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RESEARCH PAPER



# Immuno-, Phagocytosis-Modulating, and Antitoxic Properties of Amino Acids and Peptide Preparations

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## ABSTRACT

*The influence of amino acids, their mixtures, and peptides on the immune response, phagocytosis in vitro, and in CBA mice, and broiler chickens, as well as on in vitro ability of listed preparations to protect animals' splenocytes from toxic action of benzene and aflatoxin B1 were studied. It was shown that amino acids (Asp, Glu, Val, Trp), amino acid mixtures (cerebrolysine, levamine, aviamine), and the dipeptides GluTrp and LysAsp stimulate the immune response to SRBC at subcutaneous and peroral application. The peptides thymopentin, thymosin α-1, and peptide mixtures (thymosin fraction 5, thymalin) stimulate the immune response only at the site of subcutaneous injections. Lys, Tyr, and bursin (LysHisGly-amide), regardless of the mode of application, do not change, but Arg inhibits the immune response. None of the preparations studied change the immune response to Vi-antigen. Levamine, cerebrolysine, and aviamine are immunoreactive only at low doses ($6.5 \times 10^{-2}$–$6.5 \times 10^{-8}$ mg/kg). At a dose of 65 mg/kg these preparations lose immunostimulating properties. Amino acids ($6.5 \times 10^{-2}$ mg/kg), which stimulate, rather than influence or inhibit the immune response, enhance phagocytosis of* S. aureus *by granulocytes, regardless of mode of application. Levamine and cerebrolysine in the range of doses of $6.5 \times 10^{-2}$–$6.5 \times 10^{-6}$ mg/kg do not influence phagocytosis; at a dose of 65 mg/kg, phagocytosis is enhanced. Aviamine stimulates phagocytosis as well at low and at high doses.*

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*The ability of preparations to protect in vitro at a low concentration ($1.3 \times 10^{-3}$ mg/ml) murine or chickens' splenocytes from toxic action of benzene and aflatoxin B1 (at 1:1000 dilution) does not correlate with their action on the immune response and phagocytosis. The protective actions revealed by the following preparations include: (a) stimulating the immune response and phagocytosis (Glu, Asp, Trp, amino acid mixture aviamine, the dipeptides LysAsp, GluTrp, the peptide mixtures thymalin, thymosin fraction 5); (b) enhancing the immune response but not influencing phagocytosis (Met, levamine, cerebrolysine); (c) influencing neither the immune response nor phagocytosis (Gly, Ile). At the same time those preparations (Lys, Arg) that stimulate phagocytosis but influence the immune response in a different way (Lys does not influence; Arg suppresses the response) are inert as antitoxic agents. Antitoxic properties of amino acid preparations levamine, cerebrolysine, and aviamine retain as well in the assays at a rather large (1.3 mg/ml) concentration like their phagocytosis-stimulating properties.*



## INTRODUCTION

Amino acids together with other biologically active compounds are important homeostasis regulating factors. The imbalance of amino acids in an organism may be the reason for the crash of homeostasis (1,2). The amino acid imbalance may be the result of the excess or the deficiency of any amino acid. The amino acid excess may arise from (*a*) the inability of tissues to metabolize one amino acid to another; for example, phenylalanine to tyrosine (3); (*b*) the parenteral introduction of an amino acid in excess (4,5) or from the addition of an amino acid in excess to the diet (6). The excess of one amino acid may cause the deficiency of another; for example, the excess of leucine (7%) in the diet inhibits the utilization of isoleucine and valine (7), and the excess of arginine in the diet (100 mg/kg) results in the deficiency of leucine and isoleucine (8). The excess of that or another amino acid in an organism may be the reason for not only amino acid but also hormonal imbalance: the excess of leucine enhances the blood corticosterone level (9) and the excess or arginine results in the enhancement of blood insulin concentration (8).

The excess or the deficiency of an amino acid as well as the disruption of the metabolic processes caused by these circumstances may suppress the functions of the immune system. Thus, the parenteral injection of phenylalanine into animals in a large dose (300 mg/kg) depresses the production of hemagglutinins (4), and the deficiency of isoleucine or valine in the diet causes the decline of leukopoiesis and thymus atrophy (10). The amino acid metabolism products may influence directly the immunocompetent cells (7). It is known that amino acids are used as trophic agents (like levamine in human medicine and aviamine in veterinary medicine). However, in that case the afore-said data on the possibility of unfavorable effects of amino acids and their mixtures on specific and nonspecific resistance indexes are not taken into account adequately.

In 1986–1990 we showed for the first time (11–13) that it was possible to use amino acids not only as trophic agents, but also as immunomodulators by their application in very low concentrations which were near 100 or more times lower than physiological (near 20 mg/ml concentrations in mice) (14), and the immunomodulating activity of short peptides was determined by the presence of immunoactive amino acids (13,15). Later, our data about the immunomodulating properties of separate amino acids were confirmed by other investigators (6). Thereafter we found that like separate amino acids, low doses ($6.5 \times 10^{-2}$–$6.5 \times 10^{-8}$ mg/kg) of amino acid mixtures presented with a brain hydrolysate (cerebrolysine), a hen's blood hydrolysate (aviamine), or an artificially composed amino acid mixture (levamine) revealed immunostimulating activity (17,18). There is little information about the influence on the indexes of specific and nonspecific resistance of these amino acid preparation doses that cause the trophic effects (18). But any data of that kind are important for clinicians using amino acid preparations as trophic agents in different pathological processes, in particular, for bacterial and nonbacterial intoxication that often accompany the acute decline of organism resistance, with an addition of intercurrent infections. Because of this demand, the use of preparations with combined action on specific and nonspecific organism resistance is being studied.

The present investigation is a comparison study of the experiments on animals and in vitro of (*a*) a combination of effects on the immune response, phagocytosis,

and antitoxic action of low doses of amino acid and peptide preparations; and (*b*) the influence of low and relatively high (trophic) doses of amino acid preparations on the immune response, Thy-1 antigen expression on T-precursors, phagocytosis, and antitoxic effects.

## MATERIALS AND METHODS

The following preparations were used in this study: amino acids (Sigma, St. Louis, MO); levamine 70 (Leiras, Finland); the mixture of alanine 0.179 mole, arginine 0.034 mole, glycine 0.213 mole, histidine 0.014 mole, isoleucine 0.021 mole, leucine 0.033 mole, lysine 0.022 mole, methionine 0.029 mole, phenylalanine 0.027 mole, proline 0.026 mole, threonine 0.017 mole, tryptophan 0.005 mole, valine 0.027 mole), cerebrolysine (Ebeve, Austria; from hydrolyzed brain tissues that contained 18 amino acids); aviamine (The Factory of Medicinal Preparations, St. Petersburg, Russia), which contains hydrolyzed hens' blood that contains 18 amino acids (alanine 0.026 mole, arginine 0.006 mole, aspartic acid 0.034 mole, cysteine 0.004 mole, glutamic acid 0.023 mole, glycine 0.028 mole, histidine 0.018 mole, isoleucine 0.002 mole, leucine 0.002 mole, lysine 0.022 mole, methionine 0.002 mole, phenylalanine 0.004 mole, proline 0.010 mole, serine 0.014 mole, threonine 0.006 mole, tryptophan 0.001 mole, tyrosine 0.007 mole, valine 0.007 mole); thymalin (The Factory of Medicinal Preparations); the acetic acid extract of fresh calf thymus (a mixture of more than 50 peptides of molecular weights 1000–10,000 D); the thymosin fraction 5 (a mixture of 50 peptides of molecular weights 1000–12,000 D) prepared from fresh calf thymus by the method of A. Goldstein and co-workers (19); and synthetic peptides: thymogen (GluTrp), TP-5 (ArgLysAspValTyr), bursin (LysHisGly-amide), and thymosin α-1. All the synthetic peptides used except for thymosin α-1 were prepared at St. Petersburg State University by classical peptide synthesis methods in solution (20). Thymosin α-1 was the generous gift of Dr. A. Azmuko (The Institute of Immunology, Moscow).

Male CBA mice (5 weeks old, body weight 14–16 g) were purchased from Rappolovo (Russia); 1103 animals were used. Broiler chickens (2 weeks old, body weight 120–125 g) were purchased from farm Russko-Vysotskoe (Russia); 60 chickens were used.

Peptide mixtures (thymosin fraction 5 and thymalin) and amino acid mixtures (cerebrolysine and aviamine) were dosed according to protein content (0.91, 0.64, 1.0, and 0.032 g/ml, respectively); levamine was dosed according to total amino acid content (0.53 g/ml); and short peptides (LysAsp, GluTrp, bursin, and TP-5) and separate amino acids were dosed in accordance with dry weight samples. All were converted to kilograms of body weight. The preparations were dissolved in pyrogen-free saline (Polfa, Poland) just before applications, and were injected into mice subcutaneously or per os via sound in 0.5 ml of sterile pyrogen-free saline or fed to chickens for 10 days in doses of $65–6.5 \times 10^{-8}$ mg/kg a day. Control animals were given sterile pyrogen-free saline. The mice were then intravenously (tail vein) immunized once with $2 \times 10^6$ SRBC or with Vi-antigen (polysaccharide from *Salmonella typhi abdominalis*, 0.001 μg per animal). Chickens were immunized intravenously (underwing vein) with $5 \times 10^{-6}$ SRBC. On the fourth day after the immunization, the mice or the chickens were decapitated and IgM-PFC was determined in the spleens by the method of local hemolysis (21) in 0.7% agarose (Sigma) in accordance with the previous report (15). To reveal IgM-PFC, a fresh complement of guinea pigs (1:10) for murine spleen cells and the fresh hens complement (1:3) for chicken spleen cells were used. The IgM-PFC values were calculated per $10^6$ nucleated cells (not chicken nucleated erythrocytes).

The in vivo assay of phagocytosis by peritoneal granulocytes has been performed by the method published earlier (15). Peptides or amino acids were injected in mice subcutaneously for 10 days; amino acid preparations were fed to chickens and mice for the same period. The exudates for the phagocytosis assays were collected 2.5 hr after the intra-abdominal injections in mice or chickens of 10% sterile peptone (Nutritional Biochemicals Corporation) solution. The exudate cells were used at the final concentration of 12.5 million/ml. The phagocytosis experiments were performed with a 24-hr culture of *Staphylococcus aureus* at the final concentration of 250 million/ml. The peritoneal exudate cells were collected and incubated at 37°C for 15 min, then the suspension of *S. aureus* was added at the above-mentioned concentration; the mixture was incubated at the same conditions for 15 min more. For in vitro assays the peritoneal exudate cells were co-incubated with the preparations to be studied at 37°C for 15 min, followed by the addition of *S. aureus* suspension and an additional incubation at 37°C for 15 min. The cell suspension was centrifuged for 7 min at 1200 rpm and smears were made from the precipitate. The smears were stained with the Romanowski–Giemsa reagent. No

less than 300 cells for each preparation were calculated to evaluate phagocytosis in mice and chickens. Each assay was repeated three times. The phagocytic index—the percentage of granulocytes taking part in phagocytosis—and the phagocytic number—the average number of microbe cells captured by one granulocyte—were estimated.

The induction assays of T-cell differentiation in mice by levamine, cerebrolysine, or aviamine has been fulfilled in vitro in the following manner. Cells of chest, thigh, and leg bone marrow were set free of erythrocytes by 0.65% solution of ammonium chloride (22). This procedure produced a leukocyte suspension which contained no less than 85–90% of the viable cells, unlike the effect of 0.83% ammonium chloride solution, in which the leukocyte viability was no more than 70% (23). Then the cells were washed with a cold Hanks' solution five times at 1200 rpm for 7 min and were mixed with the preparations to be studied so that 1 ml of the solution contained $3 \times 10^7$ nucleated cells and $1.3 \times 10^{-3}$–$1.3 \times 10^{-9}$ mg/ml of a preparation. The mixture of the preparation with the cells was incubated with periodic shaking at 37°C for 1.5 hr, then it was washed with Hanks' solution five times and the cell susceptibility to the anti-mouse brain serum was evaluated in the complement-dependent cytotoxicity assay by the method of Niederhuber and co-workers (23). The antiserum was raised by the immunization of rabbits with the CBA mouse murine cortex tissue, in which Thy-1 antigen identical to Thy-1 antigen of T-cells was mostly localized (24,25). The immunization was carried out subcutaneously without Freund's adjuvant by the method of Golub (26). The antiserum was heated at 56°C for 30 min and then twice absorbed subsequently with murine liver homogenate and murine and sheep red blood cells (1 ml of the antiserum for 0.1 ml of the dense sediment of each kind of cells) for 1 hr at room temperature by the method of Feiglova and co-workers (27). After the absorption, the antiserum was diluted with cold distilled water (1:10) and the antigen–antibody complexes were deleted by re-precipitation with carbon dioxide. The anticortex serum was used at the 1:50 dilution; in this case the antiserum caused the death of 88.0 ± 1.3% of thymocytes in the presence of complement—fresh guinea pig serum (1:3)—and did not interact with the bone marrow cells of CBA mice. In the control cells of Hanks' solution in presence of complement (1:3), viability of lymphocytes was no less than 85–90%, as usual.

The number of Thy-$1^+$ cells in chicken bone marrow after feeding with various doses of aviamine, cerebrolysine, or glutamic acid was estimated in vitro with the use of antiserum against chicken thymocytes. The antiserum was prepared by intravenous immunization of rabbits with chicken thymocytes at a dose of $1.7 \times 10^8$ cells for 3 consecutive days, 4 days apart, during 3 weeks. In 1 week after the last immunization, rabbits were bled, the serum obtained was heated at 56°C for 30 min and absorbed with chicken liver homogenate and chicken and sheep red blood cells, as in procedures used for antiserum against murine cortex (27). The removal of antigen–antibody complexes was carried out as in the deletion in experiments on mice. The antiserum was used at dilution 1:100 whereby it caused in presence of chicken complement (1:2.5) the death of 92 ± 2.5% of thymocytes and 1.2 ± 0.7% of chicken bursa cells. To determine the number of Thy-$1^+$ cells, no less than 200 cells were calculated; viability was tested by exclusion trypan blue (0.2%, Sigma). Each assay was repeated no less than 2–3 times.

For the estimation of antitoxic properties of amino acid and peptide preparations, benzene (Reanal, Hungary) and aflatoxin B1 in benzene (The Institute of Nutrition RAMS, Russia), which contained $1 \times 10^{-2}$ mg/ml of the toxin, were used as cytotoxic substances. Murine and chicken splenocytes were freed from erythrocytes by 0.65% and 0.7% ammonium chloride solution, respectively. Spleen cells were washed three times with cold Hanks' solution and then equal volumes of cell suspension (at a concentration of $2.5 \times 10^7$ cells/ml) and solutions of preparations to be studied (at a concentration of $1.3 \times 10^{-3}$ mg/ml) were mixed. Mixtures of preparations with cells were incubated under constant shaking at 37°C for 30 min and then washed three times with cold Hanks' solution. The splenocytes treated with preparations were mixed with benzene and aflatoxin B1, again incubated for 30 min, and washed five times with cold Hanks' solution. For control, splenocytes were treated in vitro only with Hanks' solution. Results were expressed as cytotoxicity indexes (percent) that were calculated according to a known formula (26): [(percent of dead cells in an experiment – percent of dead cells in a control)/100 percent of dead cells in a control] × 100. No less than 200 cells were calculated to evaluate each preparation. Each assay was repeated 2–3 times.

## RESULTS

The assays of amino acid preparations levamine, cerebrolysine, and aviamine at subcutaneous injections

at a low dose ($6.5 \times 10^{-2}$ mg/kg/day) showed that these preparations enhanced IgM-PFC production in the response to SRBC like the peptides TP-5, thymosin α-1, thymosine fraction 5, and thymalin (Table 1). At the same time the above-listed peptides, unlike the amino acid preparations, did not influence the immune response at the peroral application (Table 1).

The assays of the shorter as compared with TP-5 peptides (dipeptides GluTrp [thymogen], LysAsp, and tripeptide LysHisGly-amide [bursin]) and their constituent amino acid mixtures in the same conditions and at the same dose ($6.5 \times 10^{-2}$ mg/kg) revealed the difference in their effects on the immune response. The dipeptides GluTrp, LysAsp, and their constituent amino acid mixtures turned out to be active both at the subcutaneous and at the oral application. At the same time bursin did not reveal any activity. The biological effects of the amino acids taken separately turned out to be independent of the route of application. Aspartic acid, glutamic acid, tryptophan, and valine stimulated the immune response subcutaneously as per os, whereas lysine and tyrosine did not reveal any such activity. Arginine inhibited the immune response (Table 1).

Levamine, cerebrolysine, aviamine, and all of the above-listed amino acids and peptides assayed at a dose of $6.5 \times 10^{-2}$ mg/kg did not influence the level of the thymus-independent immune response to Vi antigen at subcutaneous application in mice for 10 days: the IgM-PFC number varied in the range of $8.3 \pm 0.9$–$9.2 \pm 0.7$ in the experiment and $8.8 \pm 0.5$–$9.0 \pm 0.6$ in the control (8–10 mice in each group for each preparation were used; the data are not shown in tables).

The study of the action of preparations at a low dose ($6.5 \times 10^{-2}$ mg/kg a day) on phagocytosis of *S. aureus* by murine peritoneal neutrophils also revealed differences in their effects. The immunoactive mixtures of amino acids presented by levamine and cerebrolysine did not influence phagocytosis independently of the route of application, whereas the immunoactive preparation aviamine enhanced phagocytosis at both routes of introduction. At the same time dipeptides (GluTrp, LysAsp) their constituent amino acid mixtures (TP-5), and separate amino acids that stimulate the immune response (aspartic acid, glutamic acid, tryptophan, and valine) do not influence the immune response (lysine, tyrosine) or even inhibit the (arginine) enhanced phagocytic indexes independently of the mode of application (Table 1).

Phagocytosis-modulating properties of amino acid and peptide preparations assayed in vitro at a dose of $6.5 \times 10^{-2}$ mg/kg were also found in vitro when assayed at a low concentration ($1.3 \times 10^{-3}$ mg/ml, Table 2). At the same time the in vitro ability of amino acid and peptide preparations to protect murine and chicken splenocytes from the toxic action of benzene or aflatoxin B1 in vitro at a low concentration ($1.3 \times 10^{-3}$ mg/ml) does not correlate with the influence of preparations on the immune response and phagocytosis. The antitoxic properties were manifested by (*a*) preparations stimulating the immune response as well as phagocytosis (glutamic and aspartic acids, tryptophan, amino acid mixture aviamine, the separate peptides LysAsp, GluTrp, their constituent amino acid mixtures, and peptide mixtures thymalin, thymosin fraction 5); (*b*) preparations stimulating the immune response without influencing phagocytosis (methionine, levamine, cerebrolysine); (*c*) amino acids influencing neither the immune response, nor phagocytosis (glycine, isoleucine). Preparations that stimulate phagocytosis in vivo and in vitro (lysine, arginine) and influence the immune response ambiguously (lysine does not effect and arginine suppresses the response), are not active as antitoxic agents (Tables 2 and 3).

The ability of amino acid preparations to protect in vitro chicken splenocytes from toxic action of benzene and aflatoxin B1 also persists when assayed at a relatively large concentration (1.3 mg/ml). The treatment of cells in vitro with levamine or cererbrolysine at a prescribed concentration reduces the indexes of cytotoxicity of benzene and aflatoxin B1 respectively from $20.4 \pm 2.0\%$ and $38.5 \pm 2.4\%$ in the control to 0%, $18.4 \pm 2.0\%$, $6.9 \pm 1.3\%$, and $11.0 \pm 1.6\%$ in the experiment, respectively. The treatment of splenocytes in vitro with aviamine (1.3 mg/ml) at a low concentration ($1.3 \times 10^{-3}$ mg/ml, Table 3) protects cells from toxic action of aflatoxin B1 but not of benzene: the indexes of cytotoxicity of benzene and aflatoxin B1 comprise $20.4 \pm 2.0\%$ and $38.5 \pm 2.4\%$ in the control and $18.3 \pm 2.6\%$, and $5.1 \pm 1.1\%$ in the experiment ($p < 0.01$), respectively.

Antitoxic activity of amino acid preparations revealed in vitro holds true in vivo. Thus, feeding of aviamine to chickens for 10 days ($6.5 \times 10^{-2}$ mg/kg a day) enhances chicken splenocyte resistance in vitro to benzene and aflatoxin B1: the index of cytotoxicity reduces from $26.6 \pm 3.1\%$ and $47.7 \pm 3.5\%$ in the control to $11.3 \pm 2.2\%$ and $25.0 \pm 3.0\%$, respectively, in the experiment ($p < 0.01$; the data are not shown in tables).

The comparative analysis of the action on the immune response of low (immunostimulating) and respectively high (trophic) doses of different amino acid preparations at their subcutaneous application in mice revealed

*Table 1*

*The Effect of Amino Acid and Peptide Preparations on the Immune Response and Phagocytosis at Different Routes of Application in Mice* (M ± m)

| Preparation | IgM-PFC per $10^6$ Spleen Cells | | Phagocytic Index, % | |
|---|---|---|---|---|
| | Subcutaneously | Per Os | Subcutaneously | Per Os |
| Levamine | 24.5 ± 3.5** (20) | 24.7 ± 2.0* (10) | 18.2 ± 3.4 (6) | 22.2 ± 1.9 (6) |
| Control[b] | 11.0 ± 1.6 (20) | 13.2 ± 1.4 (8) | 17.3 ± 2.3 (8) | 19.5 ± 1.8 (8) |
| Cerebrolysine | 34.5 ± 3.8* (10) | 20.3 ± 1.4* (10) | 18.0 ± 1.5 (6) | 22.2 ± 1.9 (6) |
| Control | 11.0 ± 1.6 (20) | 13.2 ± 1.4 (8) | 17.3 ± 2.3 (8) | 19.5 ± 1.8 (8) |
| Aviamine | 19.0 ± 2.3** (8) | 27.2 ± 5.5** (8) | 27.9 ± 0.9* (5) | 30.0 ± 1.3** (8) |
| Control | 11.0 ± 1.6 (20) | 14.5 ± 3.5 (8) | 17.5 ± 0.7 (6) | 25.9 ± 0.8 (8) |
| Arginine (Arg) | 6.0 ± 0.7* (20) | 5.5 ± 0.2* (8) | 48.1 ± 1.8* (10) | 43.8 ± 4.3* (6) |
| Control | 10.5 ± 0.6 (20) | 8.6 ± 0.7 (10) | 26.3 ± 1.0 (8) | 20.1 ± 0.6 (8) |
| Aspartic acid (Asp) | 38.4 ± 1.0* (20) | 18.8 ± 1.9* (30) | 31.2 ± 1.9** (10) | 33.4 ± 4.4* (9) |
| Control | 12.0 ± 1.0 (20) | 8.7 ± 0.9 (30) | 26.3 ± 1.0 (8) | 20.1 ± 0.6 (8) |
| Lysine (Lys) | 10.0 ± 1.8 (10) | 10.6 ± 2.2 (20) | 49.1 ± 2.7* (10) | 25.3 ± 1.9** (8) |
| Control | 11.1 ± 1.0 (10) | 9.6 ± 0.9 (20) | 26.3 ± 1.0 (8) | 20.1 ± 0.6 (12) |
| Tyrosine (Tyr) | 12.0 ± 2.0 (12) | 12.9 ± 2.5 (8) | 44.5 ± 1.0* (10) | 34.1 ± 0.8* (6) |
| Control | 11.8 ± 1.0 (12) | 8.6 ± 0.7 (10) | 26.3 ± 1.0 (8) | 20.1 ± 0.6 (8) |
| Valine (Val) | 23.0 ± 2.6* (10) | 17.6 ± 1.3* (10) | 57.6 ± 3.6* (10) | 47.3 ± 0.9* (6) |
| Control | 10.8 ± 0.6 (10) | 8.6 ± 0.7 (10) | 26.3 ± 1.0 (8) | 20.1 ± 0.6 (8) |
| Glutamic acid (Glu) | 19.3 ± 2.8* (10) | 12.7 ± 1.6* (10) | 48.3 ± 3.6* (8) | 40.5 ± 2.5* (8) |
| Control | 11.1 ± 0.8 (20) | 8.6 ± 0.7 (10) | 18.5 ± 2.5 (8) | 18.6 ± 0.8 (8) |
| Tryptophane (Trp) | 18.5 ± 1.4* (10) | 17.5 ± 2.6* (20) | 44.7 ± 1.9* (10) | 38.5 ± 2.3* (8) |
| Control | 12.0 ± 1.0 (10) | 8.6 ± 0.7 (10) | 18.1 ± 2.3 (10) | 20.1 ± 0.6 (6) |
| Glu + Trp | 23.5 ± 1.9* (18) | 19.5 ± 1.6* (12) | 46.0 ± 2.6* (9) | 39.5 ± 2.4* (9) |
| Control | 8.4 ± 0.6 (20) | 8.6 ± 0.7 (10) | 18.5 ± 2.5 (8) | 20.1 ± 0.6 (8) |

*Table 1 Continued*

| Preparation | IgM-PFC per $10^6$ Spleen Cells | | Phagocytic Index, % | |
|---|---|---|---|---|
| | Subcutaneously | Per Os | Subcutaneously | Per Os |
| Thymogen (GluTrp) | 18.8 ± 2.4* (10) | 16.3 ± 3.6* (10) | 31.5 ± 0.4* (8) | NA |
| Control | 8.6 ± 0.8 (12) | 8.8 ± 1.3 (10) | 18.8 ± 0.3 (8) | |
| LysAsp | 18.5 ± 2.5* (11) | 16.7 ± 2.2* (10) | 31.2 ± 0.8* (9) | 33.2 ± 3.1* (9) |
| Control | 8.6 ± 0.8 (12) | 8.8 ± 1.3 (10) | 18.6 ± 2.3 (10) | 19.6 ± 0.8 (9) |
| Lys + Asp | 20.0 ± 1.8* (20) | 18.5 ± 1.6* (12) | 28.1 ± 1.6* (10) | 26.4 ± 1.8* (9) |
| Control | 8.4 ± 0.6 (20) | 8.6 ± 0.7 (10) | 17.3 ± 0.8 (10) | 18.3 ± 2.3 (8) |
| Bursin | 9.5 ± 1.5 (10) | 9.4 ± 1.5 (10) | NA | NA |
| Control | 9.2 ± 1.3 (12) | 9.2 ± 1.3 (10) | | |
| Thymopentin | 20.5 ± 2.4* (11) | 14.7 ± 1.7 (8) | 33.1 ± 4.1* (8) | 31.2 ± 3.3* (8) |
| Control | 8.3 ± 0.6 (12) | 13.2 ± 1.4 (10) | 17.3 ± 2.3 (8) | 19.6 ± 0.8 (8) |
| Thymosin α-1 | 14.1 ± 1.3* (10) | 19.4 ± 3.7 (10) | NA | NA |
| Control | 7.2 ± 0.2 (21) | 13.2 ± 1.4 (10) | | |
| Thymosin fraction 5 | 22.9 ± 1.6* (12) | 15.2 ± 1.7 (10) | NA | NA |
| Control | 11.0 ± 1.6 (12) | 13.2 ± 1.7 (10) | | |
| Thymalin | 15.4 ± 2.5* (10) | 8.7 ± 1.5 (10) | NA | NA |
| Control | 7.9 ± 0.6 (10) | 9.4 ± 1.5 (10) | | |

*$p < 0.01$; ** $p < 0.05$ by Student's $t$-test; in parentheses, the number of animals used for the assays.
[a]Assays were performed for a dose of $6.5 \times 10^{-2}$ mg/kg/day.
[b]Control: sterile pyrogen-free saline 0.5 ml/mouse/day.
NA: not assayed.

the following. The immunostimulating activity of levamine, cerebrolysine, and aviamine varied in the range of acting doses of TP-5 ($6.5 \times 10^{-2}$–$6.5 \times 10^{-4}$ mg/kg) and more. Levamine enhanced PFC production to SRBC 2–2.2 times in the range of doses of $6.5 \times 10^{-2}$–$6.5 \times 10^{-8}$ mg/kg as compared with the control. Cerebrolysine enhanced PFC production 1.6–3.1 times in the range of doses of $6.5 \times 10^{-2}$–$6.5 \times 10^{-8}$ mg/kg. Aviamine raised IgM-PFC production 1.7–3.2 times in a more narrow range of doses $6.5 \times 10^{-2}$–$6.5 \times 10^{-6}$ mg/kg. An increase of levamine dose to $6.5 \times 10^{-1}$ mg/kg caused the enhancement of PFC values (18.4 ± 2.7 as compared with 11.0 ± 1.6 in the control, $p < 0.01$; 12 mice in each group; the data are not shown in tables) but this value was reliably ($p < 0.05$) less than at the injection of levamine at a dose of $6.5 \times 10^{-8}$ mg/kg.

*Table 2*

*The Phagocytosis-Modulating and Antitoxic Activity of Amino Acid and Peptide Preparations for Murine Cells In Vitro* (M ± m)

| Preparation | Phagocytic Index (%) with Preparation | Phagocytic Index (%) with Hanks' Solution (concentration) | Phagocytic Index (%) with Benzene and Preparation | Cytotoxic Index (%) with Benzene in Hanks' Solution (concentration) | Cytotoxic Index (%) with Aflatoxin and Preparation | Cytotoxic Index (%) with Aflaxton in Hanks' Solution (concentration) |
|---|---|---|---|---|---|---|
| Levamine | 17.4 ± 2.2 | 20.6 ± 0.6 | 0* | 21.1 ± 1.4 | 0* | 31.0 ± 2.3 |
| Cerebrolysine | 18.2 ± 2.4 | 20.6 ± 0.6 | 4.3 ± 1.4* | 21.1 ± 1.4 | 21.2 ± 2.9** | 31.0 ± 2.3 |
| Aviamine | 30.3 ± 0.7* | 17.3 ± 0.4 | 12.9 ± 2.4** | 21.4 ± 1.4 | 21.1 ± 2.9** | 31.0 ± 2.3 |
| Arginine | 30.5 ± 2.2* | 18.2 ± 1.2 | 25.3 ± 3.0 | 21.1 ± 1.4 | 27.0 ± 1.2 | 31.0 ± 2.3 |
| Aspartic acid | 27.2 ± 1.6* | 18.2 ± 1.2 | 14.1 ± 1.7** | 21.1 ± 1.4 | 9.1 ± 1.4* | 31.0 ± 3.3 |
| Lysine | 44.7 ± 2.9* | 19.1 ± 0.8 | 21.5 ± 2.0 | 21.1 ± 1.4 | 32.9 ± 3.1 | 31.0 ± 2.3 |
| Tyrosine | 35.1 ± 1.5* | 18.7 ± 0.7 | NA | NA | NA | NA |
| Valine | 41.0 ± 2.3** | 18.7 ± 0.7 | NA | NA | NA | NA |
| Glutamic acid | 45.8 ± 3.6* | 18.2 ± 1.2 | 8.0 ± 1.9* | 21.1 ± 1.4 | 12.0 ± 2.3* | 31.0 ± 2.3 |
| Tryptophan | 48.3 ± 1.4* | 20.7 ± 4.8 | 11.8 ± 2.3* | 21.1 ± 1.4 | 18.8 ± 2.8* | 31.0 ± 2.3 |
| Glycine | 21.3 ± 1.7 | 23.5 ± 1.5 | 0* | 21.1 ± 1.4 | 0* | 31.0 ± 2.3 |
| Methionine | 22.5 ± 2.2 | 22.9 ± 0.1 | 0* | 21.1 ± 1.4 | 7.5 ± 1.3* | 31.0 ± 2.3 |
| Isoleucine | 20.6 ± 1.9 | 18.0 ± 0.7 | 6.7 ± 1.2* | 21.1 ± 1.4 | 8.4 ± 1.3* | 31.0 ± 2.3 |
| GluTrp (thymogen) | 35.2 ± 0.6* | 17.3 ± 0.4 | 0* | 21.1 ± 1.4 | 15.3 ± 2.5** | 31.0 ± 2.3 |
| Glu + Trp | 37.4 ± 2.6* | 17.3 ± 0.4 | 0* | 21.1 ± 1.4 | 15.3 ± 2.5** | 31.0 ± 2.3 |
| LysAsp | 30.7 ± 3.2* | 17.3 ± 0.4 | 15.1 ± 1.3* | 21.4 ± 1.4 | 12.9 ± 2.4* | 31.0 ± 2.3 |
| Lys +Asp | 25.1 ± 1.8* | 17.3 ± 0.4 | 12.9 ± 2.4* | 21.1 ± 1.4 | 15.1 ± 2.5** | 31.0 ± 2.3 |
| Thymopentin | 40.5 ± 2.2* | 21.6 ± 2.1 | NA | NA | NA | NA |
| Thymosin fraction 5 | 22.4 ± 1.7** | 17.3 ± 0.4 | 0* | 21.1 ± 1.4 | 10.8 ± 1.6* | 31.0 ± 2.3 |
| Thymalin | 34.0 ± 3.9** | 17.3 ± 0.4 | 0* | 21.1 ± 1.4 | 6.4 ± 1.2* | 31.0 ± 2.3 |

*$p < 0.01$; **$p < 0.05$ by Student's $t$-test; assays were performed for a concentration of $1.3 \times 10^{-3}$ mg ± ml.
NA: not assayed.

kg. Further increasing of the levamine dose 100 times (to 65 mg/kg) was accompanied by the loss of the immunostimulating activity of the preparation. When the preparation dose of cerebrolysine was increased to 65 mg/kg, it lost the ability to enhance the IgM-PFC number (similar to levamine) (Table 4). Aviamine, unlike levamine and cerebrolysine, inhibited the immune response when assayed at the same dose (65 mg/kg) at analogous conditions (Table 4).

When aviamine was fed to broiler chickens the same regularity was observed: feeding with the preparation at doses of $6.5 \times 10^{-2}$ or $6.5 \times 10^{-4}$ stimulated the immune response (the IgM-PFC number was 62.5 ± 6.2 and 66.0 ± 6.6, respectively, as compared with 27.0 ± 7.7 in the control; $p < 0.01$) and did not change at a dose of 65 mg/kg (the IgM-PFC number comprised 35.0 ± 3.2 in the experiment and 27.0 ± 7.7 in the control; 5 chickens were used in each group; the data are not shown in tables).

The studies of the influence on phagocytosis in vivo of the preparations to be investigated at the subcutaneous injections in mice showed (Table 5) that levamine and cerebrolysine did not reveal any activity in the range of doses of $6.5 \times 10^{-2}$–$6.5 \times 10^{-8}$ mg/kg. Aviamine, like TP-5, enhanced the phagocytic activity of murine neutrophils at a dose of $6.5 \times 10^{-2}$ mg/kg. The increase

***Table 3***

*Antitoxic Activity of Amino Acid and Peptide Preparations for Chicken Splenocytes in Vitro* (M ± m)

| Preparation | Cytotoxic Index (%) of Benzene or Aflatoxin B1 in Benzene with Amino Acid Preparations | Aflatoxin B1 in Benzene |
|---|---|---|
| Levamine | 10.3 ± 2.2** | 23.6 ± 2.8** |
| Cerebrolysine | 3.5 ± 1.3* | 16.5 ± 2.6* |
| Aviamine | 18.3 ± 2.3 | 17.5 ± 2.7* |
| GluTrp | 3.5 ± 1.3* | 13.0 ± 3.0** |
| Glu + Trp | 8.7 ± 2.0* | 15.9 ± 2.6* |
| Glu | 1.6 ± 0.6* | 7.4 ± 1.8* |
| Trp | 3.5 ± 1.3* | 23.0 ± 3.0** |
| Gly | 0* | 11.5 ± 1.6* |
| Ile | 10.6 ± 1.5* | 14.1 ± 1.7* |
| Met | 9.3 ± 1.4* | 24.5 ± 2.1** |
| Control (splenocytes in Hanks' solution) | 19.3 ± 2.3 | 34.3 ± 3.4 |

*$p < 0.01$; **$p < 0.05$ by Student's *t*-test.
Assays were performed for a concentration of $1.3 \times 10^{-3}$ mg/ml.

***Table 4***

*The Effect of Different Doses of Amino Acid Preparations and Thymopentin on the Immune Response to SRBC at the Subcutaneous Injection in Mice* (M ± m)

| Dose (mg/kg/day) | Preparation, IgM-PFC per $10^6$ Spleen Cells | | | |
|---|---|---|---|---|
| | Levamine | Cerebrolysine | Aviamine | Thymopentin |
| 65.0 | 8.4 ± 2.2 (8) | 6.5 ± 1.5** (8) | 18.4 ± 6.4 (8) | NA |
| $6.5 \times 10^{-2}$ | 2.5 ± 3.5* (20) | 34.5 ± 3.8* (10) | 19.0 ± 2.3* (8) | 20.5 ± 2.4* (11) |
| $6.5 \times 10^{-4}$ | 21.2 ± 3.4** (10) | 24.1 ± 2.8* (10) | 35.9 ± 8.7** (8) | 20.7 ± 3.3* (8) |
| $6.5 \times 10^{-6}$ | 22.6 ± 1.2* (10) | 18.0 ± 2.8* (10) | 14.6 ± 2.2 (8) | 6.9 ± 1.2 (12) |
| $6.5 \times 10^{-8}$ | 25.1 ± 1.7* (10) | 13.0 ± 2.0 (10) | 11.4 ± 3.2 (8) | NA |
| $6.5 \times 10^{-10}$ | 12.0 ± 1.4 (10) | NA | 12.5 ± 2.6 (8) | NA |
| Control[a] | 11.0 ± 1.6 (20) | 11.0 ± 1.6 (20) | 11.1 ± 1.0 (8) | 8.3 ± 0.6 (12) |

*$p < 0.01$, **$P < 0.05$ by Student's *t*-test.
In parentheses: number of animals used for IgM-PFC determination.
[a]Sterile pyrogen-free saline, 0.5 ml/mouse/day.
NA: not assayed.

of a dose to 65 mg/kg that assisted in the loss of the immunostimulating activity of levamine and cerebrolysine and in inhibiting this ability of aviamine (Table 4), influenced the phagocytic activity of neutrophils in a diverse way: levamine and cerebrolysine gained the capacity to stimulate phagocytosis but the phagocytosis-stimulating activity of aviamine did not change (Table 5). Aviamine ($6.5 \times 10^{-2}$ mg/kg) revealed the phagocytosis-stimulating activity when the preparation was fed to chickens (the phagocytic index was $33.9 \pm 2.2$ in the experiment and $21.6 \pm 1.81$ in the control; $p < 0.05$; 5 chickens were used in each group; the data are not shown in tables). Aviamine maintained the phagocytosis-stimulating activity at a dose of 65 mg/kg also when the preparation was fed to mice and chickens: the phagocytic index was respectively $31.2 \pm 0.8$ and $33.9 \pm 2.2$, as compared with $25.9 \pm 0.8$ and $21.6 \pm 1.8$ in the control ($p < 0.01$; 7–8 mice and 5 chickens were used in each group; the data are not shown in tables).

None of the preparations, when assayed at different concentrations and at different routes of application, changed the phagocytic number in mice or in chickens, which was $1.7 \pm 0.06$–$1.9 \pm 0.08$ as compared with $1.7 \pm 0.05$–$2.0 \pm 0.09$ in the control (the data are not shown in tables).

To elucidate the mechanism of the many diverse effects of preparations used at low and high doses on the immune response, we have studied the influence of different concentrations of amino acid preparations on their ability to express Thy-1 antigen on murine bone marrow T-cell precursors. The in vitro treatment of the bone marrow cells with levamine, cerebrolysine, or aviamine in the concentration range of $1.3 \times 10^{-3}$–$1.3 \times 10^{-9}$ mg/ml stimulated the Thy-1 antigen expression on the cell membranes. The increase of the Thy-$1^+$ cell number varied from $16.6 \pm 0.8\%$ to $20.7 \pm 2.8\%$ as compared with 0% in the control (cells in Hanks' solution; the data are not shown in tables). The ability of preparations to express Thy-1 antigen on T-cell precursors disappeared as the concentration of preparations increased to 1.3 mg/ml (corresponding to a dose of 65 mg/kg for the in vivo assays of preparations).

The vitral phenomenon of the effect of low and high concentrations of amino acid preparations on T-cell differentiation was confirmed in vivo when aviamine was fed to mice or chickens at doses of $6.5 \times 10^{-2}$ and 65 mg/kg. At a dose of $6.5 \times 10^{-2}$ mg/kg the preparation furthered the production of Thy-$1^+$ cells in the bone marrow of mice or chickens ($14.3 \pm 1.6\%$ and $13.9 \pm 1.7\%$, respectively, as compared with 0% in mice and $1.2 \pm 0.5\%$ in chickens in the control; $p < 0.01$), and did not reveal any activity at a dose of 65 mg/kg (the number of Thy-$1^+$ cells in mice and chickens does not differ in the experiment and in the control and comprised 0% and 0% in mice and $2.3 \pm 0.7\%$ and $1.2 \pm 0.5\%$ in chickens, respectively (8–9 mice and 5 chickens were used in each group; the data are not shown in tables).

***Table 5***

*The Effect of Different Doses of Amino Acid Preparations and Thymopentin on Phagocytosis at the Subcutaneous Injection in Mice* (M ± m)

| Dose (mg/kg/day) | Preparation | | | |
|---|---|---|---|---|
| | Levamine | Cerebrolysine | Aviamine | Thymopentin |
| 65.0 | 24.0 ± 0.4* (5) | 25.0 ± 0.6* (6) | 24.6 ± 0.4* (5) | NA |
| $6.5 \times 10^{-2}$ | 18.2 ± 3.4 (6) | 18.0 ± 1.5 (5) | 27.9 ± 0.9* (5) | 33.1 ± 4.1* (8) |
| $6.5 \times 10^{-4}$ | 19.6 ± 0.8 (6) | 20.3 ± 1.4 (5) | 14.8 ± 1.0 (4) | NA |
| $6.5 \times 10^{-6}$ | 20.6 ± 1.7 (5) | 20.2 ± 1.9 (5) | 18.3 ± 0.2 (5) | NA |
| Control[a] | 17.5 ± 0.7 (6) | 17.5 ± 0.7 (6) | 17.5 ± 0.7 (6) | 17.3 ± 2.3 (8) |

*$p < 0.01$, by Student's $t$-test.
In parentheses: number of animals used for the assay.
[a]Pyrogen-free sterile saline, 0.5 ml/mouse/day.
NA: not assayed.

## DISCUSSION

The presented data indicate that amino acids provide necessary protective functions of an organism. The results obtained confirm and widen literary data about the role of amino acids in providing the vitally important biological processes in organisms (such as nitrogenous balance and protein synthesis). The presence of eight amino acids (Lys, Val, Thr, Met, Trp, Leu, Ile, and Phe) is necessary according to literature (28) for nitrogenous balance maintenance. A few more (14) amino acids are necessary for protein synthesis: Cys, Gln, Arg, His, Tyr, and Ser in addition to those mentioned above (29). The proliferation of PHA-stimulated lymphocytes requires 12 amino acids: those enumerated above, without Phe and Ser (30). According to Chuang and co-workers (30), Asp, Glu, and Asn (which are the most immunoactive amino acids according to our data) are not at all necessary for the realization of the cell proliferative response to PHA. The literature and our own data show that amino acids take part in the processes of nitrogenous balance maintenance, lymphocyte proliferation, and immune response in different shares. The fact that the most immunoactive amino acids (Asp, Asn, and Glu) are not necessary for nitrogenous balance maintenance or for the lymphoproliferation emphasizes the difference between the mechanisms of these processes. The data presented in this paper show that peptide mixtures (thymosin fraction 5, thymalin) as well as separate dipeptides (GluTrp, LysAsp) when assayed at low doses and concentrations ($6.5 \times 10^{-2}$ mg/kg or $1.3 \times 10^{-3}$ mg/ml), reveal the combined effect on the indexes of specific and nonspecific resistance: they enhance antibody production, phagocytosis, and antitoxic action. The combination of effects of dipeptides is determined by the combination of amino acid influence of one amino acid (Asp) in the peptide LysAsp or of both amino acids (Glu and Trp) in the peptide GluTrp. The combination of influences of peptides with more amino acid residues on the indexes of specific and nonspecific resistance likely depends not only on the presence of amino acids acting in a combined mode in a peptide, but is determined by special features of this peptide spatial arrangement. Thus, pentagastrin and cholecystokinin that contain amino acids of the combined action, stimulate the immune response (22,31), but do not influence the phagocytic indexes (31); neurotensin, conversely, enhances phagocytosis (31), but does not influence the immune response (13,31), in spite of the fact that neurotensin structure contains amino acids with combined effects.

The comparative analysis of the influence of low and relatively high (trophic) doses of different amino acid preparations on the immune response to SRBC reveals the inverse dependence between trophic and immunostimulating activities. Thus, levamine is highly active as immunostimulator in the range of doses of $65 \times 10^{-2}$–$6.5 \times 10^{-8}$ mg/kg/day. The increase of a dose to $6.5 \times 10^{-1}$ mg/kg results not in the enhancement but in the reliable decrease of the PF number 1.2–1.3 times as compared with the application of the minor doses ($6.5 \times 10^{-2}$–$6.5 \times 10^{-8}$ mg/kg). Further increase of a dose of levamine 100 times (to 65 mg/kg) elicits not the increase but the marked decline in the immunostimulating activity of levamine: PFC number is 2.5–3.0 times lower as compared with the above-mentioned minor doses. Cerebrolysine and aviamine demonstrate the analogous properties; moreover, aviamine inhibits reliably the immune response at a dose of 65 mg/kg. The inverse dependence of the trophic and immunostimulating activities is characteristic not only for amino acid mixtures but also for separate amino acids, for example, for aspartic acid (12).

The immunostimulating effects of low doses of levamine, cerebrolysine, and aviamine as well as the effects of low doses of separate amino acids (Asp, Glu, Ser, Thr, Val), of thymic (thymalin, T-activin, thymosin fraction 5, TP-5), and non-thymic gastrointestinal (pentagastrin, cholecystokinin) or brain (cortexin) peptides (11,15,31–34) are connected with the functioning of T-, but not B-cells, since all these preparations induce the Thy-1 antigen expression on the T-precursor membranes and enhance the thymus-dependent immune response without influencing the thymus-independent response. The inability of amino acid preparations to increase the immune response at the trophic dose (65 mg/kg) might be explained, as it was shown in our work, by the loss of their ability to express Thy-1 antigen on T-precursors when assayed at a relatively high concentration (1.3 mg/ml).

Unlike the effects on the immune response, amino acid preparations do not diminish, but on the contrary enhance the phagocytic activity of murine neutrophils and chicken pseudo-eosinophils as the doses or concentrations increase. Thus, the large dose of levamine, cerebrolysine, and aviamine which causes the loss of immunostimulating activity of these preparations, stimulates phagocytosis. The decrease of a dose 1000 times (to $6.5 \times 10^{-2}$ mg/kg) leads to the disappearance of phagocytosis-stimulating activity of levamine and cerebrolysine, and a further decrease in dose makes all preparations inactive. Thus, the effect of the large doses

of amino acids preparations on phagocytes, unlike their influence on the immune response, appears to be defined by the philogenetically determined trophic properties of granulocytes. At the same time it is noteworthy that low doses of levamine and cerebrolysine do not influence phagocytosis. These facts are all the more important because these amino acid mixtures contain predominantly those amino acids which stimulate phagocytosis at low doses ($6.5 \times 10^{-2}$–$6.5 \times 10^{-10}$ mg/kg): alanine, arginine, asparitic acid, asparagine, cysteine, isoleucine, lysine, proline, serine, threonine, tryptophan, tyrosine, and valine (15). It should be noted that the effects of amino acids and of aviamine are very similar. The similarity might be explained by the higher content of immuno- and phagocytosis-stimulating amino acids in aviamine than in levamine (52% and 40%, respectively). Another reason for the observed phenomenon might be that aviamine contains the most immunoactive (11,13) glutamic and aspartic acids (near 24% of total amino acid quantity), which are absent in levamine. It is also important that levamine and cerebrolysine, as well as aviamine, normalize the index of phagocytosis completeness impaired under the benzene influence (Popova, Belokrylov, and Derevnina, unpublished results). TP-5, thymosin α-1, thymosin fraction 5, and thymalin increase the immune response in subcutaneous but not peroral application. At the same time TP-5 is able to enhance the phagocytic activity of granulocytes at both routes of introduction. The manifestation of the peptides' immunomodulating properties exclusively during parenteral use is true only for the peptides of a definite length. Thus, dipeptides which are known to penetrate the gastrointestinal tract (in particular, the peptide thymogen, GluTrp) unlike TP-5, thymosin α-1, thymosin fraction-5, or thymalin, are active both at the parental (subcutaneous) and at the oral application. Bursin (LysHisGly-amide), the molecule of which includes three amino acids residues only, proves to be immunoinert at subcutaneous and at peroral introduction. This fact might be explained in the following way: all three amino acids of bursin (Lys, His, and Gly) are immunoinactive (13); those peptides which consist of the immunoinactive amino acids only are not able to influence the immune response (13,15). Moreover, Lys and His are not necessary for the realization of the immune response according to Jose and Robert (35). Hence the ability of peptides to increase the immune response at per os application depends not only upon the length of the peptide chain, but also upon the amino acid composition, whereas the phagocytosis-stimulating activity of peptides is not always connected with their ability to penetrate the gastrointestinal tract as, for example, in the case of TP-5.

As the data presented in this paper show, not only peptides and their constituent amino acids (15), but amino acid mixtures also influence the immune response and phagocytosis in a different manner. This evidence supports the previous suggestion (15) about the relative independence of the specific and nonspecific forms of the organism protection. The phenomenon of the diversity in the effects of amino acids taken separately and of amino acid preparations on the immune response and phagocytosis at low and high doses shows that variations in bioactive compound concentrations may be those of homeostasis relating mechanisms. This phenomenon exhibits the general biological character. The effect also shows up in mammals (mice) and birds (chickens). It is important to emphasize that composition, doses, and routes of preparation introduction play the essential role in the realization of the immuno-effects of peptides, separate amino acids, and their mixtures; the directed action of immuno-correcting drugs might be achieved by varying these factors.

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