditions on the ordinary animal house diet. Animals with HF were killed by decapitation on the 14th day, and those with MF on the 28th day after the beginning of the experiment. The times of decapitation were determined by the writers previously, in experiments to study morphological and functional changes in the liver, which showed that HF associated with HIF appeared in the second week, whereas when associated with MIF, it appeared in the 4th week of functioning of the small intestinal fistulas [4, 5]. Control animals (10 rats at each time of the investigation) were killed parallel with the experimental animals. Blood was collected from each animal at decapitation in centrifuge tubes (10 ml), incubated at 37°C for 40 min, and then cooled at 4°C for 20 min. The serum was centrifuged at 3,000 rpm for 15 min and deproteinized with 3% sulfosalicylic acid. The resulting supernatant was kept at −70°C until required for analysis. Sixteen free AA in deproteinized sera were analyzed by a modified method of single-column ion-exchange chromatography [3, 9]. The analyses were done on a KLA-3B amino-acid analyzer (Hitachi, Japan) with 9 × 500 mm column, packed with strong cation-exchange resin from the same firm (Mark 2612). The rate of flow of the buffer was 60 ml/h. The data were subjected to statistical analysis on the ACBT-40-30 computer, using the standard TRAN-4 statistical analysis program.

EXPERIMENTAL RESULTS

The total AA content in the stage of HF in rats with both HIF and MIF was 20-30% below normal. In rats with MIF this decrease in the AA content resulted from a uniform fall in the level of nearly all AA observed (Fig. 2). In the group of gluconeogenetic AA the average fall in their levels was 25% compared with normal (Fig. 2). About 40% of the fall in the content of branched-chain AA took place on account of a decrease in Val and Leu concentrations.

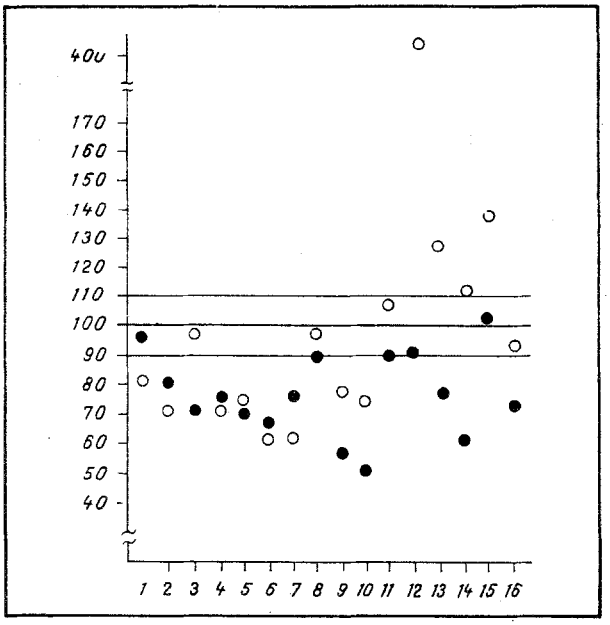

Fig. 2. Percentage deviation of free AA content from normal in HF developing as a result of functioning of small-intestinal fistulas. Empty circles — HIF, filled circles — MIF.

TABLE 1. Concentration (in mmoles/liter) of Free Amino Acids in Blood Serum of Rats with HF Associated with Total Small-Intestinal Fistulas (M ± m).

Amino acid	Control ($n=20$)	MIF ($n=20$)	HIF
Ala	0,628±0,027	0,615±0,026	0,520±0,026*
Lys	0,523±0,12	0,428±0,014*	0,383±0,04*
Glu	0,442±0,017	0,315±0,005*	0,424±0,037
Gly	0,434±0,035	0,312±0,004*	0,313±0,015*
Ser	0,441±0,033	0,314±0,008*	0,294±0,03*
Pro	0,441±0,015	0,299±0,007*	0,280±0,022*
Thr	0,364±0,025	0,275±0,01*	0,234±0,01*
Arg	0,258±0,035	0,227±0,01	0,227±0,011
Val	0,176±0,011	0,102±0,003*	0,139±0,009*
Leu	0,178±0,009	0,093±0,003*	0,136±0,009*
Ile	0,109±0,011	0,090±0,005	0,121±0,007
His	0,090±0,011	0,085±0,004	0,353±0,029*
Asp	0,092±0,004	0,071±0,002*	0,127±0,007*
Tyr	0,075±0,006	0,047±0,008*	0,087±0,002
Phe	0,076±0,002	0,083±0,011	0,125±0,013*
Met	0,059±0,002	0,043±0,004*	0,052±0,002
Total	4,386±0,023	3,399±0,008*	3,815±0,017*

Legend. *$p < 0.01$.

The concentrations of essential AA in the rats with MIF were reduced on average by 20% below normal. The content of AA with an aromatic chain (Phe, Tyr) was reduced on account of a 40% decrease in the Tyr concentration in MIF (Fig. 2). Fisher's coefficient for MIF was 2.19. The numerical results are given in Table 1.

In rats with HIF, against the background of a fall in the concentration of gluconeogenetic and essential AA on average by 20-30%, just as was observed in MIF, a statistically significant increase was observed in the Asp, Phe, and His levels. Whereas the Asp and Phe concentrations were raised by 30 and 40%, respectively, the Tyr concentration was only 13% above normal. The His concentration was 400% above normal. By contrast with rats with MIF, in these animals the Ala and Lys concentrations were 30 and 40% respectively below normal (Fig. 2). Fischer's coefficient for HIF was indistinguishable from that for MIF (1.87).

In the presence of high catabolic activity resulting from the enormous losses and reduced assimilability of nutrients through small-intestinal fistulas, the energy expenditure

of the animal was met by high activity of gluconeogenesis [2, 8]. Whereas Glu, Gly, Ser, Pro, Thr, Val, and Leu play the role of substrate in this process, Ala and Lys are modulators of gluconeogenesis. The fall of 20-30% which we recorded in the concentration of both glucogenetic and essential AA in the animals of both experimental groups, is an indicator of high activity of gluconeogenesis in the presence of considerable losses of intestinal contents, leading to reduction of the quantity of substrate available for synthesis. There is no doubt that a distinguishing feature of the metabolic relations in the model which we studied, compared with models used by other workers, is the higher level of activity of gluconeogenesis. Integrity of the mechanism of gluconeogenesis in cirrhosis, hepatitis, and poisoning by hepatotoxic poisons, even in a state of profound HF and its insufficiency on account of a shortage of substrate in the model which we used are the main causes of the disparity between our results and those obtained by other workers for this group of AA. We consider that the fall in the level of aromatic AA by 20-30% in MIF and the moderate increase in their content on account of Phe in HIF can be attributed also to the particular features of the pathology which we investigated. Efflux of Phe as the substrate for Tyr synthesis through the fistula, low enzyme activity in HF, and a high level of its utilization may be the reasons for disparity between our data and those obtained by other workers [4, 12]. Irrespective of which mechanism or combination of mechanisms is responsible for the fall in the Tyr level, they are connected with the pathogenic features of HF in the presence of small-intestinal fistulas. The fall in concentration of aromatic AA explains the disparity between the ratios of branched-chain AA with aromatic-chain AA, taken by some workers [6, 11] as an indicator of HF (Fischer's coefficient), in HF which develops in our own experiments. The question of the cause of the sharp rise in the His level in rats with HIF, despite its normal concentration in animals with MIF — rapid proteolysis or a different pattern of metabolism of this amino acid, connected with the local tissue reaction under conditions of developing pathology, requires further investigation.

Thus when the diagnostic value of the content of free amino acids in HF is examined, the loss of substrate and activity of gluconeogenesis must be taken into account.

LITERATURE CITED

1. É. I. Gal'perin, M. I. Semendyaeva, and E. A. Neklyudova, Hepatic Failure [in Russian], Moscow (1978).
2. I. N. Kendysh, Regulation of Carbohydrate Metabolism [in Russian], Moscow (1985).
3. A. R. Kushelev, Chromatography in Biology and Medicine [in Russian], Moscow (1983), pp. 119-120.
4. V. I. Yukhtin, R. O. Labotkin, and L. P. Pashintseva, Khirurgiya, No. 12, 151 (1985).
5. V. I. Yukhtin, R. O. Labotkin, and N. N. Safronov, Khirurgiya, No. 5, 153 (1986).
6. P. Bouletreau, B. Delafosse, and C. Auboyer, Liver: Anaesthesia and Critical Care, Paris (1979), pp. 239-254.
7. P. M. Daniel, E. R. Love, S. R. Moorhouse, and O. E. Pratt, Lancet, 2, 179 (1975).
8. D. Dolif and P. Jürgens, Parenteral Nutrition, ed. by F. W. Ahnefeld et al.. Berlin (1976), pp. 54-65.
9. R. S. Erasser, J. Chromatog., 15, 612 (1975).
10. J. E. Fischer, N. Yoshimura, A. Aguirre, et al., Am. J. Surg., 127, No. 1, 40 (1974).
11. J. E. Fischer, J. Funovics, A. Aguirre, et al., Surgery, 78, 276 (1975).
12. E. Holm, J. P. Striebel, R. Munzenmaier, et al., Leber, Magen. Darm., 7, 241 (1977).
13. B. Joelsson, B. Hultberg, A. Alwmark, et al., Scand. J. Gastroent., 19, 547 (1984).
14. H. G. Knauff, D. Seyhold, and B. Miller, Klin. Wschr., 7, 326 (1964).
15. F. Steigmann, A. Dubin, and P. B. Szanto, 9th International Congress of Gastroenterology, Abstracts, Hamburg (1980), p. 214.