Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Askerov F, Çakmak G, Karadağ H (2023).** Features of the Spectra of Medium-Molecular Peptides of the Liver with Protein-Deficient Nutrition. *Van Vet J*, 34 (1), 18-24. DOI: https://doi.org/10.36483/vanvetj.1192729

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

Features of the Spectra of Medium-Molecular Peptides of the Liver with Protein-Deficient Nutrition

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Received: 21.10.2022 Accepted: 25.01.2023

ABSTRACT With this study, the determination of three spectra of medium molecular peptides (SMP) in liver tissue can provide a basis for ideas about the relationship between exogenous and endogenous proteins, the degree of damage to nucleotides and proteins, and enable certain judgments to be made. The work was carried out on 45 outbred white rats against the background of full-fledged protein-vitamin nutrition and under conditions of 60% protein deficiency in the diet. The experimental animals were divided into 3 groups, 15 rats in each group. The first group (Group I) of rats in all subgroups served as controls, kept in a vivarium; rats of the 2nd group (Group II) received a complete protein-vitamin diet; Group III received the same diet, but with a 60% protein (casein) deficiency in the diet. Experimental animals of groups II and III received food for 20, 30 and 40 days. The results of studies on the background of a monthly feeding of animals with a protein-vitamin complex with a deficiency of 60% protein in the recipe showed that the content of medium molecular peptides (MMP) fractions at a wavelength of 230 nm significantly increases compared with the control group and is 124%. The results of these studies indicate that 40-day feeding of animals with a full-fledged protein-vitamin diet and 60% protein deficiency causes significant changes in the MMP content at 254 and 280 nm wavelengths, and in the MMP subfractions at 230 nm wavelength, there is a moderate increase in the MMP subfractions, which is possibly due to the peculiarities of the substitution of the genetic apparatus of hepatocytes.

Keywords: *Liver, Peptides, Protein, Spectrum.*

ÖZ

Protein Eksikliği Olan Karaciğerde Medium Moleküler Peptitlerin Spektrumlarının Özellikleri

Bu çalışma ile karaciğer dokusunda medium moleküler peptitlerin (SMP) üç spektrumunun belirlenmesi, eksojen ve endojen proteinler arasındaki ilişki, nükleotidler ve proteinlere verilen hasarın derecesi hakkında fikirler için bir temel oluşturabilir ve belirli yargılarda bulunmayı mümkün kılabilir. Çalışma, protein-vitamin beslenmesinde diyette %60 kazein eksikliğinde 45 beyaz sıçan üzerinde gerçekleştirilmiştir. Deney hayvanları her grupta 15 rat olacak şekilde 3 gruba ayrılmıştır. Tüm alt gruplardaki ilk sıçan grubu (Grup I), bir vivaryumda tutulan kontrol grubu olarak belirlenmiştir. İkinci grubun (Grup II) sıçanlarına tam bir protein-vitamin diyeti verilmiştir. Grup III'e ise aynı diyet %60 protein (kazein) eksikliği ile uygulanmıştır. Grup II ve III'teki deney hayvanları 20, 30 ve 40 gün boyunca beslenmiştir. %60 protein eksikliği olan bir protein-vitamin kompleksi ile beslenen hayvanların aylık beslenmesi üzerine yapılan çalışmaların sonuçları, 230 nm dalga boyundaki medium moleküler peptitlerin (MMP) fraksiyonlarının içeriği ile karşılaştırıldığında kontrol grubuna göre bu grupta önemli ölçüde %124 oranında artış gösterdiği belirlenmiştir. Bu çalışmaların sonuçları, protein-vitamin diyeti ve %60 protein eksikliği olan hayvanların 40 günlük beslenmesinin, 254 ve 280 nm dalga boylarında MMP içeriğinde ve 230 nm dalga boyunda MMP alt fraksiyonlarında önemli değişikliklere neden olduğunu göstermektedir. Muhtemelen hepatositlerin genetik özelliklerinden dolayı MMP alt fraksiyonlarında orta derecede bir artış söz konusudur.

Anahtar Kelimeler: *Karaciğer, Peptitler, Protein, Spektrum.*

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INTRODUCTION

It is no accident that the liver, which affects the metabolic state of the body, performs a complex digestive function in the body, physiologists call the liver the laboratory of the body. In the liver, the main amount of blood plasma proteins is synthesized - albumin, globulins, blood coagulation factors (prothrombin, afibrinogen, etc.). In hepatocytes, glycogen is synthesized and deposited, from which, with an increase in energy costs (for example, muscle activity), glucose is formed to maintain a constant level of its concentration in the blood. This occurs under the influence of activation of the sympatho-adrenal system (adrenaline, glucagon). In hepatocytes, fats are also broken down into fatty acids. The liver has an indirect effect on metabolism, deactivates intermediate toxic metabolites, hormones. Protein-peptide hormones are deactivated by proteinases, steroid-hydroxylases, catecholamines and monoxidases (Ibragimova et al. 2011)

The liver protects the body from poisonous digestive products coming from the intestines - indole, phenol, skatole and from foreign substances. Neutralization of toxic substances by hepatocytes is carried out due to their oxidation. The restoration of the connection with glucuronic and sulfuric acids, glycine and glutamine, leads to the formation of non-toxic products. When deamination of intermediate products of protein metabolism in hepatocytes, toxic ammonia is formed, which is inactivated due to the synthesis of urea from it, etc. (Tkachenko 2005).

Currently, it is believed that 80% of SMP are products of protein metabolism disorders, and an increase in the content during restorative-destructive processes is associated with enhanced proteolysis (Bergstom and Turst 1983; Drukker et al. 2012). It is possible that individual fractions of polypeptides of this group are the products of the vital activity of bacteria and viruses, and the spectra of SMP obtained during the biodegradation of fibrinogen, albumin, α2 -macroglobulin, and other proteins have a structure similar to known bioregulators: angiotensin, bradicanin, enkephalin, etc. (Jornvalin et al. 1981). In recent years, neurobiology has developed an understanding of the important role of relatively low and medium molecular weight peptides in processes specific to the central nervous system (CNS), such as learning, memory, emotional behavior, attitude to pain and in sleep processes (Koroleva and Ashmarin 2005).

Our previous biochemical, morphological and morphometric studies in various tissues (liver, heart, kidneys, brain and blood) have shown that protein-free nutrition causes a significant increase in the content of lipid peroxidation products (LPO) and SMP, a decrease in live weight and organ weight, an increase in the organ index and is accompanied by morphological changes of a dystrophic nature, i.e. protein-free food has a prooxidant effect. Protein nutrition, on the other hand, lowers LPO and SMP, increases live weight and organ weight, lowers the organ index and leads to morphological changes of a reparative nature, accompanied by functional hypertrophy of the cellular elements of internal organs, i.e., has an antioxidant effect (Kafarova and Askerov 2009).

To date, the biological effect of SMP has been studied in sufficient detail. Many of them have neurotoxic activity, inhibit the processes of protein synthesis, are able to suppress the activity of a number of enzymes, uncouple the processes of oxidation and phosphorylation, disrupt the mechanisms of regulation of the synthesis of adenyl nucleotides, act on phagocytosis, are able to block receptors of any cell and can interact with components of homeostasis systems (Nikonorow et al. 1973). Due to the presence of peptide bonds and cyclic amino acids in the structure of the SMP, the content of the SMP can be established by registering the effect of absorption of monochromatic ultraviolet light flux caused by it. In this case, it is possible to isolate three fractions of SMP molecules: a) bound to nucleic acids (at 230 nm), b) not containing amino acids - products of incomplete degradation of proteins, having a toxic effect (at 254 nm), containing non-toxic aromatic amino acids (mediators, hormones) (Kafarova and Askerov 2009).

Thus, various fractions of SMP can serve as a basis for judging the degree of damage to nucleoproteins and proteins of various tissues of the body, and also allow one to make certain judgments about the degree of oxidative modification of protein metabolites in various compartments of the cells of the body (Nikonorov et al. 1973).

The aim of this work was to study the effect of high-grade protein-vitamin nutrition and under conditions of 60% protein deficiency in the composition of the diet specially prepared for experimental animals according to Nikonorov's recipe in our modification on the MMP content in the liver tissue of white rats in within 20, 30 and 40 days (Nikonorov et al. 1973; Oyarzabal et al. 2019; Li et al. 2022).

MATERIAL AND METHODS

The study protocol was approved by the Azerbaijan Republic National Committee on Bioethics, Ethics of Sciences and Technology on the date 28 December 2021 with the decision number 03/21. The work was carried out on 45 outbred white rats against the background of full-fledged protein-vitamin nutrition and under conditions of 60% protein deficiency in the diet. The experimental animals were divided into 3 groups, 15 rats in each group. Each group was also subdivided into 3 groups of 5 rats each. The first group of rats in all subgroups served as controls, kept in a vivarium; rats of the 2nd group received a complete protein-vitamin diet; Group 3 received the same diet, but with a 60% casein deficiency in the diet. Experimental animals of groups II and III received food for 20, 30 and 40 days.

The content of medium-molecular peptides in the homogenate of the liver tissue of white rats was determined by the method of Ermakov at various wavelengths (230 nm, 254 nm and 280 nm) on a spectrophotometer (Cary WIN UV NON 21 CFR11, America) (Ermakova 2006).

The results were expressed in conventional units of optical density. Statistical processing of the results was carried out by the method according to the Student's t-test.

RESULTS

As can be seen from Table 1, against the background of 20 daily feeding of experimental animals with a full-fledged protein-vitamin complex, the content of fractions of MMP at a wavelength of 230 nm in comparison with control animals does not change and is 106%. And at 254-280 nm, the content of MMP tends to increase and is, respectively, 114-116% ($p<0.05$) compared to the control level. In rats fed food with 60% protein deficiency in the diet, the content of MMP fractions at 230nm significantly decreases to 74% ($p<0.5$), and at 254nm, the content MMP fractions is within the normal range, i.e. 100% ($p<0.05$), at 280 nm

the content of MMP fractions decreases to 85% ($p<0.01$) compared to control animals.

These data indicate that the observed changes in all MMP subfractions at 20-day feeding of animals with full-value protein-vitamin and 60% protein deficiency in the diet are within physiological limits. A significant decrease in the SMP of subfractions at a wavelength of 230 nm against the background of 60% protein deficiency in the diet is of a compensatory nature and is possibly associated with the detoxic properties of nucleoproteins in the nuclear apparatus of hepatocytes, and changes at a wavelength of 254 nm in subfractions, changes at the level of the norm of the MMP indicate that microsomal the level of protein renewal is not disturbed, and as for a certain significant decrease ($p<0.001$) at 280 nm in subfractions of the MMP to 85% is evidence of the normal course of intracellular mediator-hormonal regulatory processes in hepatocytes during these feeding periods of experimental animals.

Thus, with a 20-day high-grade protein-vitamin diet and under conditions of 60% protein deficiency in the diet, intracellular protein plasticity is not significantly impaired. At the same time, first of all, intracellular regulatory mechanisms of plasticity of protein synthesis are connected, in particular the content of peripheral and integral proteins in the membranes of hepatocytes of the liver of vertebrates (Medvedeva 1993), and there is also evidence of the important role of exogenously introduced low molecular weight and medium-molecular peptides in processes specific to the central nervous system, such as learning, memory, emotional behavior, attitude to pain and in sleep processes (Koroleva and Ashmarin 2005). A slight decrease in the content of SMP in subfractions 230 and 280nm is possibly associated with some disturbance of the transmembrane transport of proteins and protein biosynthesis against the background of 60% protein deficiency in the diet (Tanadenko and Guly 1973).

Based on the analysis of the data obtained, it can be concluded that 20 days of complete protein-vitamin nutrition and 60% of protein deficiency in the diet do not cause significant changes in protein biosynthesis at the microsomal level of hepatocytes and do not disrupt the exchange of aromatic amino acids, the synthesis of peptide hormones and mediators in hepatocytes, but lead to a moderate activation of nucleoproteins in the nuclei of hepatocytes, which is in good agreement with the literature data (Sushkova and Guly 1981).

As can be seen from Table 2, against the background of 30-day feeding of experimental animals of the 2nd subgroup with a high-grade protein-vitamin feed, the content of all 3 fractions of medium-molecular peptides at a wavelength of 230nm does not undergo significant changes in comparison with control animals, which, respectively, is 101%, and the content of 254 nm fractions is reduced to 70% in comparison with the control animals. The MMP content at 280 nm increases moderately.

The results of these studies indicate that against the background monthly full-fledged protein-vitamin feeding of animals the genetic apparatus functions within a genetically determined program. Therefore, there are no significant changes in the content of nucleotides. The observed decrease in the MMP at a wavelength of 254 nm indicates that no changes are observed in the products of defective protein breakdown with a toxic effect (amino acids) for this period of feeding. These data give reason to conclude that against the background of 30-day feeding of animals with a protein-vitamin complex, the mechanism of protein renewal at the microsomal level occurs within physiological limits, i.e. within the physiological deterministic program.

Moderate increase in aromatic amino acid content, i.e. non-toxic substances MMP (mediators, hormones) MMP in the spectrum of 280nm indicates that for this period of feeding, the inducing effect of exogenously introduced casein-vitamin nutrition is triggered in the liver tissues. We observed this natural mechanism in our early experiments with general starvation (Askerov 1991), where a phased mobilization of the hypothalamic-insular-adrenal and thyroid endocrine regulatory systems was found to mobilize metabolites from various tissues of the body. It should be noted that we observed this pattern during protein starvation in the structures of the central nervous system (Askerov and Akimova 2008). With prolonged protein starvation, a noticeable increase in the content of toxic fractions of MMPs was observed, and against the background of protein nutrition, a noticeable decrease in toxic fractions of MMPs was observed in comparison with control animals.

It should be noted one important point in our experiments against the background of 30 days of feeding, the content of 254 nm and 280 nm of MMP subfractions in both groups of experimental animals are within the normal range and lower in comparison with control animals. This state of subfractions 254 nm and 280 nm MMP in both groups indicates that for this period of nutrition, intracellular protein plasticity and the detoxification mechanism function within physiological limits. This state of the MMP can also be explained by the fact that the experimental animals simultaneously receive the entire set of fat-soluble and water-soluble vitamins in the feed. Therefore, for this period of nutrition, almost all intracellular processes associated with protein and energy plasticity and detoxification mechanisms function within the physiological limits of the norm, which is in good agreement with the literature data (Boldyrev et al. 2011; Fletcher et al. 2018) on the role of carnosine in restorative and protective processes. In the tissues of the body, especially in the neurodegenerative processes of the brain.

The results of studies on the background of a monthly feeding of animals with a protein-vitamin complex with a deficiency of 60% protein in the recipe showed that the content of MMP fractions at a wavelength of 230 nm significantly increases compared with the control group and is 124%. These data indicate that the genetic apparatus of hepatocytes senses a protein deficiency in the diet. Apparently, due to the lack of protein in the body, hepatocytes by modifying the genetic apparatus of protein synthesis switches to another level of protein plasticity. If, with a full-fledged protein diet, the microsomal level of protein synthesis from the MMP is directed towards detoxification, then at the level of protein deficiency in the diet it is directed towards the modification of intermediate products of protein metabolism and the normalization of the MMP content. The observed changes in subfractions at a length of 254 nm showed that the content of MMP is within the normal range compared to the control animals. The results obtained indicate that, against the background of a 60% protein deficiency in the diet, intracellular protein plasticity is not significantly impaired. These data show that the products of incomplete breakdown of proteins, which have a toxic effect, do not exceed the norm. However, at a wavelength of 280 nm, the content of MMP fractions significantly decreases, which may be a consequence of a significant weakening of the inducing effect on the mediator-hormonal status against the background of protein deficiency, and intracellular

adaptive-compensatory mechanisms are compensated by intracellular protein plasticity of hepatocytes.

As can be seen from Table 3, against the background of 40 daily protein-vitamin feeding of animals of the 3rd subgroup, the content of MMP subfractions increases to 114.9% in the liver compared to control animals at a wavelength of 230 nm. In the content of subfractions of MMP at a wavelength of 254 nm, almost the same regularity remains. At 280 nm, the content of subfractions increased significantly and amounted to 180.9% compared to the control animals. It should be noted that almost the same pattern persists against the background of protein deficiency in the recipe, because at 230 nm, the content of MMP increases (118%), and the content at 254 nm and 280 nm increases to 146.5 and 149.2%, respectively, compared with control animals.

The results of these studies indicate that 40-day feeding of animals with a full-fledged protein-vitamin diet and 60% protein deficiency causes significant changes in the MMP content at 254 and 280 nm wavelengths, and in the MMP subfractions at 230nm wavelength, there is a moderate increase in the MMP subfractions, which is possibly due to the peculiarities of the substitution of the genetic apparatus of hepatocytes. This indicates that 40-day feeding in both groups of experimental animals does not cause significant changes in the content of MMP, i.e., nucleoproteins of the nuclear apparatus of hepatocytes do not undergo oxidative modification in comparison with control animals. Thus, the obtained data indicate that that both types of nutrition contribute to an increase in the functions of the genetic apparatus within the physiological norm, i.e., the genetically determined function of hepatocytes is not disturbed after feeding the animals with a full-fledged protein-vitamin diet. And as for a more noticeable increase in the content of subfractions at 254 nm in both types of feeding, an increase in the products of incomplete breakdown of proteins, which have a toxic effect within certain limits, ie. 146.5-147% in comparison with control animals indicate that in the microsomal apparatus of hepatocytes there are apparently certain limits of enzymatic activity of proteolytic enzymes that contribute to the renewal of the pool of microsomal proteins. We encountered such cases in our previous experiments, carried out at different periods of protein starvation in the central nervous system (Ibragimova et al. 2011). A more noticeable increase in the content of MMP subfractions at 280 nm (up to 180%) in comparison with control animals indicates that against the background of a longer period of feeding with a protein-vitamin diet, in addition to participation in the normal course of metabolic processes, exogenous casein behaves as an inducing factor for mobilization endocrine glands and metabolites from other body tissues. A number of authors have proven that selective induction of enzymatic activity closely depends on hormonal activity and dietary factors (Kritsman and Konkova 1968).

Table 1. The content of MMP in the liver of white rats against the background of a 20-day full protein-vitamin complex and 20-day deficiency of 60% protein in the diet (conventional unit).

No	LIVER			
	Experience condition	**Wavelength - γ**		
		230 nm	**254 nm**	**280 nm**
	I group	0.427 ± 0.07	0.818 ± 0.035	0.196 ± 0.004
	II group	0.453 ± 0.065	0.940 ± 0.009	0.227 ±0.007
1.	**%**	106	114	116
2.	**p**	<0.5	<0.05	<0.01
	III group	0.316 ± 0.02	0.821 ±0.045	0.167 ±0.004
3.	**%**	74	100	85
4.	**p**	>0.05	<0.05	<0.01

1, 3 is the percentage of comparison of II and III groups with I group; 2, 4 - reliability of comparison of II and III groups with I group.

Table 2. The content of MMP in the liver of white rats against the background of a 30-day full protein-vitamin complex and 30-day deficiency of 60% protein in the diet, (conventional unit).

No	LIVER			
	Experience condition	**Wavelength - γ**		
		230 nm	**254 nm**	**280 nm**
	I group	0.416 ± 0.09	1.114 ± 0.02	0.133 ± 0.0002
	II group	0.421 ± 0.003	0.783 ± 0.20	0.147 ± 0.03
1.	**%**	101	70	111
2.	**p**	>0.05	<0.01	<0.05
	III group	0.517 ± 0.05	1.156±0.0	0.07± 0.003
3.	**%**	124	103	52
4.	**p**	<0.01	>0.0	<0.001

1, 3 is the percentage of comparison of II and III groups with I group; 2, 4 - reliability of comparison of II and III groups with I group.

Table 3. The content of MMP in the liver of white rats against the background of a 40-day full protein-vitamin complex and 40-day deficiency of 60% protein in the diet (conventional unit).

No	LIVER			
	Experience condition	**Wavelength - γ**		
		230 nm	**254 nm**	**280 nm**
	I group	0.408±0.005	0.760±0.02	0.142±0.004
	II group	0.469±0.03	1.1178±0.18	0.257±0.06
1.	**%**	114.9	147	180.9
2.	**p**	>0.05	>0.05	>0.05
	III group	0.482±0.02	1.1134±0.119	0.212±0.029
3.	**%**	118	146.5	149.2
4.	**p**	>0.05	<0.05	<0.01

1, 3 is the percentage of comparison of II and III groups with I group; 2, 4 - reliability of comparison of II and III groups with I group.

DISCUSSION AND CONCLUSION

The inductive effect of dietary factors on various enzymes of amino acid metabolism has been discovered by a number of authors. Thus, feeding rats for 7 days with protein food containing 90% casein leads to a significant increase in the activity of tyrosine dehydrogenase. The authors, continuing experiments in this direction, showed that even a single administration of growth hormone or testosterone to thyroidectomized, hypophysectomized, and castrated rats causes a significant increase in the activity of Mg+2 activated by RNA polymerase of the liver nuclei. In this case, there is a proportionality between the degree of changes in liver growth and the activity of enzymes. A large number of works are devoted to the study of induced enzymes in the animal body, the inducible enzymatic activity of tryptophan hydroxylase in the liver and tyrosine aminotransferase, which play a key role in the synthesis of aromatic amino acids; as well as urea and other non-toxic amino acids. As these authors show, hydrocortisone is an almost universal inducer. It has superconjugated bond systems, which make it possible to be an active carrier of electrons (Kritsman and Konkova 1968).

Thus, aromatic amino acids play a special role in the protein molecule. They are separate islands of conjugated structures in the saturated chain of a protein molecule. These include phenylalanine, tyrosine, histidine, and tryptophan. All of these amino acids have either a benzene or indole ring as a component (Kritsman and Konkova 1968).

When studying the electron-donor properties, the ability of this amino acid to interact with substances such as the redox coenzymes flavin mononucleotide (FMN), nicotinamide adenine dinucleotide (NAD), nicotinamide adenine dinucleotide phosphate (NADP), which has a low free molecular orbit (Kritsman and Konkova 1968).

It is interesting to note that the product of the conversion of tryptophan arising from the cleavage of its side chain, indoleacetic acid, is a strong stimulator of cell growth. It is highly likely that this function is due to its special electronic structure. Also necessary is the electronic properties of the imidosal ring of histidine, which is characterized by a special behavior of the tertiary and secondary nitrogen atoms. The tertiary nitrogen atom of this ring, giving off a p-electron (p-electron) to a conjugated orbit, has a total negative charge and is therefore an electrophilic attack bridge, while another nitrogen atom, which has an indivisible pair of n-electrons in a conjugated orbit, has a positive charge and tends to replace the proton with other groups. The subsequent rearrangement of electrons inside this molecule leads to the fact that the initial pyridine nitrogen atom becomes parallel and vice versa. These properties of the nitrogen atoms of the imidazole ring play an important role in the formation of high-energy phosphate bonds and mainly in the catalytic function of that amino acid residue, since the histidine residue is the main part of the hydrolytic amino acids. Therefore, when assessing the value of the electronic structure of aromatic amino acids, it is necessary to take into account this feature of the electrical processes occurring in complex molecules, combined with a cooperative effect, conformational shifts, and a number of other structural transformations of the protein that enhance the effect of the electron donor function of these conjugated chemical compounds. An essential feature of such groups is that the atoms that are part of them, i.e. carbon and nitrogen have a positive charge and, consequently, are ruptured by the action of hydrolyzing enzymes. A striking example of double-positive bonds is the peptide bond (Kritsman and Konkova 1968). It should be noted that the double-positive relationship is an insignificant factor in determining enzymatic hydrolysis. This process is influenced by the geometry of the molecules, the degree of polarization, electrical interactions within the molecule, and electrical forces acting at a distance. All these factors, in the case when they contribute to an increase in the positive charge, complement each other's actions in the direction of increasing the activity of the enzyme. It is likely that the interaction of the substrate with the enzyme involves not only the active center of the enzyme, but also other parts of its molecule, which in turn can change the rate of enzymatic catalysis. At the same time, the leading moment that determines the enzymatic hydrolysis reaction as such is the deficiency of electrons conjugated with the two-positivity of atoms that are involved in the formation of bonds. It is possible that during transreactions this moment is also essential, determining the initial stage of this reaction, which may end not with the breaking of the bond, but with the formation of a new connection between the parts of the reacting molecules (transamination reaction).

Induction is always associated with qualitative and quantitative changes in the induced enzyme, therefore the

mechanism of this phenomenon is closely related to the laws that determine the dynamism of the action of enzyme systems (Kritsman and Konkova 1968). In this regard, induction may depend on an increase in the reactivity of the enzyme protein molecules, since the catalysis carried out occurs with their direct participation in the formation of an intermediate complex with a substrate.

The reactivity of complex protein molecules is largely determined not only by the dynamics of submolecular processes, in particular, by the features of the electronic structure, but also largely by the spatial arrangement of their constituent parts.

Thus, in protein molecules in which double bonding to carbon-oxygen atoms occurs, a change in the rotation at the level of peptide bonds can occur; this is apparently very important for the manifestation of the variability of biological, including catalytic, properties of protein molecules.

The observed increase in the number of MMP fractions at 280 nm can be associated with limited proteolysis at the level of protein molecules in hepatocytes in animals that received complete protein nutrition in the form of a protein (casein) - vitamin complex and 60% protein deficiency.

The scientific positions put forward by us are supported by literary and our research (Kritsman and Konkova 1968; Bernhard 1971; Askerov et al. 1990; Askerov et al. 1991) which note that almost all proteolytic enzymes have some of the same type of polypeptide chains. It has been shown that trypsin, chemotrypsin, trypsin, and elastase contain the same peptide chain, which is directly related to the active center of these enzymes. In addition, they all contain a histidine residue, which is located in a different part of the polypeptide chain, but which approaches the above fragments due to the geometry of the molecules, due to the secondary and tertiary structure of the protein. Due to this, the imidazole ring of histidine contributes to the high reactivity of the serine residue, which is an integral part of the active center, probably by increasing its nucleophilicity. It is quite possible that factors that change the secondary and tertiary structures of proteolytic enzymes, for example, changes in the ratio of conformational forms of peptide fragments that make up the active center of enzymes, as well as hydrophobic bonds and a number of other phenomena associated with a change in the balance of processes within the molecule can affect the manifestation of the activity of these enzymes, i.e., on the rate of reactions catalyzed by them. From this point of view, all influences leading to an increase in the nucleophilicity of active centers participating in the attack can be considered as prerequisites for the induction of these biocatalysts (Kritsman and Konkova 1968). We also believe that a partial deciphering of the mechanism of action of proteolytic enzymes in terms of the concepts of quantum mechanics is one of the significant advances in this area. Much more developed thanks to a series of studies by Pullmann (Pullmann and Pullmann 1965), the quantum-biochemical basis of the mechanism of action of coenzymes. Almost all enzymes work in conjunction with coenzymes, although there are significantly fewer coenzymes than enzymes (Bezerra et al. 2022; de Paula Junior et al. 2022). Considering the fact that in our experiments the recipe we compiled included the entire set of fat-soluble and water-soluble vitamins, it becomes necessary to give some clarification about the role of vitamins in the oxidative modification of peptides in various tissues of the body. In this aspect, let us dwell on the role of folic acid in the catalysis of many reactions. This compound is a condensation product of three components: a substituted pterizin ring, a para-aminobenzoic acid residue, and a glutamic acid residue. The author believes that when catalyzing the transformation of a number of substrates with the participation of folic acid into various hydroforms, this, in turn, can change the degree of manifestation of the catalytic activity of both proteids and other folic acid derivatives. Under conditions when the less active form of this coenzyme is converted back into a more active one, which can take place under the action of various environmental factors, the activity of the enzyme system increases, i.e. the reaction rate increases, and this phenomenon can be characterized as induction (Puhlmann and Puhlmann 1965). The observed effect in the subfractions of the MMP at 280 nm of changes in substrate-enzyme relationships is associated with the content of non-toxic aromatic amino acids (mediators, hormones), which play an important role in synchronizing and desynchronizing factors in the intercellular environment of hepatocytes - as monoamines and other factors (Nechaeva et al. 2005). Brodsky et al. (2013) believe that dopamine disorganizes the rhythm of protein synthesis and disrupts the self-organization of hepatocytes (in vitro). The authors believe that dopamine disorganizes the conditioning of the extracellular environment by gangliosides. The lack of this endogenously synchronizing effect in the intercellular environment blocks the self-organization of the protein synthesis rhythm (Brodsky et al. 2005).

Thus, unlike (previously studied) norepinephrine and serotonin, gangliosides, these regulate the population rhythm of protein synthesis, while dopamine disrupts the rhythm and causes direct intercellular interactions. And also in the literature there is evidence that there is a relationship between the level) of circulating corticosterone and the level of carbolation of proteins in the liver during short-term hypokinesia. An increase in the duration of hypokinesia led to an aggravation of violations of the negative feedback mechanism in the hypothalamus-pituitary-adrenal axis and an increase in protein carbolation in the liver (Tseilikman et al. 2013).

All these data indicate that a longer use (40 days) of a full-fledged protein-vitamin complex contributes to a noticeable violation of genetically determined programming of protein metabolism in the body. Longer use of a full-fledged protein-vitamin complex leads to a significant increase in the number of subfractions at 280 nm, changes in substrate-enzyme relationships (apparently, the intracellular fund of proteolytic enzymes begins to deplete). Therefore, the exogenously introduced protein (casein) is not fully hydrolyzed to amino acids, and against this background, the amount of under-oxidized proteins in the liver increases, which contributes to an increase in the number of subfractions at a wavelength of 280 nm. This state of non-toxic amino acids (phenylalanine, tyrosine, tryptophan, and histidine) of mediators and hormones is apparently necessary to connect the inducing effect of the protein-vitamin complex on the secondary and tertiary structures of enzymes that change the ratio of conformational forms of peptide fragments at the level of microsomal and cytosolic peptides in hepatocytes. Due to the presence of peptide bonds and cyclic amino acids in the structure, the content of average weight molecules can be established by registering the effect of absorption of monochromatic ultraviolet light flux caused by it. Isolation of three SMP fractions at different wavelengths 230 nm, 254 nm, and

280 nm, i.e. spectra of SMP can serve as a basis for judging the degree of damage to nucleoproteins and proteins of various tissues of the body, as well as (allow us to make certain judgments) oxidative modification of protein metabolites in various compartments of hepatocytes.

These scientific provisions set out in this article are in good agreement with our earlier studies (Askerov et al. 2018a; Askerov et al. 2018b; Bakshaliyeva 2020; Ibrahimova 2020; Askerov et al. 2021).

CONFLICTS OF INTEREST

The authors declare that there were no conflicts of interest in the realization of this research.

AUTHOR CONTRIBUTIONS

Idea / Concept: FA
Supervision / Consultancy: FA, HK, GÇ
Data Collection and / or Processing: FA
Analysis and / or Interpretation: FA
Writing the Article: FA, HK, GÇ
Critical Review: HK, GÇ

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