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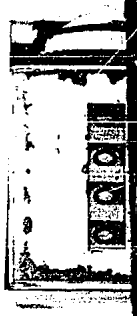

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## RECENT USSR DEVELOPMENTS IN THE FIELD OF PROTEIN CHEMISTRY

[Comment: Work on atomic energy has had a considerable influence on recent USSR research dealing with proteins. First, the availability of radioactive isotopes has enabled USSR investigators to use newer and more precise methods for the investigation of the behavior and biological synthesis of proteins under normal and pathological conditions. Secondly, radiation damage as it affects the tissue proteins of the body has apparently been subjected to intensive investigation. In order to explain why proteins suffer very little apparent [illegible] in vitro on exposure to ionizing radiation, while relatively severe local injury is inflicted in vivo, this injury being greater in the case of higher animals than of those which are low on the evolutionary scale, an attempt has been made to study functional effects of proteins and changes in these functional effects produced by the action of radiation. Obviously, damage to a single functional group or to a relatively small number of functional groups would have a greater effect both centrally and locally on a complex organism with many interdependent functions than on a more primitive and less highly integrated organism.

Because of the well-known protective effect exerted by cysteine and other thiol compounds and by reason of the significance attached to sulfhydryl groups in the transmission of nerve impulses by mediators, the study of changes in these groups and in their functioning under the effect of damaging radiation or because of other influences has received a great deal of attention. Clear results along these lines have not been obtained, because ionizing radiation may even increase the number of free sulfhydryl groups by causing denaturation of tissue proteins, while at the same time the active sulfhydryl groups which are of functional importance may be inactivated. Under the circumstances there is a tendency in USSR work to develop more precise chemical, biophysical, and physiological methods for the determination of sulfhydryl groups and, by using these methods, to attempt differentiation between more active and less active groups.



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In addition to the work on such methods outlined below, research by A. I. Smirnova-Zamkova on the so-called "argyrophilic substance," which in this investigator's opinion is identical with the sum total of active sulfhydryl groups present in proteins, may be mentioned as an example (Kh. S. Koshtoyants, "Sulfhydryl Compounds in Theory and Practice," Meditsinskiy Rabotnik, Vol 18, No 85, 18 October 1955). Besides contributing to a better understanding of radiation effects, work along these lines has also furnished data that have a bearing on the interdependence between the action of acetylcholine as a nerve mediator and the availability of free and active sulfhydryl groups.

Since the full syndrome of radiation injury cannot be assumed to result solely from the action of toxic substances formed in the process of denaturation of body proteins, local damage to tissue proteins must play a role in the development of this syndrome in addition to central effects (see Frank's discussion of the subject in the text of the report). Damage to the active groups of enzymes may take place and cause serious injury, because the total mass of proteins is assumed to possess enzymatic activity (see Oparin's statement to that effect and the account of Engel'gardt's work in the text of the report). Results of the type mentioned and reasoning along these lines have induced USSR investigators to pay increased attention to damage inflicted by radiation on the polynucleic acid moiety of nucleoproteins and to inactivation of prosthetic groups of enzymes resulting from exposure to damaging doses of radiation.

To counteract the inactivation of enzymes, vitamins capable of restoring the prosthetic groups can be applied. Some vitamins may exert a direct chemical effect on proteins in vitro and thus protect them against damage caused by radiation (see Oparin's statement to that effect in the text of the report).

If it is assumed that radiation inactivates sulfhydryl groups, the activity of ATP, which depends on the presence of sulfhydryl groups, will also be impaired. Under the circumstances, it ought to be possible to restore the function lost because of partial inactivation of sulfhydryl groups by treating the tissue with ATP, particularly if simultaneous replacement of sulfhydryl groups by adding thiol compounds such as cysteine is also attempted. Experiments along this line have actually been carried out by USSR workers in the case of partially denatured actomyosin (see description of work by I. I. Ivanov and Yu. Torchinskiy in the text of the report).

According to experiments carried out by V. A. Engel'gardt and members of his group in the Laboratory of Biochemistry, Physiological Institute imeni I. P. Pavlov, Academy of Sciences USSR, myosin solutions, on being photosensitized by means of methylene blue and exposed to the action of light, undergo changes which bring about an increase in viscosity. Addition of ATP reduces the viscosity, restoring it to the original value. In view of the fact that no increase of viscosity takes place in the absence of oxygen, Engel'gardt interprets his results by assuming that the combined action of light and of oxidation agents impairs certain active groups of the myosin, possibly the sulfhydryl groups, with the result that the capacity of myosin to combine with itself, just as it combines with actin, is increased. At the same time, the adenosinetriphosphatase activity of the myosin is reduced. Addition of ATP reverses the changes produced by the photosensitized oxidation (V. A. Engel'gardt, N. S. Demyanovskaya, T. V. Venkstern, "The Photosensitizing Action of Methylene Blue on Myosin," Doklady Akademii Nauk SSSR, Vol 72, No 5, 11 June 1950, pp 923-926).

Engel'gardt's investigation is only a single instance of rather extensive research done by USSR workers on the interaction between myosin, ATP, dyestuffs, and light. At a later stage of work on this subject extending over the period 1950-1954, no connection between sulfhydryl groups and the mechanism of photooxidation could be found (cf. Study of Soviet Biochemistry, DSI Report No 5/55,

Ottawa, May 1, 55, p 11.) Although the intention to investigate the mechanism of radiation injuries is not mentioned explicitly in any statement made by the USSR investigators, results obtained in work of this type and in this general field have a direct bearing on the action of penetrating (ionizing) radiation on body tissues.

Numbers in parentheses refer to appended sources.]

General Aspects of Research on Proteins

Recent progress and current trends in USSR research on protein chemistry were outlined as follows by V. Orekhovich subsequently to the 8th All-Union Congress of Physiologists, Biochemists, and Pharmacologists, which was held in the first part of 1955.

He stated that although a considerable amount of work has been done on proteins and protein metabolism, important questions such as the exact manner in which proteins are synthesized in the body and undergo metabolic trnsformation there have not been clarified as yet. While some scientists assume that inclusion of individual amino acids takes place only in the process of neoformation of protein molecules, others maintain that individual amino acids may be taken up or eliminated independently, thus keeping the composition of the protein molecules constant by restoring them rather than synthesizing them anew.

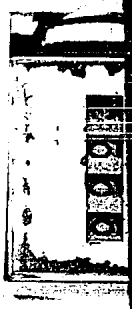
An extreme view is held by A. S. Konikova, M. S. Kritsman, and members of their group, who assume than even isolated, pure proteins are capable of restoring their amino acid composition, i.e., that these proteins possess properties of living matter. Konikova et al. arrived at this conclusion on the basis of the observation that amino acids containing tracer atoms readily form compounds with proteins. They disregarded the fact that proteins form complexes with a great number of other substances and that formation of these complexes has nothing to do with the synthesis of proteins or their restoration in the body.

Important data on the capacity of amino acids to form polypeptides in vitro were presented at the congress. Scores of polypeptides have been synthesized, e.g., polyglutamic acid (a peptide with a molecular weight of 15,000) polylysine (a peptide consisting of 32 lysine residues), and polyalanylglycylglycine (a peptide with a molecular weight of about 6,000). It was found possible to combine a protein enzyme such as chymotrypsin with scores of glycine molecules without impairing its biological activity.

The polypeptides which have been mentioned are readily split by enzymes. The X-ray analysis of these polypeptides (e.g., polyalanine and polyphenylalanine) has demonstrated that they are similar in structure to some proteins. It follows from this that even formation of peptides from amino acids or their derivatives cannot be regarded as manifestation of vital activity.

It was shown in a number of reports presented at the congress (those by V. A. Engel'gardt, M. M. Sisakyan, R. B. Khesin, A. Ye. Braunshteyn, S. Ya. Kaplanskiy, and others) that the vital activity of living substance does not come into play in solutions of individual proteins, but in complex systems which contain various proteins and many other substances besides.

Experiments conducted by M. M. Sisakyan and R. B. Khesin demonstrated in a particularly striking manner that the biosynthesis of proteins takes place in definite structural elements of the cell and that microsomes, mitochondria, and plastids play an important part in the processes involved in this biosynthesis. Khesin reported that the secretory granules of pancreas cells are capable of synthesizing proteins, specifically amylase, outside of the organism. In order that this synthesis may take place, the medium must contain substances



that are formed by mitochondria in the presence of adenosine triphosphoric acid and some other substances. Khesin also demonstrated that in liver cells special granules are found, the principal function of which is the synthesis of proteins.

V. A. Engel'gardt emphasized in his report that the ideas which have been developed on the functioning of the biologically active proteins which act as enzymes presuppose that the enzymes are present in the cell not merely as ingredients of a mechanical mixture, but as components of functional systems in the formation of which other substances besides proteins take part.

The problem of the restoration or regeneration of proteins in the body, which has already been mentioned above, formed the subject of an extensive discussion at the congress. If an amino acid containing radioactive carbon as a tracer in introduced into the body, the amino acid which has been labeled in this manner can soon be found as a component part in the proteins of all tissues of the body.

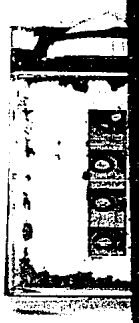
This led US investigators to the conclusion that proteins are restored by the elimination and subsequent inclusion of individual amino acids without prior decomposition and renewed formation of the protein molecules. Some USSR scientists also adhere to this point of view. However, experimental data which have been obtained in recent years refute the assumption that there can be any restoration of proteins without decomposition and resynthesis.

Such data have been obtained in particular by workers at the Institute of Biological and Medical Chemistry, Academy of Medical Sciences USSR. They found that when amino acids labeled with radioactive carbon and radioactive sulfur are introduced into a fertilized chicken's egg, and the egg is then incubated, only the embryo acquires radioactivity, while the yolk and the egg-white remain inactive. The reason is that synthesis of proteins takes place in the embryo but not in the yolk or egg-white. When radioactive tyrosin had been administered to a chicken 16 hours prior to the laying of an egg, i.e., at a time when the egg itself (ovocyte) had already formed, but the egg-white was just beginning to form, the egg-white and not the yolk of the egg was found to be radioactive.

Experiments carried out on animals suffering from scurvy also speak against the hypothesis of protein restoration. In the bodies of animals which have scurvy formation of collagen fibers does not take place, because no procollagen is formed. When labeled amino acids are administered to such animals, they enter into all tissues of the body with the exception of skin collagen and procollagen.

According to Brekhovich, the information presented at the congress shows how carefully one must use and evaluate the results of work with labeled amino acids. When a protein precipitate is found to be radioactive after incubation of a protein solution with a labeled amino acid, this does not mean that the amino acid has been embodied in the protein; formation of a complex has taken place or else contamination with bacteria may have occurred, with the result that the labeled amino acid has been utilized by these bacteria in the synthesis of their own proteins.

Orekhovich continued his account of the congress by discussing recent work on the structure of proteins. He stated that although the polypeptide theory has been fully proven at present, some long protein molecules (e.g., insulin) must be regarded as consisting of several parallel chains, mentioning as an example that there are four parallel chains in insulin, a protein the amino acid composition of which has been completely clarified. He added that the amino acid composition of the proteid pituitary hormones oxytocin and



vasopressin has also been completely clarified, with the result that the synthesis of these hormones could be accomplished. Orekhovich concluded by saying that although research on the chemistry of proteins is carried out on an extensive scale at the Institute of Biological and Medical Chemistry, Academy of Sciences USSR, work in this important field must be pursued to a more adequate extent elsewhere. To indicate the inadequate extent of work on the structure of proteins, Orekhovich mentioned that only one paper in this field was given at the congress, i.e., "Concerning the Structure of Silk Fibroin" by K. G. Ioffe. (1)

The data on the restoration of proteins given by Orekhovich in this instance were also reviewed by him in another paper of which he was the author and which was presented by A. M. Kuzin at the Geneva Conference on the Peaceful Utilization of Atomic Energy. (2)

Radiation Effects and the Structure and Biological Functions of Proteins

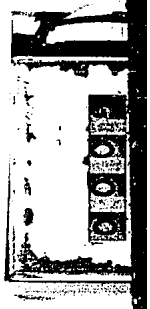
At the Moscow Conference on the Peaceful Utilization of Atomic Energy, the chemical changes produced in proteins by ionizing radiation were discussed. In view of the fact that no peptide linkages are broken in proteins exposed to ionizing radiation in vitro (A. S. Pasynskiy), the biological effect of radiation on tissue proteins can be explained most logically by assuming that active groups are damaged. These active groups may be the nucleic acids of nucleoproteins (A. M. Kuzin) or sulfhydryl groups, to which a specific role is ascribed in the transmission of nerve impulses (Kh. S. Koshtoyants, G. M. Turpayev, and D. Ye. Rivkina) and which are transformed into disulfide groups in the process of keratinization (S. D. Balakhovskiy and I. V. Kuznetsova). In Kuzin's opinion, an important aspect of the action of radiation consists in the damage inflicted on enzymatic systems. (3)

A. I. Oparin, in reviewing the work done during 1954 at the Department of Biological Sciences, Academy of Sciences USSR, stated that it follows from work done at the Institute of Biochemistry that the harmful action of X rays on proteins is determined not only by the presence of sulfhydryl groups, as some foreign investigators believe. He added that work done on the subject of radiation damage led to the finding that vitamins P and $B_6$ protect proteins against "aggregation" [i.e., apparently denaturation] when solutions of these proteins are exposed to radiation.

In outlining USSR progress in work on proteins, Oparin also mentioned inclusion of individual enzymes or groups of enzymes into artificial proteins and work on isolated mitochondria and plastids.

Commenting further on the functions of sulfhydryl groups contained in proteins and amino acids, Oparin stated on the basis of work carried out at the Institute of Animal Morphology, Academy of Sciences USSR, that substances containing free sulfhydryl groups counteract the toxicity of streptomycin, particularly its specific neurotoxic action, and exhibit a pronounced chemotherapeutic activity in diseases brought about by either gram-positive or gram-negative bacteria.

According to Oparin, it has now been established that the biological activity with which some protein enzymes are endowed is not the exclusive property of substances which were regarded as enzymes hitherto: proteins that were considered inert also possess enzymatic activity. As stated by Oparin, it follows from this that all proteins possess catalytic properties, i.e., enzyme activity, to a certain extent. (4).



As far as the enzymatic activity of proteins is concerned, V. A. Engel'gardt in a recently published article cites new experimental data supporting the view that ordinary muscle myosin exerts the enzymatic activity of adenosinetriphosphatase and is, in fact, identical with this enzyme. The purpose of the investigation described by him was confirmation of the results obtained by K. Bailey and S. V. Perry Acta, Vol 1, p 506, 1947), who found that the sulfhydryl groups of myosin must be preserved in order that this protein retain its capacity to decompose adenosinetriphosphate (ATP) and to combine with actin.

In their investigation Bailey and Perry inactivated the sufhydryl groups with parachloromercuribenzoate. By using different thiol poisons (silver and cadmium ions) and applying other methods of inactivating the myosin, Engel'gardt arrived at results which indicate a complete parallelism between the capacity of myosin to decompose ATP and its capacity to combine with actin. This confirmed the data to that effect obtained by Bailey and Perry.

Engel'gardt concludes from his results that both types of activity pertain to the same protein, which he calls eromyosin, and expresses the opinion that the reaction of myosin with the free nucleotide ATP must be similar to its reaction with the adenine nucleotide which forms the prosthetic group of actin. He further draws a distinction between the desaminase activity of muscle myosin, which is not a property of eromyosin, but that of another protein bound to it, and its adenosinetriphosphatase activity, which is a property of eromyosin itself, and states that this distinction is valid, although the protein having desaminase activity is so firmly bound to myosin that only denaturation of the myosin destroys the desaminase activity just as it destroys the adenosinetriphosphatase activity. (5)

Functions of Sulfhydryl Groups and the Role of These Groups in Radiation Damage

G. M. Frank points out that the activity of adenosinetriphosphatase is strongly affected by ionizing radiation, because this activity depends on the presence of free sulfhydryl groups in view of the fact that adenosinetriphosphatase is a sulfhydryl enzyme. It has been established that the ionic yield (i.e., the number of molecules of the enzyme inactivated by one pair of ions) is 0.93 for the HS-enzyme adenosinetriphosphatase, while it is only 0.025 for trypsin, an enzyme the activity of which is not connected with the presence of sulfhydryl groups.

As a further argument in favor of the assumption that the inactivation of sulfhydryl groups plays an important role in damage caused by radiation, Frank mentions the fact that thiol compounds such as cysteine protect animals against harmful effects produced by radiation. He states that in cases when cysteine has been administered to experimental animals prior to irradiation, a 100% rate of survival is achieved, although the dose of radiant energy to which the animals have been exposed would otherwise have been lethal.

Frank goes on to say, however, that data have been obtained recently which conflict with the view in regard to the exclusive role of the inactivation of sulfhydryl groups in radiation damage. To substantiate this statement, he says that although in model experiments oxidation of HS-groups proceeds with high ionic yields when solutions of substances are exposed to radiation, inactivation of HS-groups in vivo could for all practical purposes not be established. Even in laboratory tests the ionic yields are sharply lowered when other substances are admixed to the solutions. Furthermore, other substances besides cysteine exert a protective action and this action is even stronger than that of cysteine.

Frank concludes from this that although inactivation of HS-groups plays a certain role in the biological effects produced by radiation, other substances besides sulfhydryl compounds are also oxidized in vivo. He refers to the view held by B. N. Tarusov and other investigators that the presence of strongly acting oxidizing agents, such as the hydrogen peroxide which is assumed to form under the action of ionizing radiation, leads to the formation of toxic substances in the course of a self-accelerating chain reaction. That such toxic substances are present in the blood of animals subjected to radiation could be shown in experiments in which the blood of irradiated animals was transfused to animals that had not been irradiated or the blood circulation systems of two animals were joined and one animal was irradiated while the other was shielded from the radiation.

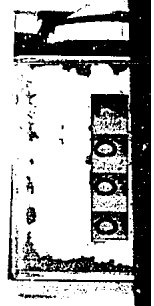
However, in view of the fact that the full syndrome of pathogenic raidation effects could not be reproduced when the toxic substances that had formed were introduced into the blood stream of healthy animals by these means, Frank assumes that the changes which take place in the body as a result of the direct action of radiation on the tissues are also of importance. (6)

Workers at the Bacterio-Serological Laboratory, Central Scientific Research Roentgeno-Radiological Institute of the Ministry of Health USSR, investigated the antigenic specificity of the denatured proteins formed in the serum of experimental animals (rabbits) under the effect of X rays. They found that the proteins denatured by X rays have no antigenic specificity: the antibodies formed in their presence are identical with those formed when the proteins have been denatured by other means. (7)

To establish with precision whether a protein has been denatured or not, a method whereby the activity acquired by the protein as a result of the absorption of methionine labeled with radioactive sulfur is measured has been developed at the Institute of Biochemistry imeni A. N. Bakh, Academy of Sciences USSR. Denaturation of proteins with X rays and ultraviolet rays was carried out in the course of the experimental work leading to the development of this method and the denaturation produced in this manner studied. The authors believe that the sulfhydryl groups of the proteins which are exposed or activated in the process of denaturation react with the sulfur atoms of the methionine, thus binding the latter compound. (8,9)

In view of the functional importance which free sulfhydryl groups are assumed to have in many biochemical processes (cf. Kh. S. Koshtoyants's work, who found that tissue HS-groups are essential for the realization of the most diverse functions of the animal organism, including those of the nervous system), an attempt was made to develop a reliable method for the detection of these groups in histological work. The following procedure, by means of which surfaces where free sulfhydryl groups are present can be stained, was found satisfactory for this purpose: the sulfhydryl groups are reacted with beta-hydroxynaphthylmercuric chloride and the mercaptide which has been formed brought into reaction with doubly diazotized ortho-dianisidine.

The number of HS-groups which are present influences not only the depth of the staining, but also the shade: when only a few HS-groups are present, and these are rarely spaced, a red-colored monoazo-dyestuff results, while a dark-violet bis-azodyestuff is formed when the HS-groups are closely spaced. When tissue slices had been treated with thiol poisons (mercuric chloride, iodoacetate, or allyl isothiocyanate), staining by this method could not be accomplished. On the basis of work done by Kh. S. Koshtoyants and T. M. Turpayev, experiments were carried out to determine the localization of the HS-groups in a frog's heart the blocking of which produces elimination of the suppression of contractions in response to perfusion with acetylcholine and suppression of rythmic contractions culminating in a complete stoppage of the heart in the systolic phase.



It was found that the HS-groups which are responsible for both phases of heart action are located in the endocardium and the trabeculae nearest to it. However, the HS-groups of the structures which respond to the action of acetylcholine (i.e., to impulses originating from the vagus) are more reactive and fewer in number than those which are responsible for the automatic contractions of the heart. (10)

By using as a thiol poison mercuric chloride that contained the radioactive isotope $Hg^{203}$, T. M. Turpayev of the Laboratory of General and Comparative Physiology, Institute of Animal Morphology imeni A. N. Severtsov, Academy of Sciences USSR, arrived at a quantitative estimate of the number of sulfhydryl groups which must be blocked in order that the heart muscles become insensitive to acetylcholine. (11)

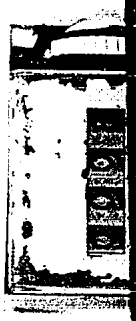
E. Ya. Grayevskiy and L. I. Korchak of the Institute of Animal Morphology imeni A. N. Severtsov used the ferricyanide method and determined sulfhydryl groups photocolorimetrically in extracts in their work on changes in the content of sulfhydryl groups under the effect of lethal doses of radiation. They observed no significant changes immediately after irradiation, but found that a considerable reduction of the sulfhydryl group content took place in the spleen, liver, and lungs of the animals 3 hours after exposure to lethal doses of radiation. They regard this reduction of the sulfhydryl group content as a secondary effect and conclude from their findings that relatively small, but biologically active doses of ionizing radiation cannot be assumed to produce selective damage of sulfhydryl enzymes in vivo.

As far as the protective action of cysteine and some other thiol compounds is concerned, Grayevskiy and Korchak are of the opinion that this action is not bases on replacement of damaged sulfhydryl groups, but most probably on chemical reduction. They point out that the protective action of a substance does not depend solely on the presence of a free sulfhydryl group in it, because other compounds which are similar to cysteine in composition and also contain sulfhydryl groups (e.g., dimethylcysteine, beta-mercaptopropionic acid, BAL, and thiourea) exert no protective action. (12)

According to a statement made by Yakovlev and Sokolovskiy, the ferricyanide method for the determination of free sulfhydryl groups (used by Grayevskiy and Korchak) has been found unreliable in histological work when used for the staining of tissue slices. (10)

Using the ferricyanide method and also the method of staining with Nile blue for the determination of HS-groups, N. N. Demin of the Institute of Animal Morphology imeni A. N. Severtsov investigated the effect of acetylcholine on the availability of free HS-groups in animal tissues. He found that although acetylcholine did not exert any denaturing effect on tissue proteins (as he originally assumed), thus exposing additional sulfhydryl groups it did increase the reactivity of these groups as measured by their capacity to be oxidized with ferricyanide. (13)

I. I. Ivanov and Yu. Torchinskiy established that stretched and partly denatured actomyosin preparations regain their capacity to contract under the effect of ATP after they have been reactivated with cysteine. The paper on the subject written by these two investigators has been published as a joint contribution from the Chair of Biochemistry, First Moscow Medical Institute, and the Chair of Radiation Biochemistry, Central Institute of Advanced Training for Physicians, Moscow. (14)



Resynthesis of Proteins Under Pressure

Workers at the Pasteur Institute in France have attempted to duplicate S. Ye. Bresler's results on the resynthesis of proteins under pressure in the presence of enzymes. Their attempts to achieve a resynthesis under the conditions described by Bresler were unsuccessful. According to Bresler, the experimental arrangement used by the French workers closely duplicated his, but the experiments failed by reason of the insufficient purity of the enzyme used by them. He states that the commercial "chemically pure" trypsin is actually a mixture of various proteases and peptidases: the pure crystalline proteases trypsin and chymotrypsin prepared according to Northrop et al. must be used and adequate attention paid to the purity of the substrate. In Bresler's opinion, these precautions have not been observed by the French workers. (15)

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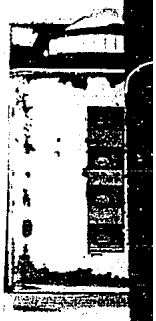

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