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Mixtures of Aminoacids in Human Nutrition

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The use of mixtures of individual aminoacids instead of proteins in human nutrition has been the subject of numerous recent investigations. The aim was not only to investigate the process of aminoacid assimilation itself but also to elucidate the question of their possible long term use in food. The large scale and prolonged employment of aminoacid mixtures in therapeutic feeding shows that they constitute an adequate replacement of food proteins and their consumption does not apparently entail any undesirable consequences. Various aspects of the use of aminoacids to improve the nutritional value of second-class proteins by adjusting their [aminoacid] balance are also discussed. However, before reaching final conclusions concerning the ways of utilising aminoacids in nutrition, more prolonged and comprehensive studies are necessary. The review deals with work carried out in the last 10 years.
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I. INTRODUCTION

A considerable proportion of the earth's population, particularly in certain countries in Asia, Africa, and Latin America, suffers nowadays from a chronic deficiency of foodstuffs, mainly protein, which leads to high child mortality both from the malnutrition itself and from the reduced resistance of the organism to infectious diseases and possible genetic damage caused by malnutrition. Owing to the high rate of increase of the populations of these countries, the situation is continually deteriorating[1].

The intensification of agriculture, the use of protein products previously not employed in food, and the tapping of the resources in the ocean are the most obvious ways of solving this problem. These ways are based on an extensive agriculture, i.e. harvesting the crop from larger areas. In addition they inevitably interfere with the earth's ecological system[2].

A new and promising method of obtaining foodstuffs is chemical synthesis. Where protein is concerned, this involves the synthesis of aminoacids and their mixtures and if necessary of polymers from inorganic non-food raw materials[3,4]. Apart from other considerations, this procedure has the advantage that it gives a high yield of production per unit productive area.

However, in an examination of the possible utilisation in food of such products, a number of problems concerning the physiology and biochemistry of nutrition arise. Naturally, before introducing into human nutrition any kind of synthetic diet, one must develop compositions of the latter such that the normal functioning of the human organism, accustomed throughout evolution to food of plant and animal origin, is ensured. This is an exceptionally complex problem.

Body-tissue material and energy are introduced into the organism with food. Although the adaptive capacity of the organism to various sources of food is high, it is nevertheless limited. Therefore excessive abuse of this capacity can eventually lead to the breakdown of the functions of the regulating mechanisms in digestion, which is revealed in a wide variety of diseases associated with different types of food deficiency and when the proper proportions of the nutrient substances in the diet are not maintained. Owing to its internal reserves, the organism itself can correct many errors in the rations, but it does appear to be more satisfactory to extend our knowledge in the field of nutrition in order to provide the organism with its essential requirements to the maximum possible extent. The nature of food to some extent induces the secretion of digestive enzymes—their activity and amount.

The requirement for essential individual food components, including aminoacids, is due to the fact that the organism lacks enzyme systems which can synthesise them at a sufficient rate from non-specific metabolic intermediates. Therefore a thorough investigation of the effect of essential nutrient factors as such on the organism as a whole is necessary before they are introduced into human diet.

The aim of the present review is to examine work concerning the use of synthetic or more precisely monomolecular nutrient substances, primarily aminoacids, in human nutrition.

II. NITROGEN BALANCE AND BIOLOGICAL VALUE OF PROTEINS, AMINOACIDS, AND THEIR MIXTURES

On entering the human organism, protein is fully hydrolysed in the digestive tract to aminoacids, which pass into the blood only in this form and are supplied to all the tissues and body organs where protein is resynthesised. There are data showing that proteins introduced parenterally also break down into aminoacids before being utilised by the organism[5].

Table 1. Soluble diets for the feeding of human patients *per os*.

Substance	Diet according to Winitz*	Diet of Schwartz Bioresearch Inc.
Carbohydrates	(g)	(g litre^{-1})
Glucose	550.0	418.1
Glucono-δ-lactone	17.2	–
Gluconoaldehyde	–	12.8
Aminoacids		
Lysine.HCl	3.58	2.64
Leucine	2.83	2.82
Isoleucine	2.42	1.78
Valine	2.67	1.96
Phenylalanine	1.75	1.29
Methionine	1.75	1.29
Threonine	2.42	1.78
Tryptophan	0.75	0.55
Alanine	2.58	1.90
Arginine.HCl	2.58	1.90
Histidine.HCl.H_2O	1.58	1.16
Glycine	1.67	1.23
Glutamine	9.07	5.0
Na aspartate	6.40	4.05
Na glutamate	–	3.83
Proline	10.33	7.58
Serine	5.33	3.92
Tyrosine ethyl ester.HCl	6.83	5.03
Cysteine ethyl ester.HCl	0.92	–
Water-soluble vitamins	(mg)	(mg litre^{-1})
Ascorbic acid	62.50	46.0
(+)-Biotin	0.83	0.58
B_{12}	1.67	1.25
Inositol	0.83	0.59
(+)-Aminobenzoic acid	416.56	307.1
Thiamin.HCl	1.00	0.74
Nicotinamide	10.00	7.36
Pyridoxine.HCl	1.67	1.25
Ca (+)-pantothenate	8.33	6.11
Folic acid	1.67	1.25
Choline hydrogen tartrate	213.25	–
Choline chloride	–	92
Riboflavin	1.50	1.10
Fat-soluble vitamins	(mg)	(mg litre^{-1})
Vitamin A acetate	3.64	5.0
Vitamin D	0.057	–
α-Tocopheryl acetate	57.29	25.0
Calciferol 40 ME/MG	–	3.5
Menadione	4.58	2.1
Polysorbate 80	–	3.0
Ethyl linoleate	200	260
Salts and microelements	(mg)	(mg litre^{-1})
Manganese acetate.$4H_2O$	18.30	13.48
Copper acetate.H_2O	2.50	1.84
Cobalt acetate.$4H_2O$	1.67	1.23
Ammonium molybdate.$4H_2O$	0.42	0.31
Zinc benzoate	2.82	1.49
Zinc acetate	–	1.49
Potassium iodide	0.25	0.18
	(g)	
Potassium hydroxide	3.97	–
Magnesium oxide	0.38	–
Sodium chloride	4.77	–
Na glycerophosphate	5.23	–
Iron(II) gluconate	0.83	–
$CaCl_2.2H_2O$	2.44	–
Sodium benzoate	1.00	–

* All the dry substances were dissolved in distilled water to give a concentration of 50–70%.

Therefore many investigators decided to test directly the effect of introducing into the organism various amino-acid compositions instead of proteins. In experiments on rats, Greenstein et al.[6] showed that diets comprising chemically pure monomers can support growth, reproduction, and life of the experimental animals throughout several generations. Subsequently Winitz et al.[7] demonstrated the possibility of using such diets also to support normal life in healthy man. During a 19-week experimental period 24 male volunteers aged between 24 and 39 were kept on a synthetic diet, whose composition is presented in Table 1.

Physiological observations showed that they all became accustomed to such diets; throughout the experimental period, a positive nitrogen balance was maintained in the majority of the individuals tested.

It is believed that the subject is in nitrogen balance when the amount of nitrogen received with food (I) is equal to the amount eliminated from the organism in urine (U), faeces (F) and via the skin (S):

$$I = U + F + S\,.$$

If I exceeds the sum $U + F + S$, a positive nitrogen balance is obtained, otherwise the balance is negative.

Thus the nitrogen balance is the fundamental criterion in the estimation of the protein state of the organism. If the organism is maintained for a long time in a negative nitrogen balance, its protein reserves are exhausted with an eventual lethal outcome.

In order to maintain a nitrogen equilibrium, different amounts of different proteins are required. If the amount of a protein required to maintain equilibrium is small, the protein is said to have a high biological value. In general the nutrient value of protein is determined by two different factors: (1) digestibility and (2) biological value[8].

Digestibility is determined by the ratio of the absorbed protein elements to the amount introduced. It depends on the exocellular and endocellular proteolythic activity of the intestine and the capacity of all the intestines for absorption. Digestibility is related to the necessity for the simultaneous introduction of all essential aminoacids into the reaction medium during synthesis. The biological value (BV) indicates the ratio of the protein elements absorbed and retained by the organism.

According to the classification adopted by FAO/WHO [9],

$$\mathrm{BV} = \frac{\text{retained nitrogen}}{\text{absorbed nitrogen}}$$

The biological value depends both on the aminoacid composition of the protein and on the digestion and transport of the nutrient substances. In terms of nutrient value, the proteins of mother's milk (90–97%), eggs, cow's milk, cheese, meat, fish (80–90%), and yeast (77%) come first, are followed by grain proteins, and finally by wheat flour (54%).

The studies on the effects of individual aminoacids in proteins on the biological value of the latter begun as early as the beginning of the 20th century by Kellner, Willsk [Wills ?], Hopkins, Abderhalden, Osborn, Mendel, and others, were completed by the systematic work of Rose, who, as it were, summed up the studies concerning the elucidation of the role of protein in nutrition. The nutritive value of protein is closely related to its composition, namely to the content and relative proportions of essential aminoacids. Rose was the first investigator who studied systematically the effect of individual amino-acids on the nitrogen balance. The nitrogen balance technique, which he was the first to employ, still remains the foundation of all metabolic experiments involving human nutrition by aminoacid mixtures[10].

Hegsted[11] quotes different versions of the human requirement for aminoacids (Table 2).

The norms in aminoacid requirement were proposed by the FAO committee in 1957 and were then revised by FAO/WHO experts in 1965.[9] The same organisations put forward as a criterion of protein nutrition the ratio of essential aminoacids (E) to the total amount of nitrogen in a particular protein (T). The E/T ratio is expressed in milligrammes of essential aminoacids per gramme of total nitrogen. Proteins with high, medium, and low nutrient values have E/T = 3, 2, and below 2.

Table 2. Average aminoacid requirements of adult humans.

Aminoacid	For women (according to D.M.Hegsted)		Published data (according to W.C.Rose)		
	mg/day		for women	for men	
			mg/day		
	amount of aminoacids required to maintain a zero nitrogen balance	amount of aminoacids required to maintain an aminoacid balance of +0.5 g/day		minimum	optimum
Tryptophan	168	270	157	250	1200
Phenylalanine in the presence of tyrosine	258	550	220	300	—
Threonine	375	1750	305	500	3600
Isoleucine	550	1700	450	700	3000
Lysine	545	1750	500	800	6000
Methionine (+ 500 mg of cystine)	194	375	180	200	—
Methionine (+ 10 ml of cystine)	700	3450	550	1100	—
Valine	622	1000	650	800	4200
Leucine	727	2600	620	1100	5400

According to Rose, the requirement for protein reduces to 2 sources of nitrogen: (1) essential aminoacids which the organisms cannot synthesise and (2) non-specific additional nitrogen for the synthesis of other aminoacids and nitrogen-containing compounds. The latter can consist either of a single aminoacid or a mixture of non-essential and essential aminoacids. It can be provided also in another form, for example in the form of urea, diammonium citrate, etc. Thus animal and human organisms require both essential and non-essential nitrogen.

Comparing the biological value of natural proteins and the equivalent aminoacid mixtures imitating their compositions both qualitatively and quantitatively, some investigators suggested that the effectiveness of such mixtures is lower than that of protein[12-21]. This conclusion was made largely on the basis of the decrease of the nitrogen balance, loss of appetite, the shortening of the feeding period, and the arrest of growth in young animals. There arises the valid question to what extent one can rely on conclusions based on experiments on laboratory animals with the aim of transferring them to human beings.

In an early paper Hegsted[22] noted that there is a close relation between the aminoacid requirements of man and animals, since the differences in their body structures are not all that great. The rat, being a more rapidly growing animal than man, has a higher overall requirement for nutrient substances. Moreover, the rat's requirement for methionine is higher, due to the growth of its hair covering. It also has a higher requirement for lysine (lysine is known to be required more for growth than for the maintenance of the nitrogen balance). However, the assertion that aminoacid mixtures tested on animals are less effective cannot be regarded as fully reliable, since there are many studies with the opposite findings[23-32].

Guggenheim and Szmelcman[33] actually demonstrated a higher effectiveness of the mixture compared with protein. When rats were maintained on a diet consisting solely of an aminoacid mixture, they gained more in weight and had a higher protein content in their serum and also more nitrogen in RNA and in the liver than the animals receiving the same amount of protein as casein. Sauberlich[32] suggests that correctly balanced aminoacid mixtures can fully replace proteins in diet without an arrest of growth.

III. CAUSES RESPONSIBLE FOR DIFFERENT MODES OF UTILISATION OF PROTEINS AND AMINOACID MIXTURES

These conflicting data led to a number of hypotheses explaining the possible cause of the different ways of utilisation of proteins and aminoacid mixtures. Thus it has been suggested that the rates of absorption of monomeric and polymeric substances are different. Rose and Clark believe that monomers are absorbed faster. However, the studies in recent years showed that the hydrolysis products of food proteins are absorbed at the same rate as the equivalent monomer solutions[34]. Absorption then takes place at an equal and constant rate, is rigorously coordinated, and is of a competitive type[35].

The idea of the possible utilisation of the aminoacids from mixtures by the bacterial flora of the intestine has also been put forward. Abderhalden suggested that the period during which aminoacids derived from proteins remain in the intestine is much shorter than when the free mixtures are introduced. Therefore they are "captured" by bacteria and are at the same time converted into toxic substances. Abderhalden failed to take into account the fact that the diameter of the pores in the filaments of the so called "brush border" of intestinal epithelium, where the concluding stage of the formation of free aminoacids and hexoses takes place, is too small (of the order of 100–200 Å) to allow penetration by bacteria. Therefore aminoacids from proteins cannot be available to bacteria. However, when a complete mixture of monomers is provided, they must be inevitably supplied to bacteria. This view is supported by Ugolev[36]. The reason for undesirable effects in this case may be the multiplication of bacteria, the entry of ammonia and toxic amines into the bloodstream, and the breakdown of dietary balance. Fears were voiced that the gastric intestinal tract may degenerate owing to the reduced functional requirements.

Another consequence of the low molecular weight of the aminoacid may be the so called "osmotic effects" expressed in digestive disorders, vomiting, diarrhoea, etc.[37,38]

Shishova-Kasatochkina et al.[21] suggest that the growth factors, vitamin B_{12}, vitamin A_1, streptogenins, etc. contained in natural proteins, ensure a higher nutrient effectiveness of protein. Moreover, it may be that certain protein-like bonds, providing the synthetic processes in the organisms with additional energy evolved on their dissociation, are absent from the mixtures. This results in a higher calorie requirement in the aminoacid diet compared with the protein diet. In the same study the authors demonstrated on sterile animals that the intestinal microflora does not constitute an obstacle to the utilisation of aminoacid mixtures.

Other workers[39] believe that protein may have an effect on the production of enzymes in the small intestine

and also may act as an agent preventing the inactivation of these enzymes.

A number of investigators relate the effectiveness of the utilisation of mixtures to the intake of the total energy, total nitrogen, and its quality. It was established long ago that, when an aminoacid mixture is ingested by man, it is necessary to introduce more energy into the organism than in the ingestion of casein[40]. In later studies on rats this observation was not confirmed[41]. However, six years later, Akrens et al.[39] undertook a study of the same effect. They carried out a comparative feeding of two groups of rats of different ages with casein and an aminoacid mixture imitating it. For both old and young animals, there was no difference in the gain of weight as a function of the source of nitrogen (from the protein or from the aminoacid mixture) at a low-calorie level of intake. On the other hand, when the rats received a high-calorie diet, the gain in weight was greater with the casein feed.

Kies and Linkswiler[42] showed on man a significant relation between the requirement for a specific amount of essential aminoacids and the level of calorie intake. Thus with increase of the daily intake from 35 to 55 kcal per kilogramme body weight, the fraction of 8 essential aminoacids required to maintain a positive nitrogen balance was reduced.

Swendseid[43] examined the problem of the effect of the total amount of dietary nitrogen on the requirement for essential aminoacids. A number of studies on 60-year-old men showed that their aminoacid requirement increased with the amount of nitrogen in the diet. On the other hand, this type of relation was not observed in experiments on young men and girls. Swendseid notes that this problem is of interest in connection with the establishment of a relation between the total nitrogen, essential aminoacids, and the requirements for them associated with age.

Young and Zamora[44] showed that the level of nitrogen in the diet has little effect on growth when the intake of essential aminoacids is low; for relatively high intakes, in excess of 10% of the total diet, growth is better maintained for higher total nitrogen levels in the diet. The positive results of the introduction into the aminoacid mixtures of an additional amount of arginine, which reduces the toxicity of the ammonia produced on feeding aminoacid mixtures, were noted. Ranhotra and Johnson[30] found that, when newly born rats are fed with a pure aminoacid mixture equivalent to protein, an approximately similar gain in weight is achieved as on feeding with a protein diet. This gain was obtained as a result of an increase in the amount of dietary arginine compared with that employed previously. The optimum requirement for arginine hydrochloride is apparently 0.5% of the total weight of the diet.

Hepburn and Bradley[31] emphasised the protective role of glutamine in aminoacid diets. It also has a detoxicating effect.

The best effect as regards the increase in weight of growing animals was produced by the following mixture of essential aminoacids (expressed in percentages of the dry rations, all in the L-form): histidine 0.25, lysine 0.90, tryptophan 0.11, isoleucine 0.55, valine 0.55, leucine 0.70, threonine 0.50, methionine 0.16, cystine 0.34, phenylalanine 0.42, and tyrosine 0.30.[45]

Despite the proven suitability of aminoacid mixtures in the feeding of experimental animals, the compositions tested directly on human beings are nevertheless of particular interest.

Examination of the literature shows that, in all cases where the suitability of aminoacid mixtures in nutrition was investigated by direct experiments, there was no doubt about the equivalence of aminoacids mixtures and protein.

IV. DETERMINATION OF THE REQUIREMENTS OF THE ORGANISM FOR AMINOACIDS

As already mentioned above, the principal technique adopted to discover the requirement for a particular aminoacid is the nitrogen balance method. In addition, many investigators, in particular Almquist, believe that there is a close relation between the plasma aminoacids and the protein level in the diet. This might in turn serve as a direct method for the determination of the aminoacid requirement.

Exact methods for the quantitative determination of aminoacids (ion-exchange and gas chromatography, sensitive aminoacid analysers, etc.) make it possible to compare the aminoacid composition of the plasma with that of the aminoacid mixtures or protein employed. Morrison et al.[46] established a relation between lysine-deficient diets and the level of lysine in the plasma. Analogous investigations of the plasma and dietary levels of lysine, arginine, and valine were reported by Zimmerman and Scott[47].

McLaughlan and coworkers[48-53] have been concerned with the same problem for many years. They demonstrated an almost linear relation between the amount of free plasma aminoacids and their level in the diet. The same investigators concluded that the method used to detect plasma aminoacids can be applied to determine the human aminoacid requirement.

However, later the same workers[54] established that the method used to determine the blood aminoacids is suitable for the determination of the limiting aminoacids in the diet only when there is a deficiency of one or two limiting aminoacids but is virtually unsuitable for the prediction of a multiple aminoacid deficiency in the diet.

Interesting data in this respect were presented by Swendseid in her report delivered in the summer of 1968 in the Institute of Organic Derivatives of the Elements of the USSR Academy of Sciences. The experiments were performed both on young people—college students (men and women)—and middle-aged people. Measurements were made of the plasma aminoacid level in the post-absorptive period in individuals receiving a series of controlled diets with different amounts of total nitrogen. With 7 g of nitrogen (i.e. 45 g of protein) in their diet, both age groups showed the same average plasma aminoacid level and also equal proportions of essential aminoacids. However, with 3.5 g of nitrogen in the diet, the amount of essential plasma aminoacids gradually fell during the 55 days of the test period. In this case, as the fraction of essential aminoacids (E) decreased, that of non-essential aminoacids (N) rose to such an extent that the total amount of aminoacids in blood plasma remained the same as before or slightly higher. The E/N ratio fell.

On the basis of these experiments, the author concludes that the decrease of the E/N ratio in plasma is associated with prolonged feeding on low-protein diets.

Other workers[55] also showed (on 6 women) that a decrease of the E/N ratio in the plasma to 0.3 and below is evidence of inadequate protein intake. In general, the E/N ratio can serve as an indicator of the protein nutritional status of man[57]. Czech biologists[56] proposed a simplified method for the determination of the E/N ratio in blood plasma (the so called Whitehead test) in healthy and sick people.

The experiments of Nakagawa and Masana[58] on healthy young people showed definite changes in the aminoacid composition of plasma due to an increase in the amount of free aminoacids associated with a deficiency of protein or free aminoacids in the diet. This phenomenon may serve as a test for a subclinical nitrogen deficiency, indicating an enhanced decomposition of tissue proteins.

On the other hand, a specific deficiency of a particular essential aminoacid was not determined. Swendseid investigated this problem too. Measurements were made of the amount of plasma aminoacids after the intake of a diet with a deficiency of any one aminoacid. It was found that, when the amount of lysine in the diet was reduced to about 50% of the requirement, the lysine level in the plasma remained unchanged. Even in its complete absence (less than 100 mg in the total diet), there was no change either in the amount of plasma lysine or in the E/N ratio.

Analogous results were obtained for phenylalanine and tyrosine[59]. Valine deficiency was investigated on two subjects. In one a decrease in plasma valine after the intake of 60% of the level of control valine was observed after 5 days, while in the other the same decrease was noted towards the end of the second day of the experiment. From this, one may conclude that the dietary deficiency of some aminoacids has a more rapid effect on their level in the plasma than for others, which is probably associated with the different metabolic pathways of these aminoacids.

When Rose tested on human beings various aminoacid mixtures, he noted that the deficiency of certain aminoacids did not have an appreciable effect, while the deficiency of others caused vomiting, loss of appetite, irritability, etc.

It is interesting to elucidate whether the deficiency of dietary aminoacids causing these symptoms in man is linked to a change in their plasma level. Then, by controlling the level of aminoacids in plasma, it might be possible to eliminate these undesirable phenomena. A considerable influence of the correct ratio of essential and non-essential aminoacids (E/N) in the diet on the aminoacid composition of the plasma and the growth of animals has been noted.

Young and Zamora[44] found that the optimum E/N ratio in the diet is in the range 1.0–2.66. Harper and Stucki[70] believe that a range between 1 and 4 is possible.

In their extensive review dealing with the role of essential aminoacids in nutrition, Swaminathan and Daniel[61] quote numerous data in favour of the ratio E/N = 1, i.e. the same as that found in chicken egg protein. This E/N ratio is preferable in the diet of children and growing animals, while in adults the nitrogen balance is maintained with E/N = 2:1.

It has been established[62] that, when the total nitrogen content in food is higher, maximum growth is attained with a smaller fraction of essential aminoacids. Thus by increasing the total nitrogen level with the aid of cheap non-specific nitrogen sources, it is possible to achieve the "preservation" of essential nitrogen, i.e. essential aminoacids.

V. THE ROLE OF NON-SPECIFIC NITROGEN AND ITS FORMS

The relatively low requirement by man for essential nitrogen permits the "dilution" of first-class protein by cheap forms of non-essential nitrogen. Kremer[63] emphasises the dual function of non-essential nitrogen in the organism: (1) the provision of material necessary for the synthesis of nitrogen compounds; (2) the prevention of the use of essential nitrogen for these purposes.

The question arises of the form in which non-essential nitrogen is most fully utilised by the organism. It has been found that it can be provided either as a mixture of all the non-essential aminoacids which enter into the composition of a particular natural protein, or as various ammonium and urea salts, or as a single aminoacid, etc.

Watts et al.[64] investigated the relation between the retention of nitrogen, the excretion of ammonia, and the source of non-essential nitrogen. The retention was best on feeding with a mixture of 8 non-essential aminoacids: alanine, arginine, glycine, histidine, proline, serine, and aspartic and glutamic acids, in proportions corresponding to those in beef. Large losses of nitrogen with urine were observed during the intake of proline, histidine, or arginine. The losses with urine were higher during feeding with arginine alone and lower during feeding with histidine, compared with the mixture of non-essential aminoacids. The level of blood ammonia was higher on feeding with glycine alone than on feeding with the aminoacid mixture.

These data are consistent with those presented in the report by Swendseid quoted above. The unsuitability of the replacement of an aminoacid mixture by glycine alone was clearly demonstrated on four adult subjects. However, a 1:1 mixture of glycine and diammonium citrate proved to be a complete replacement for a mixture of 7–8 non-essential aminoacids.

On the other hand, Snyderman et al.[65] demonstrated on unweaned infants that the replacement of 74% of the milk nitrogen by glycine alone does not have a negative effect on the rate of their growth.

Huang et al.[66] investigated on 9 male students the degree to which beef protein can be replaced by an equivalent amount of non-specific nitrogen. The latter (equal amounts of glycine and diammonium citrate) could constitute up to 20% of the basic diet in all 9 subjects without undesirable consequences.

A dependence of the effectiveness of the utilisation of non-specific nitrogen on the total nitrogen level has been noted[67]. This observation was confirmed also by Kies et al.[68]: each of 6 young men received 4 g, 6 g, and 8 g of total nitrogen per day during 3 experimental periods, each lasting 12 days, 1.12 g of nitrogen being in the form of all 8 essential aminoacids, the non-essential aminoacids cystine and glycine, and diammonium citrate. The nitrogen balance was negative with 4 and 6 g of total nitrogen. An increase of nitrogen intake from 4 to 6 g reduced the nitrogen deficiency in 4 out of 6 cases, while an increase of the intake from 6 to 8 g improved the balance in all cases. Kies et al.[69] investigated the optimum amounts of non-specific nitrogen for the retention of nitrogen by the organism of adult human beings under conditions where the essential aminoacids were provided by whole cereal protein. Best retention of nitrogen was achieved when the level of total nitrogen was 12.68 g per day, 6.0 g comprising non-essential nitrogen.

Many investigators have paid more attention to the qualitative composition of non-essential nitrogen than to

its quantitative amount in the diet. It has been shown that not all sources of non-essential nitrogen are equivalent[70]. The key position of glutamic acid in nitrogen metabolism has long been known, while the role of hydroxyproline is insignificant. In many cases the addition of serine and cystine actually has a toxic effect[71].

The inclusion of asparagine in aminoacid mixtures plays a favourable role in their effective application[72,73]. Urea as a possible source of food nitrogen is also of interest. There are data showing that, like animals, man has the enzyme urease, which cleaves urea to ammonia and carbon dioxide[74].

Snyderman et al.[65] investigated the nitrogen balance and the gain in body weight in children receiving 1.1 g of milk protein per kilogramme of body weight with the addition of urea as a source of non-essential nitrogen. The results showed that the urea additive favoured an increase in body weight and in the retention of nitrogen. At the same time urea has been found to have a toxic effect on the human organism[75]. Despite these findings, urea will probably not find wide application in synthetic diets for man.

One may assume that, although the role of non-essential aminoacids in protein nutrition has been investigated in fair detail on animals and man[65,68,76], the determination of a quantitative relation between essential and non-essential aminoacids in the diet requires further work.

VI. AMINOACID MIXTURES TESTED ON MAN

Harker et al[77] suggested that, in a study of the effect of the balance in mixtures, the latter must be constituted on the basis of the composition of the tissues and body plasma. The investigation of various combinations of aminoacid mixtures, made up in proportions according to the natural proteins in human nutrition selected as models, has been the subject of numerous publications. Thus, in experiments on 6 women, Kirk et al.[78] demonstrated a higher effectiveness of a mixture of aminoacids in the proportions in which they are contained in milk proteins and in peanut oil compared with the effectiveness of the set of aminoacids proposed by FAO. In analogous experiments on the same number of female patients, Leverton and Steel[26] did not find any difference in the retention of nitrogen for two aminoacid diets: one based on the FAO standard and the other based on the composition of oat protein with the addition of limiting aminoacids. A similar comparison was also made by Swendseid et al.[79] They investigated on 10 young women (18–25 years) the effect of diets made up of a mixture of pure aminoacids according to the FAO standard and on the basis of the composition of the chicken egg. The results are presented in Table 3.

Best retention of nitrogen was observed for the aminoacid mixture based on the egg composition but the differences in the balances are small for the two mixtures. A clear difference between men and women as regards the requirement for total essential nitrogen has been found. In addition, the authors confirmed in experiments on women their previous observations on men, namely that the requirement for essential nitrogen is in no way related to body weight. The limits of the requirement for essential aminoacids established are between 0.65 and 0.85 g per day for women and between 1.0 and 1.3 g per day for men. However, it is so far unknown whether these differences are associated with different requirements for all these aminoacids or only with any one of them.

Clark et al.[81] showed that the requirement for lysine is the same for both sexes. Thus it is possible that the requirement for essential aminoacids in man is greater than in women owing to certain specific aminoacids.

Other investigators[80] showed that an increase in the content of essential aminoacids in the diet of 6 healthy males from 1.1 to 5.5 g per day (for an overall daily dose of 6 g of nitrogen) was accompanied by a progressive increase in the retention of nitrogen in the organism (on average by 1.9 g of nitrogen per day).

Table 3. The proportions of aminoacids and the daily intake of essential aminoacids based on the FAO composition and the set of aminoacids in egg protein.

L-Aminoacids	Aminoacid : L-tryptophan ratio		Tryptophan intake = 240 mg	
	FAO	egg	FAO, g/day	egg, g/day
Tryptophan	1.0	1.0	0.24	0.24
Isoleucine	3.0	3.7	0.72	0.89
Leucine	3.4	5.4	0.81	1.30
Lysine	3.0	5.4	0.72	1.09
Methionine and cystine	3.0	3.7	0.81	0.99
Phenylalanine and tyrosine	4.0	4.7	0.96	1.13
Threonine	2.0	3.1	0.48	0.74
Valine	3.0	3.9	0.72	0.93
Total essential nitrogen	—	—	0.63	0.86

In experiments on young men, Watts et al.[82] compared the effectiveness of three sets of aminoacid diets: (1) a diet based on the FAO standard: (2) a modified FAO diet; (3) a diet based on the wheat flour protein. The total nitrogen intake was 10 g in all cases. It was shown that the first two diets have the same biological value. When comparable amounts of lysine, a limiting aminoacid in wheat flour, were ensured in the FAO diet and in the diet based on the wheat flour model, the retention of nitrogen in both groups of men was the same. The limiting requirement for total essential nitrogen ensuring a slight positive nitrogen balance in men was established in this series of experiments as between 0.63 and 0.84 g per day, i.e. less than shown by Swendseid but higher than the minimum requirement according to FAO, which is suggested as 0.48 g per day.

According to the data of Swendseid et al.[83], the nitrogen balance in people receiving essential aminoacids in the same proportions as in plasma proved to be negative. It was established that in plasma the concentrations of valine and lysine are relatively higher than in food proteins and the contents of isoleucine and leucine are lower, and that this probably does not constitute optimum conditions for the anabolic processes in the organism.

As an example of an aminoacid diet successfully employed, we may quote that tested by Clark et al.[84] Apart from the essential aminoacids (taken in the proportions specified by Rose), the diet contained 130–140 g of white flour (in the form of biscuits and cake) and also dehydrated milk, cornstarch, sugar, and preserved fruit. The overall calorie content was 2100–3250 kcal.

Non-essential nitrogen (mg per day):

arginine	-620	aspartic acid	-850
histidine	-330	glycine	-580
alanine	-490	serine	-800
proline	-1820	glutamic acid	-5810

The amount of total nitrogen in different series of experiments was 4.5, 6, and 9 g. The amount of the basic mixture of essential aminoacids was increased by factors of 1.0, 1.5, 2.0, and 2.5 and the corresponding mean nitrogen balance was +0.07, +0.22, +0.56, and +0.72. It was found that a daily dose of total nitrogen of both 6 and 9 g is quite sufficient to maintain a positive nitrogen balance, while 4.5 g is clearly inadequate.

Table 4. An aminoacid diet for children (12 year old boys).

Aminoacid	Daily intake, g	N content, g	Aminoacid	Daily intake, g	N content, g
Essential L-aminoacids			Non-essential aminoacids		
Isoleucine	1.00	0.107	Alanine	9.22	1.449
Leucine	1.50	0.160	Arginine	10.81	3.476
Lysine	1.60	0.307	Aspartic acid	10.76	1.132
Methionine	0.80	0.075	Glutamic acid	10.76	1.024
Phenylalanine	0.80	0.068	Na glutamate	12.50	1.035
Threonine	1.00	0.118	Glycine	7.65	1.420
Tryptophan	0.12	0.017	Histidine	1.00	0.271
Valine	0.90	0.108	Proline	4.61	0.561
			Serine	6.92	0.922
			Total nitrogen		12.250 g

In their subsequent investigation Clark et al.[85] studied the effect of an increase of the amount of lysine in the mixture on the retention of nitrogen. The following amounts of lysine were added to the mixture (in place of the previous 1780 mg of lysine per day): 2900, 1800, 2700, and 3600 mg per day. The best retention of nitrogen was noted when the level of lysine was 3600 mg per day. Very valuable data have been presented by Nagakawa et al.[86], who investigated synthetic aminoacid diets on children. In earlier studies by these workers the minimum requirement by school age children for each essential aminoacid, determined separately, was established[87]. Then mixtures of the same aminoacids were made up. Tests were made on 5 boys, 10–12 years old, and were continued for 24 days. The basic diet contained a mixture of aminoacids, corn starch, corn and olive oil, mineral salts, and vitamins. The entire experiment was carried out in two periods: during the first period the total nitrogen intake was 12.25 g and during the second 10.23 g. The energy intake was between 53 and 75 kcal per kilogramme of body weight. The data are presented in Table 4. All the children remained healthy, retaining a constant weight and a good appetite. The average nitrogen retention varied from +0.68 to +1.36 per day. But, as stressed by the authors, a simple positive nitrogen balance is not sufficient for children, who require additional nitrogen for growth and development.

If one bears in mind that the body of 10–12 year old boys contains 3% of nitrogen and that they gain 3 kg in weight per year, the retention of nitrogen should increase by 90 g annually, i.e. by +0.25 g per day. However, an additional 50%, i.e. a total of +0.38 g per day, must be added as a reserve.

Comparison of the data obtained by these workers with the value +0.38 g per day shows that the aminoacid mixtures tested are quite suitable to support the life of children at the normal level.

The necessity for the presence of arginine in the diet is still questionable. The removal of arginine from the diet did not affect the nitrogen balance and also did not give rise to any clinical disturbances. As regards histidine, the authors do not share the view of Albanese, who believes that this is an essential aminoacid for children. The absence of histidine from the diet for 15 days of the experiment did not affect the nitrogen balance.

Cabalska[88] also demonstrated the successful application of aminoacid diets in the feeding of children. Mixtures of aminoacids in the feeding of unweaned infants proved to be particularly effective[89].

Abernathy et al.[90] investigated the nitrogen balance in 58 girls aged 7–9, whose daily doses of total nitrogen amounted to 86–460 mg per kilogramme of body weight. The authors present data concerning the minimum level of essential aminoacids which must be provided with the diet to maintain the nitrogen balance at the normal level (in milligramme per kilogramme of body weight per day):

isoleucine	-24	phenylalanine	-28
leucine	-45	threonine	-20
lysine	-29	tryptophan	-4.9
methionine + cystine	-21	valine	-31

These data were obtained on at least 6 subjects whose daily nitrogen intake was 104–135 mg per kilogramme of body weight.

The American Company Schwarz Bioresearch Inc. is marketing synthetic diets, which consist of aqueous solutions of chemically pure ingredients with a 50% content of dry substances. They are designed for the experimental feeding of both laboratory animals and human beings. The diets are sold in bottles or sealed tubes and if desired may be flavoured. Table 1 gives the composition of one of the diets used for human nutrition. Its calorie content is 1.9 cal ml^{-1}. The specific gravity of the solution is 1.18–1.20 and its pH is 4.85. In the preparation of diets of this kind, the order in which the components are dissolved is very important.

VII. AMINOACID IMBALANCE, ANTAGONISM, AND TOXICITY

When artificial aminoacid diets are made up, particular attention must be paid to the study of the phenomenon of the so called imbalance—the breakdown of the optimum proportions of the aminoacids in the diet, which may arise both in the presence of an excess and a deficiency of a particular aminoacid. The problem of imbalance was investigated in very great detail by Harper and Rogers[91-94]. The imbalance is expressed in a series of symptoms characteristic of a disturbance in nutrition, namely in the arrest of the growth of young organisms and a disturbance of the nitrogen equilibrium in adult organisms, in the fatty degeneration of the liver, in an increase of the number of leucocytes in the blood, in albuminuria, and frequently in convulsions, vomiting, nausea, and other undesirable symptoms.

Some workers believe that an imbalance occurs also when even a slight excess of one or several aminoacids is added to the diet. Such an imbalance is usually less dangerous than in the absence of a particular aminoacid. Harper does not regard such a state as imbalance at all but attributes it to the antagonism and toxicity of aminoacids.

Sauberlich[95] made a comparative study of the toxicity and antagonism of 19 aminoacids fed under different conditions to young weanling rats. All 19 aminoacids were added in an amount of 5% of the weight of the diet, at a low overall nitrogen level. With the exception of alanine, all the aminoacids caused an arrest of growth to different extents.

An excess of methionine caused the most acute toxic effect. It is followed in decreasing sequence by tryptophan, aspartic acid, histidine, tyrosine, phenylalanine, cystine, leucine, valine, isoleucine, glycine, asparagine, arginine, lysine, and threonine. Glutamic acid, proline, and serine caused only a slight arrest of growth.

Antagonism can also occur in the interaction of the following pairs of aminoacids: leucine–isoleucine, isoleucine–valine, isoleucine–phenylalanine, phenylalanine–valine, phenylalanine–threonine, arginine–lysine.

The inclusion of an excess of leucine in a casein diet[94] arrests growth. The addition of isoleucine and valine, neither of which is limiting in this diet, prevents or decreases the arrest of growth, while the addition of threonine, a limiting aminoacid, does not have an effect. The absence of a response to the addition of the limiting aminoacid makes it possible to distinguish antagonism from imbalance. Thus antagonism constitutes the elimination of a harmful effect of an excess of one aminoacid by another with a similar structure, which, however, is not limiting in the given diet.

By virtue of the similarity of their structures, the antagonistic aminoacids probably interfere with one another during transport into the cells and disturb the enzyme equilibrium necessary for protein synthesis.

The toxic responses of the organisms are variable, including the cessation of growth and also irreversible functional and organic disturbances in many organs. Thus a dietary excess of tyrosine (more than 3%) causes an acute affection of the eyes and paws of young growing rats[94]. An excess of methionine completely suppresses growth and causes the atrophy of the cells in certain organs. An excess of phenylalanine interferes with the function of the brain[95].

Doolan et al.[96] observed a severe toxic response in man, expressed in ammonia intoxication following the intravenous injection of a 5% solution of glycine.

There are data[97] showing that sodium monoglutamate, widely used in the Chinese and Japanese cuisine as a taste improving additive to various dishes, causes an illness called the "Chinese restaurant syndrome" expressed in headache, a burning sensation in the stomach, and an increased [blood] pressure. This effect of sodium glutamate is associated with a specific dose. There exist appreciable deviations in the oral threshold doses for different individuals. The syndrome is caused by the following substances: sodium monoglutamate (3 g), potassium mono-L-glutamate (4 g), DL-glutamic acid (5 g), and L-glutamic acid (5 g). The syndrome is not caused by sodium mono-D-glutamate (7 g), sodium mono-L-aspartate (5 g), sodium chloride (10 g), and glycine (5 g). After the intravenous administration of sodium monoglutamate the symptoms occur even with a dose of 50 mg. However, there are doubts whether these may not be the effects of the enhanced sensitivity of the test subjects.

Bearing in mind that L-glutamic acid is a normal metabolic product, the official international medical organisations object to any limitations to the use of sodium glutamate as a taste additive[98].

The above examples of the effect of the toxicity of aminoacids on animal and human organisms make it necessary to refine the dosage in such a way that acute toxicity does not occur. Acute toxicity is understood as entailing the death of all the animals receiving the lethal dose (LD) of aminoacids. The dose which leads to the death of 50% of the animals receiving the test diets is also regarded as lethal, is denoted by LD_{50}, and is defined as follows:

$$LD_{50}=x_s-d\,(\Sigma p-{}^1/_2)^{99},$$

where x_S is the dose for which all the animals die, d the interval between the specified doses, p the ratio of the number of dead animals to the particular dose, and Σp the sum of all the ratios of the numbers of dead animals to all the doses administered to them[99].

As long ago as 1955, Greenstein and coworkers[100] put forward the problem of acute toxicity in the comparison of L- and D-forms of aminoacids. Examples of the comparative toxicities of the DL-, D-, and L-forms of certain aminoacids are presented below:

Aminoacid	LD_{50}, g kg^{-1} for		
	DL-	L-	D-
Methionine	5.95 ± 0.04	4.3	5.2
Threonine	5.85 ± 0.04	3.1	5.3
Valine	6.25 ± 0.05	5.4	6.1

Molle[99] noted that, in respect of acute toxicity, racemic forms are less toxic than the L-forms. On the basis of numerous experiments on laboratory animals, the author concludes that even high doses of the D-isomers have a less toxic effect than the corresponding L-forms. These conclusions are in conflict with the views of Elvehjem[101], who attributes especially high toxicity to racemic aminoacids[101].

Probably D-isomers do not have any unfavourable effects and are simply unused by the organism. Sauberlich also showed that the D-isomers are less toxic than the L-forms. However, DL-aspartic acid has a more harmful effect than the L-isomer. The L- and D-forms of tryptophan have the same toxic effects[95].

Studies on these lines should be continued in the first place, since large-scale application of aminoacids in the food industry will become possible only after an accurate determination of the aminoacid norms in the diet which do not give rise to any acute or toxic effects.

Shtenberg and Ignat'ev[102], who examined the principles of the study of toxicity, recommend that for greater reliability the experiments be performed on three animal species, one of which must be a rodent. It is necessary to test a large number of animals in each group: not less than 100 mice, 50 rats, 100 chickens, 25 guinea-pigs, 3 dogs, and 15 rabbits. The concluding stage of the investigations should involve experiments on volunteers (10–20 people), who should receive the test diet over a period of 10–30 days.

The observation of the initial stage of the changes occurring in the organism under the influence of the experimental diet is of great interest. In a short experiment on rats, Sanahuja and Rio[103] carried out biochemical studies in order to distinguish the initial stage of the changes due to the intake of a non-balanced aminoacid mixture. An increase in the amount of protein, amino-nitrogen, and the content of threonine in the liver was

noted. Probably the initial imbalance is associated with a more rapid protein synthesis in the liver and also with an increase of the glycogen content in the latter. The same phenomenon was also observed by other workers[104,105].

L-Tryptophan causes the accumulation of neutral fat in the liver in the course of 2.5 h after its introduction into the rat organism. The introduction of other aminoacids such as L-methionine, L-lysine, L-tyrosine, and L-threonine did not increase the amount of fat in the liver. The introduction into the organism of ATP, ADP, AMP, adenosine, or folic acid together with L-tryptophan prevents the generation of fat in the liver. Probably the generation of fat caused by L-tryptophan is associated with a decrease of the amount of ATP, whose effect is suppressed when L-tryptophan is administered.

Hunter et al.[105] observed an increase in the amount of liver glycogen after feeding with added glycine and L-tyrosine. This was eliminated by including D-tyrosine in the diet.

Murata et al.[106] noted a favourable effect of L-threonine (but not D-threonine), introduced in an amount of 0.2% of the weight of the diet, in preventing the accumulation of fat in the liver caused by the dietary imbalance.

The metabolic rearrangement shown as a response to the consumption of a non-balanced aminoacid mixture by man was investigated by Soliman[107]. All the investigators studying the aminoacid imbalance note a favourable effect of protein added to the diet.

In experiments on weaned rats with three protein levels in the diet (6, 9, and 12%), Harper et al.[108] showed that 12% of protein prevents the harmful effect of the excess histidine and tyrosine in the diet. Added protein has a similar effect also in the presence of an excess of methionine and threonine.

A high aminoacid content in the diet does not by itself have a toxic effect[109]. In fact rats can digest large amounts of aminoacids in the presence of 80–90% of protein in the diet without an appreciable arrest in growth. This observation is confirmed by the diet of specific groups of men containing up to 200 g of protein per day. Certain Australians, the Gaucho in the South American prairie, and the Masai tribe in Central Africa retain satisfactory wellbeing on a diet of 250–300 g of protein per day.

A characteristic sign of imbalance is a reduced requirement for food. Sometimes it is difficult to conclude whether the decrease of the effectiveness of the nitrogen intake is linked directly to a toxic effect or whether this is a secondary factor, while the primary factor is the reduced requirement for food.

A stimulation of food intake and growth can be achieved by the introduction of insulin[110] and cortisol[111] and also by maintaining the animal at a reduced temperature[112]. One may conclude from this that a principal cause of the arrest of growth in animals receiving non-balanced diets is a decrease of the requirement for food.

The reduction of the requirement for food can reach up to 50%.[113] Leung and Rogers conclude that the central nervous system probably contains receptors sensitive to the concentration of the essential aminoacids in blood plasma.

Arginine plays a positive role in the utilisation of non-balanced diets[114,115]. It reduces the imbalance caused by an excess of the following aminoacids: lysine, tyrosine, histidine, glycine, methionine, cystine, valine, threonine, isoleucine, and phenylalanine. The addition of arginine in an amount of 0.3–2.0% of the weight of the diet eases the imbalance due to the aminoacids enumerated above but does not eliminate the imbalance caused by tryptophan, leucine, and glutamic acid.

Hepburn and Bradley[31] also confirm the idea that the function of arginine and glutamic acid consists in a weakening of the potential toxicity of ammonia arising on feeding large amounts of aminoacids. In this respect glutamine is more effective than glutamic acid. The attempts to determine the role of arginine in the detoxication of an excess of aminoacids have so far been unsuccessful. After a time the imbalance phenomena decrease, which is evidence of a gradual adaptation of the organism.

VIII. ENRICHMENT OF SECOND-CLASS PROTEINS BY THE ADDITION OF AMINOACIDS

It is also necessary to take into account the aminoacid balance in the addition of synthetic aminoacids to second-class plant proteins, such as those derived from wheat, corn, rice, millet, etc. with the aim of increasing their biological value. Similar additives constitute a valuable contribution by aminoacids to human nutrition.

McPherson has noted[116] that, in order to cover the deficit in food products by 1980, it will be necessary to produce an additional 18 million tons of fat, 9.9 million tons of animal protein, and 11.4 million tons of aminoacids. The total amount of aminoacids necessary for addition to human food in underdeveloped countries is 0.5–1.0 million tons per year. The total consumption of protein in Asia, Africa, and Central America reaches only 48–54 g daily per man, while the minimum requirement is 60 g. The greatest world deficiency is experienced in respect of three essential aminoacids—methionine, lysine, and tryptophan.

In 1966 the American Chemical Society[117] examined widely the problem of the sources of additional protein. The following sources of this kind have been suggested: various oil bearing plants (cotton seeds, peanuts, coconuts, sesame, sunflower, etc.), threshed wheat fractions previously employed as cattle-feed, defatted dry milk, various protein concentrates, such as, for example, fish protein or soya protein concentrate.

The problem of the aminoacid additives was specially examined and their advantages compared with the use of protein concentrates for the enrichment of second-class proteins was noted. Chemically pure aminoacids are added in small amounts and therefore cannot affect the organoleptic properties of cereal proteins. The quality of cereal proteins is then enhanced and they are more easily assimilated owing to the improved aminoacid balance. On the other hand, the amount of additive is smaller than in the case where protein concentrates are added.

The amounts of each aminoacid to be added to human food in practice still requires a more accurate determination.

Prolonged experiments on man can help discover the necessary amounts for such enrichment. Howe and coworkers[118,119] showed that aminoacid additives, including lysine, tryptophan, and threonine, improve the quality of proteins, for example those in cereals, making them equivalent to casein.

The amounts of aminoacids employed vary in the range 0.1–0.4% of the total weight of the diet. Tryptophan is an exception—not more than 0.07% should be taken.

In order to evaluate a particular protein and to establish the aminoacid in which it is deficient, Block and

Mitchell introduced the concept of the "chemical score" of the protein. With the aid of this concept, it is possible to compare the aminoacid composition of any protein with that of a protein adopted as a standard. The whole chicken egg is regarded as an ideal protein both as regards composition and its aminoacid balance. Therefore the percentage content of each aminoacid in the test protein is compared with that in the chicken egg. Then the aminoacid which, in relation to its amount in the egg protein, is present in the smallest quantity is regarded as the first limiting aminoacid of the test protein. Its ratio in the two proteins is in fact the "chemical score" of the given protein.

For example, lysine is present to the extent of 2.7% in wheat protein and 7.2% in egg protein. The ratio $\frac{\text{wheat lysine}}{\text{egg lysine}} \times 100 = 37$. Thus the deficiency of wheat lysine is $100-37 = 63\%$ relative to egg protein.

Yuyst, Arnould, and coworkers[120,121], who for many years have been concerned with the problem of the improvement of the biological value of proteins by the addition of synthetic aminoacids, suggest that, before introducing each additive into the cereal protein diet, one should establish accurately the first limiting factor of the given protein, and this should in fact be the first additive. The same authors showed the effectiveness of the addition of lysine to white flour. If the "white flour–lysine" mixture is treated further with threonine and isoleucine together, the latter have an additional improving effect, but this does not occur if the aminoacids are added separately. The addition of valine, methionine, and cystine together or without lysine did not produce any results. Consequently here a clear non-equivalence of particular aminoacids is shown, which gives rise to the problem of further improvement of the necessary norms in the addition of aminoacids to protein.

Lysine and methionine are now manufactured on an industrial scale and their addition to food does not present difficulties; on the other hand, threonine and tryptophan are obtained only in small amounts.

Nevertheless the advances in the chemical synthesis of all the essential aminoacids give rise to hope that the problem of obtaining cheap aminoacids will be solved in the near future[122]. The techniques used to enrich cereal protein by synthetic aminoacids are various[123].

When cereals are employed in the form of ground flour or granules, the enrichment is carried out directly by mixing in the aminoacids. The added aminoacids should be ground to the same particle size as the bulk of the flour. The technique becomes more complicated when the cereals are employed in the form of whole grains. Crystalline pure aminoacids can then be introduced as granules resembling particular grains. When protein concentrates are introduced, the ratio of the true and artificial grains is 20:1 or 30:1 and when aminoacids are added the ratio is 200:1.

Graham[124] investigated the enrichment of wheat used in the form of grains. He suggests various envelopes and also grain imitation. Lysine is added by spray drying and also by a "steeping" technique. Additional work is required to determine whether this lysine can withstand treatment of any kind (mechanical or thermal) during the preparation of fortified flour.

Pokrovskii et al.[125] investigated the effectiveness of the addition of L-lysine hydrochloride to improve the nutrient value of wheat bread. The optimum dose of the preparation (0.25–0.5%) relative to the total weight of the flour used to make the bread was established. The bread should be baked at a temperature reduced by 20–25°C.

Koz'mina's review[126] deals with the improvement of the nutrient value of the grains and the development of "synthetic" grains. Gololobov[127] deals with the problem of the enhancement of the nutrient value of protein by synthetic aminoacids with the aim of increasing the protein resources not only of backward but also of advanced countries. A successful use of aminoacid additives to cereal proteins in children's diet has been reported by Bressani et al.[128]

Parthasarathy et al.[129] added DL-methionine to the diet of children 8–9 years of age, which led to an appreciable improvement of the biological value of the proteins. In this respect, the hydroxy-analogue of DL-methionine is less effective[130].

Other Indian workers[131] suggest that methionine and lysine be added to children's food comprising a soya bean–sesame mixture.

Cantor and Roberts[132] reported an improvement of the quality of the protein in corn food products. They discussed mixtures of corn with various proteins and aminoacids, particularly lysine and tryptophan. Kies et al.[133] established that lysine is the limiting aminoacid in corn.

In a study of a plant mixture containing 47% of autho-clave-treated chick peas, 35% of defatted sesame flour, and 18% of soya bean flour, Guggenheim and Szmelcman[134] showed that it is less effective than casein. The addition of lysine improves its value. However, its value does not alter on addition of either methionine or tryptophan. The addition of threonine together with lysine did not affect the effect of the latter, while methionine reduced it.

Despite the abundance of studies demonstrating the value of aminoacid additives to second-class proteins, there are also others—for example, Hegsted[135] believes that the use of products of this kind is still untimely and uneconomical. The author voices the fear that there are still no quantitative data concerning the optimum content of aminoacids in a particular diet and that they should not be used, particularly in the food of children.

Apart from being used in the food industry to improve the biological value of cereals, mixtures of aminoacids can also be used to enhance and improve the taste of many food products. The literature quotes aminoacids which, when mixed in appropriate proportions, can produce the taste of the following products: onions[136], crab[137], fish[138], soya sauce[139], and chicken and meat broth[140]. Chemically pure aminoacids have the following intrinsic flavours: none (tasteless) (D- and L-alanine, D- and L-arginine, D- and L-aspartic acid, D-glutamic acid, L-histidine, D- and L-isoleucine, D- and L-lysine, D- and L-proline, D- and L-serine, D- and L-threonine, and D- and L-valine), sweet (D-tryptophan, D-histidine, D-phenylalanine, D-tyrosine, D-leucine, L-alanine, and glycine), bitter (L-tryptophan, L-phenylalanine, L-tyrosine, L-leucine), and with a slight sulphurous flavour (D- and L-cysteine and D- and L-methionine).[141]

IX. AMINOACIDS IN MEDICINE—WITH PARTICULAR REFERENCE TO PARENTERAL FEEDING

The use of aminoacids in medicine must be regarded as a special case of their employment in human food. Pokrovskii notes several applications of aminoacids in medicine[142]. Various aminoacids are used in the therapy of many diseases. L-Lysine is very effective in the feeding of premature babies. L-Methionine, which

exhibits lipotropic activity, is indicated in the treatment of the cirrhosis of the liver.

In addition methionine is used in the surgical traumas of the heart, in the myocardial infarct, and also in thyrotoxicosis. There has been a general accumulation of definite data concerning the effect of aminoacids on cardiac function. Thus DL- and L-methionine and L-leucine intensify cardiac contractions, while aspartic and glutamic acids and arginine have an inhibiting effect[143].

The cause of a number of psychoneurotic disorders is an innate disturbance of the aminoacid metabolism (phenylpyruvic oligophrenia, the glycine disease, argininosuccinicaciduria, and Hartnup disease). L-Glutamic acid is used in the treatment of many psychological illnesses, for example, in epilepsy. Efron[144] quotes a list of 14 diseases caused by a disturbance of the aminoacid metabolism; 12 of these are expressed in psychological disorders. Many of these diseases are susceptible to treatment with the aid of aminoacid diets and by vitamin therapy.

A classical example of the successful application of an appropriate aminoacid diet is the treatment of phenylketonuria[145]. The exclusion of phenylalanine from the diet of patients maintains a normal state.

Artificial aminoacid mixtures are also used to feed patients by the intravenous injection of a solution of these aminoacids, i.e. parenterally. Parenteral feeding is indicated in most cases where feeding *per os* is impossible or endangers life. Experimental clinical studies showed that total parenteral feeding using a mixture of aminoacids, glucose, and lipid emulsions can maintain a satisfactory nutritional state in the majority of patients.

In parenteral feeding one usually employs (1) enzymatic hydrolyzates of various proteins, primarily casein, and (2) mixtures of synthetic crystalline aminoacids in the L- and DL-forms containing all the essential aminoacids and some source of non-essential nitrogen (mainly glycine). The D-forms are usually not utilised by the organism and their inclusion in such mixtures is undesirable.

Aminoacid mixtures have the advantage of purity over hydrolysates (inadequately dialysed enzymatic hydrolysates can contain peptides capable of inducing an allergic response) and also there is a possibility of varying their composition depending on the physiological state of the patient.

As early as 1940, Shohl and Blackfan[146] were the first to use an aminoacid mixture for complete parenteral feeding. Since then, this field of medicine has been successfully developing in a number of countries: Sweden, Denmark, Germany, France, Italy, and Poland[147-155]. In the USSR Latvian scientists have been successfully working in this field under the supervision of A. A. Shmidt[156,157].

In 1960 Geyer[158] published an extensive review on the application of parenteral feeding. Trémolières[159] quotes examples of the most thoroughly investigated aminoacid mixtures for parenteral feeding (Table 5).

The limiting aminoacid in the majority of preparations is isoleucine[160]. The problem of the addition of non-essential aminoacids in parenteral feeding has so far been inadequately investigated.

Satisfactory proportions of the aminoacids, similar to those in first-class proteins such as those of milk, eggs, and meat, have been found in the enzymatic hydrolysate of the yeast *Candida tropicalis* and in the product obtained from it after purification and consisting of a mixture of aminoacids and lower peptides[161].

A synthetic aminoacid mixture marketed by the Japanese firm Ajinimoto Co. under the conventional name "aminoacid substances" is used as a highly nutritive food product (in the form of tablets) or as a substitute for blood plasma (in the form of a sterile solution). The mixture contains 16 aminoacids and its composition is as follows (%):

alanine	6.0-7.4	lysine	11.5-14.5
arginine	4.6-6.0	methionine	1.0-1.2
aspartic acid	5.8-7.4	phenylalanine	4.3-5.3
glutamic acid	9.0-11.8	proline	4.2-5.2
glycine	4.0-4.8	serine	3.5-4.5
histidine	3.2-4.0	threonine	3.0-3.6
isoleucine	4.4-5.4	tyrosine	1.0-1.2
leucine	8.4-10.4	valine	4.1-5.1

Table 5. The most thoroughly investigated aminoacid mixtures for parenteral feeding (aminoacids contents expressed as percentages of the dry substance).

Name	Amigène	Merck soln.	Parenamine	Aminosol	Travamine	Trophysan
Total nitrogen	12.9	13.3	13.7	13.4	12.0	15.2
Amino-nitrogen (% of total nitrogen)	75	100	93	69	65	100
Ash	5.5	1.0	—	—	—	1.2
Arginine	3.2	2.5	3.2	4.8	3.8	—
Isoleucine	4.9	8.0	4.5	3.8	3.0	3.2
Histidine	1.0	4.2	2.0	2.0	2.1	—
Leucine	9.1	16.5	6.0	5.6	7.5	5.1
Lysine	5.1	9.4	5.8	7.0	6.8	7.9
Methionine	2.6	4.0	3.7	2.8	1.1	5.2
Phenylalanine	3.9	4.3	2.8	3.0	3.2	4.4
Threonine	2.9	1.9	2.3	5.2	5.3	3.2
DL-Tryptophan	1.3	0.9	1.3	0.9	1.0	1.9
Valine	5.3	7.0	4.7	4.0	5.8	4.4
Glutamic acid	17.1	1.5	—	10.6	10.9	—
Tyrosine	4.6	0.3	1.4	2.6	0.9	—
Glycine	0.4	23.0	—	5.6	—	59.5
Aspartic acid	4.7	0.2	—	5.2	8.1	—
Proline	6.2	—	—	5.8	—	—
Serine	4.9	—	—	5.8	—	—
Alanine	4.2	—	—	3.8	—	—
Hydroxyproline	1.5	—	—	—	—	—
Cystine	0.3	—	—	2.4	2.1	—

Among non-essential aminoacids, best results are given by glycine and glutamic acid. Furst et al.[147] showed that glycine is a necessary source of non-essential nitrogen when essential aminoacids and arginine are administered. This investigator demonstrated the higher effectiveness of a mixture of crystalline aminoacids compared with hydrolysates in the parenteral feeding of surgical patients, while in healthy people a positive nitrogen balance is most pronounced when casein hydrolysates are administered. He also established that the nitrogen balance increases when a mixture of glycine, L-alanine, and L-proline serves as a source of non-essential nitrogen and therefore he believes that alanine and proline are semi-essential aminoacids, the introduction of which into the mixture for parenteral feeding is necessary.

Table 6 gives the compositions of the USSR preparations for parenteral feeding. The data show that in all the preparations an approximately 50:50 ratio of essential and non-essential aminoacids is maintained[162].

Deligné and Wretlind[163] believe it useful to employ a mixture of these non-essential aminoacids rather than other sources of non-essential nitrogen. Breinlich[164] also thinks it necessary to include in the mixture L-histidine,

L-arginine, and vitamin B, sorbitol, and mineral salts in addition to the eight essential aminoacids.

Heller et al.[150] demonstrated that the aminoacids in solutions prepared from casein hydrolysate are better assimilated than the aminoacid mixtures made up in accordance with the composition of plasma. These data are consistent with the ineffectiveness, mentioned above, of the aminoacid mixture based on the plasma model and designed for peroral feeding.

Table 6. Aminoacid composition of protein hydrolysates produced in the USSR for parenteral feeding.

Aminoacid	Aminoacid composition, g per 100 g of conventional protein				
	Amino-peptide	L-103	TSOLIPK hydrolysate	Amino-krovin	Fibrinosol
Phenylalanine	5.8	6.5	4.6	6.8	4.6
Tyrosine	1.2	2.3	3 9	2.2	3.2
Total aromatics	7.0	8.8	8.5	9.0	7.8
Methionine	1.02	1.1	2.1	1.1	2.1
Cystine	1.83	1.3	1.1	1.1	2.1
Total sulphur-containing acids	2.85	2.4	3.2	2.2	4.2
Threonine	4.6	4.9	3.9	4.7	6.8
Tryptophan	2.1	0.5	0.5	0.4	1.2
Valine	9.3	9.1	7.7	10.8	4.8
Lysine	10.1	13.1	9.3	11.3	12.8
Leucine	11.8	12.7	9.1	13.8	8.2
Isoleucine	1.80	1.4	5.9	1.10	4.0
Total essential aminoacids	49.55	52.9	48.1	53.3	49.8
Arginine	4.9	4.8	3.7	3.4	7.0
Histidine	4.7	6.4	4.1	8.3	5.9
Alanine	8.2	9.4	3.1	10.2	4.4
Glycine	4.5	4.7	2.2	4.8	7.5
Serine	4.8	5.6	3.8	3.9	6.7
Proline	3.6	3.0	11.0	4.7	7.4
Glutamic acid	8.4	6.3	15.0	3.7	5.5
Aspartic acid	9.8	6.3	4.3	4.4	1.7
Total non-essential aminoacids	48.9	46.5	47.2	43.4	46.1
Total	98.45	99.4	95.3	96.7	95.9

As in peroral feeding, when an aminoacid solution is introduced intravenously, the correct ratio of total nitrogen to carbohydrates and fats must be rigorously observed and the amounts must be sufficient for the energy needs of the organism.

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Summarising the literature data discussed above, certain fundamental conclusions may be made.

1. Aminoacid mixtures based on the composition of first-class food proteins probably constitute an adequate substitute for meat, milk, fish, etc. proteins. Man can subsist for a long time on a diet containing aminoacids with the addition of fats, vitamins, carbohydrates, and mineral salts. The physiological and biochemical parameters are then maintained at the normal level.
2. It is useful to improve the biological value of plant proteins to the level of highly effective animal proteins by the addition of chemically pure aminoacids. It is then necessary to observe the correct aminoacid balance in the overall fortified protein because the breakdown of such balance is in fact primarily responsible for the inadequacy of a particular diet.
3. The aminoacid mixtures introduced into the organism parenterally in the form of solutions constitute an excellent means for the maintenance of a sick organism incapable of food intake in the usual manner for one reason or another. This does not exhaust the applications of aminoacids in medical practice. There are many diseases which are susceptible to treatment by specially compounded aminoacid diets and by vitamin therapy.

In all cases, before the direct application of aminoacid mixtures in human nutrition, it is necessary to test them carefully on experimental animals and volunteers to avoid toxic or allergic effects of the artificial diet. However, science has now at its disposal data showing that there is no reason to over estimate the toxicity, since it can be avoided by the simultaneous introduction into the artificial mixture of appropriate amounts of arginine and glutamate and also by observing the appropriate balance of all the mixture components. When protein hydrolysates are used as food products, particular attention must be paid to the removal of impurities.

It follows from all the foregoing data that individual aminoacids can be used in human nutrition. They will probably play a significant role in increasing the food resources.

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