# PHARMACOLOGY AND TOXICOLOGY

# Ammonia Redistribution from the Gastrointestinal Tract to General Circulation after Intraperitoneal Injection of Cyclophosphamide to Rats

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Ammonia level in the blood increased within 3 h after intraperitoneal injection of cyclophosphamide in doses of 200, 600, and 1000 mg/kg: by 1.4, 1.8, and 2.5 times in the blood from *v. portae*, by 1.5, 2.1, and 3.3 times in the blood from *v. cava caud.* caudally to *vv. renales* orifice, and by 1.8, 2.7, and 4.2 times, respectively, in the same vein cranially to *vv. hepaticae*. A positive portocaval gradient of ammonia concentration was abolished. Blood concentrations of glutamine and urea increased less markedly than those of ammonia. Ammonia and glutamine accumulation in isotonic saline injected to animals intraperitoneally 2.5 h after cyclophosphamide was stimulated depending on the drug dose. Blood concentrations of cytolysis markers (lactate dehydrogenase and alanine and aspartate transaminases) increased. Ammonia, glutamine, and urea concentrations in the blood remained high 18 h after injection of cyclophosphamide, the glutamine/ammonia ratio increased. These data indicate that a single intraperitoneal injection of cyclophosphamide to rats in doses of 200-1000 mg/kg disorders detoxification and stimulates transperitoneal diffusion of ammonia from the digestive tract to the posterior vena cava basin with the development of hyperammoniemia.

**Key Words:** *cyclophosphamide; rats; blood ammonia; peritoneal lavage*

Cyclophosphamide (CP) is an alkylating substance used as a cytostatic drug. For some indications CP is injected intraperitoneally [1,2] and exhibits local and resorptive effects on the stomach, intestine, and liver. Disorders in ammonia ($NH_3$) detoxification in these organs related to cell death and inhibition of glutamine and urea synthesis in them can stimulate $NH_3$ release from the gastrointestinal tract to the systemic circulation. Interactions between the toxicities of $NH_3$ and CP can narrow the interval of tolerated doses of the drug and hence, reduce the efficiency of chemotherapy. Therefore, we studied the effect of intraperitoneal CP on the kinetics of $NH_3$ of gastrointestinal origin.

## MATERIALS AND METHODS

The study was carried out on outbred male albino rats (200-240) from Rappolovo Breeding Center of the Russian Academy of Medical Sciences. The animals received no fodder during 24 h before the experiment and were allowed a free access to water.

In experimental series 1, 2, and 3 the concentrations of $NH_3$, glutamine, and urea were measured in the blood collected from *v. portae* or *v. cava caud.*

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**TABLE 1.** Accumulation of $NH_3$ and Glutamine in Isotonic Saline Injected Intraperitoneally to Rats 2.5 h after CP ($n$=6; $M\pm m$)

| CP dose, mg/kg | Volume of solution, ml/kg | Concentration, mM | | Rate of accumulation in solution, mmol/(kg×min) | |
|---|---|---|---|---|---|
| | | $NH_3$ | glutamine | $NH_3$ | glutamine |
| 0 (control) | 40.2±2.5 | 0.112±0.014 | 0.679±0.051 | 0.146±0.013 | 0.912±0.097 |
| 200 | 42.7±0.6 | 0.099±0.009 | 0.652±0.038 | 0.141±0.014 | 0.929±0.059 |
| 600 | 51.5±2.5* | 0.235±0.046* | 0.890±0.057* | 0.407±0.082* | 1.527±0.114* |
| 1000 | 59.3±3.3* | 0.480±0.054* | 1.095±0.086* | 0.927±0.083* | 2.130±0.130* |

**Note.** Here and in Table 2: *$p\leq0.05$ compared to the control.

0.5 h (series 1), 3 h (series 2), and 18 h (series 3) after intraperitoneal injection (10 ml/kg) of CP (Biokhimik) in doses of 0, 200, 600, and 1000 mg/kg. Controls were injected with the same volume of water. Two minutes before laparotomy the animals were narcotized by sodium thiopental (75 mg/kg intraperitoneally). *V. portae* were punctured cranially to *v. pylorica, v. cava caud.* in two points: caudally to *vv. renales* flows and cranially to *vv. hepaticae* orifice at the level of the diaphragm. Blood specimens (1 ml) were deproteinated with 10% trichloroacetic acid and measurement of $NH_3$ (using Nessler's reagent), glutamine (by $NH_3$ after acid hydrolysis), and urea (with diacetylmonoxime using Allwex Diagnosticum kit) in the supernatant were carried out.

Accumulation of $NH_3$ and glutamine in 0.9% NaCl solution injected intraperitoneally (50 ml/kg) 2.5 h after CP was evaluated in experimental series 4. Laparotomy was carried out 3 h after injection of the toxicant under light chloroform narcosis. Free solution was removed completely from the abdominal cavity, its volume was measured, the solution was deproteinated, and $NH_3$ and glutamine were measured in supernatant.

In experimental series 5, activities of cytolysis markers (ALT, AST, and lactate dehydrogenase, LDH) were measured in the plasma collected from the trunk by decapitation 3 h after intraperitoneal injection of CP using Diagnostic Systems GmbH & Co kits. According to the instruction for the kit, normal values of these enzymes in human plasma are ≤45, ≤37, and ≤480 U/liter, respectively. In parallel with this, hematocrit and blood concentrations of $NH_3$, glutamine, and urea were measured.

Each series of experiments was carried out within 1 day. All groups consisted of 6 animals. The significance of differences in the means was evaluated using heteroscedastic (for unrelated samples) or paired (for related samples) Student's $t$ test.

## RESULTS

The CP dose of 200 mg/kg was inessential for animal behavior. In a dose of 600 mg/kg the drug caused slowly progressing somnolence and stupor. The dose of 1000 mg/kg caused tremor, lateral posture, loss of the audiomotor reflex, rare short-term episodes of tonic convulsions after 0.5-3 h and death of the majority of rats during 18 h after injection.

The level of $NH_3$ increased 1.4, 1.8, and 2.5 times within 3 h after injection of CP in doses of 200, 600, and 1000 mg/kg, respectively. The level of $NH_3$ in

**TABLE 2.** Hematocrit, $NH_3$, Glutamine, and Urea and Plasma Cytolysis Markers in the Blood of Rats Collected by Decapitation 3 h after Intraperitoneal Injection of CP ($n$=6; $M\pm m$)

| CP dose, mg/kg | Hematocrit, % | Blood content, mM | | | Plasma activity, U/liter | | |
|---|---|---|---|---|---|---|---|
| | | $NH_3$ | glutamine | urea | ALT | AST | LDH |
| 0 (control) | 45±1 | 0.097±0.015 | 1.204±0.054 | 9.2±0.6 | 42.7±4.8 | 89.6±6.1 | 481.7±62.4 |
| 200 | 43±1 | 0.106±0.023 | 1.309±0.045 | 9.2±0.7 | 44.9±5.0 | 123.6±18.7 | 520.4±44.0 |
| 600 | 47±1 | 0.222±0.020* | 1.739±0.072* | 15.3±0.8* | 57.7±6.7 | 186.7±11.9* | 995.9±89.7* |
| 1000 | 59±1* | 0.385±0.039* | 2.227±0.159* | 17.0±1.1* | 68.2±8.8* | 186.2±8.3* | 2338.6±327.6* |

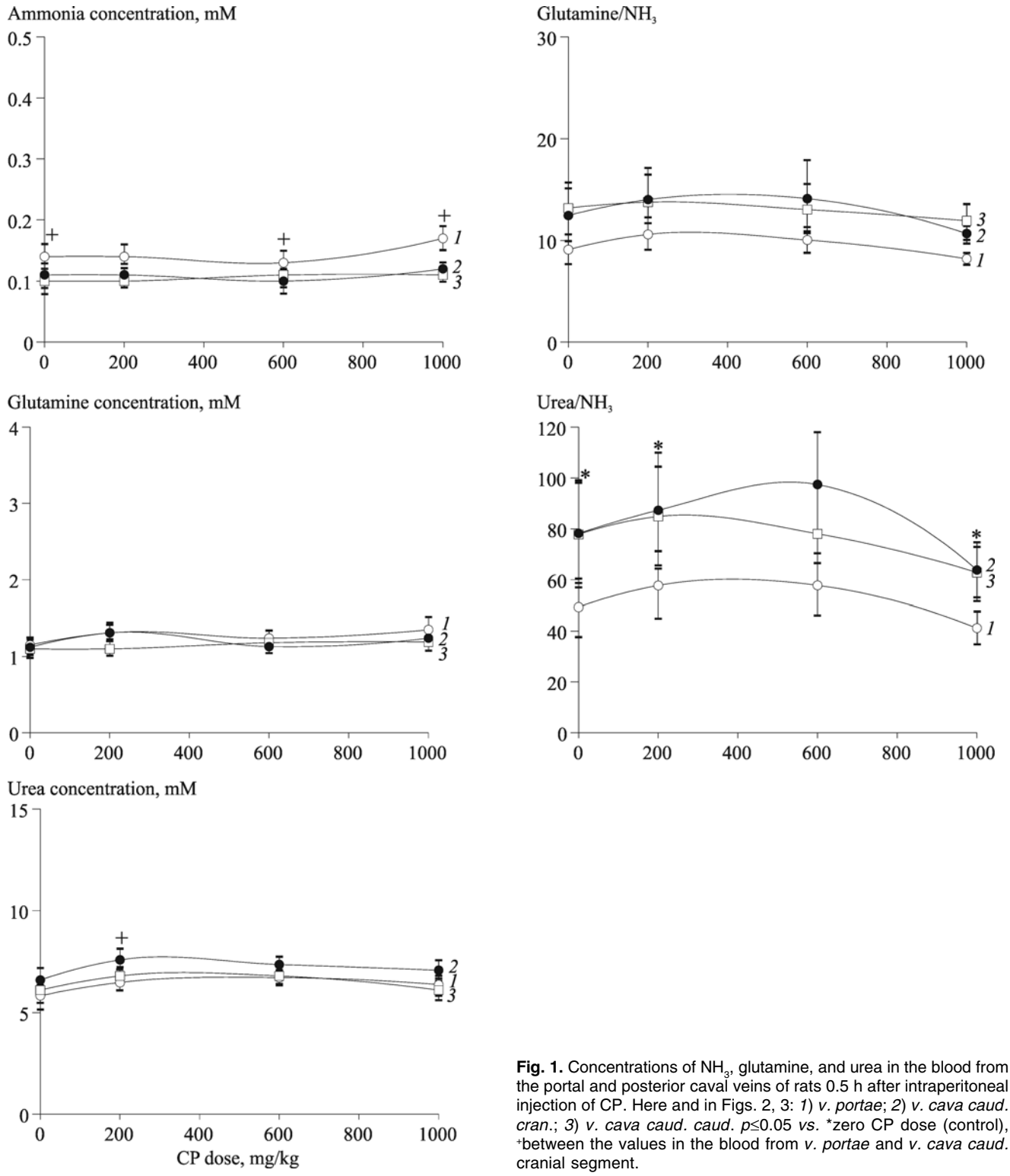


**Fig. 1.** Concentrations of $NH_3$, glutamine, and urea in the blood from the portal and posterior caval veins of rats 0.5 h after intraperitoneal injection of CP. Here and in Figs. 2, 3: *1*) *v. portae*; *2*) *v. cava caud. cran.*; *3*) *v. cava caud. caud*. $p \leq 0.05$ *vs.* *zero CP dose (control), +between the values in the blood from *v. portae* and *v. cava caud.* cranial segment.

the blood from *v. cava caud.* caudally to *vv. renales* orifice increased by 1.5, 2.1, and 3.3 times, cranially to *vv. hepaticae* by 1.8, 2.7, and 4.2 times, respectively. Cyclophosphamide in doses of 600 and 1000 mg/kg abolished the positive portocaval gradient of $NH_3$ concentration, the negative portocaval gradient of urea concentration being retained. Accumulation of glutamine and urea in the blood lagged behind hyperammoniemia: glutamine/$NH_3$ ratio in the blood from *v. cava caud.* cranial segment decreased by 1.9, 1.6, and 2 times 3 h after CP injection in doses of 200, 600, and 1000 mg/kg, while urea/$NH_3$ ratio decreased by

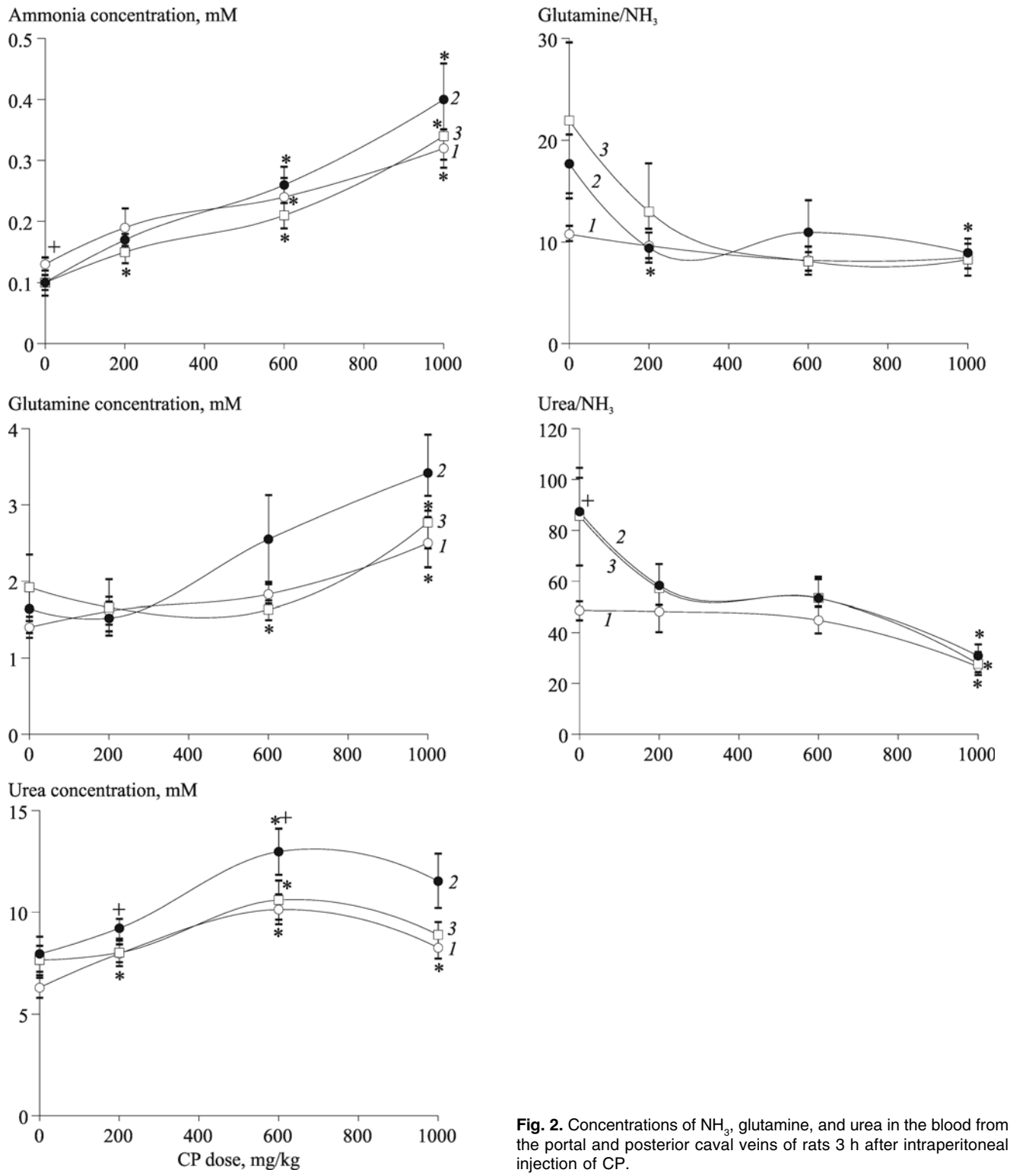


**Fig. 2.** Concentrations of $NH_3$, glutamine, and urea in the blood from the portal and posterior caval veins of rats 3 h after intraperitoneal injection of CP.

1.5, 1.6, and 2.8 times, respectively. Hyparammoniemia persisted 18 h after injection of the toxicant in a dose of 600 mg/kg and was paralleled by a significant increase in blood levels of glutamine and urea (Figs. 1-3). The glutamine/$NH_3$ concentrations ratio in the blood from *v. cava caud.* increased by 1.6-1.8 times.

Accumulation of $NH_3$ in isotonic saline injected intraperitoneally surpassed the control level by 2.8 and 6.4 times 3 h after injection of CP in doses of 600 and 1000 mg/kg, glutamine level being by 1.7 and 2.3 times higher, respectively (Table 1).

Activities of cytolysis markers AST and LDH in

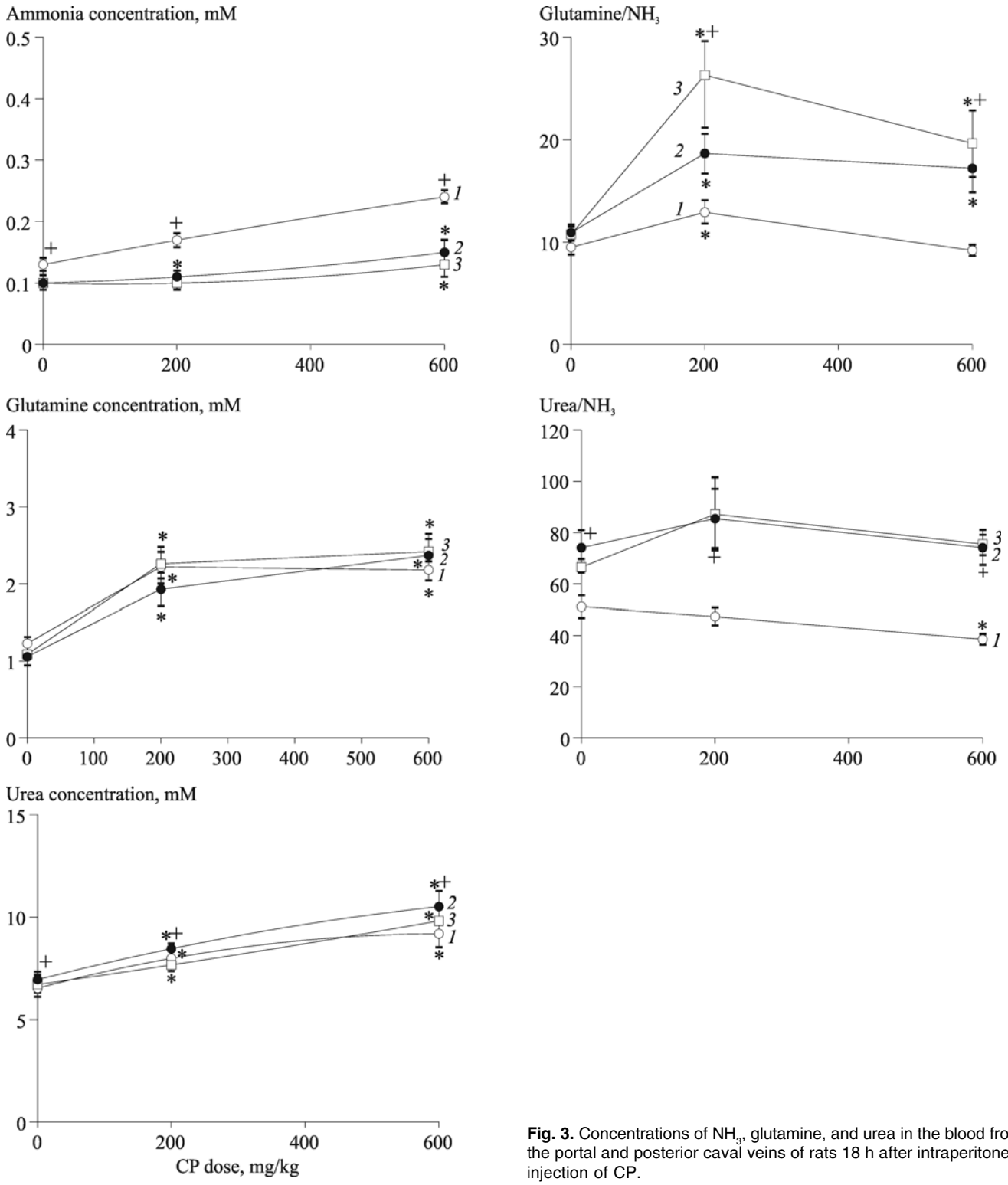


**Fig. 3.** Concentrations of $NH_3$, glutamine, and urea in the blood from the portal and posterior caval veins of rats 18 h after intraperitoneal injection of CP.

the plasma increased 2.1 times 3 h after injection of CP in a dose of 600 mg/kg. After the dose of 1000 mg/kg plasma concentrations of ALT, AST, and LDH increased by 1.6, 2.1, and 4.9 times, respectively. Hematocrit increased (by 1.3 times) only after CP dose of 1000 mg/kg, and hence, the increase in the concentrations of $NH_3$, glutamine, and urea by 4.0, 1.8, and 1.8 times, respectively, in the same blood samples could not be attributed to dehydration (Table 2).

Hence, intraperitoneal injection of CP caused a dose-dependent elevation of $NH_3$ level in the blood in parallel with clinical manifestations of intoxication.

Some of these symptoms (tremor, convulsions) could be partially due to hyperammoniemia. The leading mechanisms of hyperammoniemia were cytolysis-mediated intensification of transperitoneal diffusion of $NH_3$ from the gastrointestinal tract to the caudal vena cava by-passing the liver and delayed synthesis of glutamine and urea in the liver compared to increased release of $NH_3$ into the portal vein basin. These data attest to possible involvement of $NH_3$ in the formation of CP toxic effects in its intraperitoneal injection.

## REFERENCES

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