INTRODUCTION

[Introduction written by G. K. Skryabin, Corresponding Member of the USSR Academy of Sciences, and A. A. Pokrovskiy, Academician of the USSR Academy of Medical Sciences; Moscow, Mediko-Biologicheskiye Issledovaniya Uglevodorodnykh Drozhzhey, Russian, 1972, pp 5-8]

The Directives of the 24th CPSU Congress provided for scientifically sound prospects for further improvements in the welfare and nutrition structure of the Soviet people. Proposals in this area are directed toward further improvement in the health conditions of the population, increase in the efficiency and lengthening of the period of active life of human beings.

In solving the food problem, the socialist society bases itself on a progressive and vital concept ensuring a planned production of foodstuffs in the proportions, amounts, and conditions needed by the society. This concept is based on a single plan of the development of socialist economy whose main goals are to ensure the health and happiness of mankind.

In predicting the routes for the solution of the food problem for the following decades, it should be foreseen that the

natural productivity of our biosphere based on the accumulation of solar energy, rationally adjusted by the methods of agricultural production, and strengthened by achievements of the scientific and technological progress, first of all, by chemistry and microbiology, will continue to be the main source of nutrition for the population of our planet. This means that the main forces of science must be concentrated on how to make the agricultural production more profitable and productive, on how to increase the nutritional utilization of the overall productivity of the biosphere, and on how to strengthen more reliably the feed resources of animal husbandry.

At the same time, if we consider the food problem on a global scale, it becomes evident that the use of the traditional methods of food production will not sufficiently solve the food problem of the world in the near future. Unfortunately, the considerable progress made in the agricultural production in the last decades does not make it possible to solve the problem of the protein crisis. It is also obvious that a considerable shortage of proteins in the diets of human beings which was termed as the protein crisis in numerous UN documents is a serious detriment to the health of hundreds of millions of people. Children of early ages are the most sensitive to the shortage of proteins. It is generally known that a chronic protein deficiency during the first years of human life results in a lagging not only in

the physical, but also in the intellectual development, and leaves its aftereffects for the rest of the person's life.

In solving the strategic problems of the food policy of the future, the attention of the scientists of technologically developed countries has been increasingly concentrated on the possibility of using proteins of unicellular organisms which are natural concentrates of proteins.

The considerable materials accumulated by the Soviet and foreign researchers leave no doubt that the methods of microbiological synthesis are exceptionally promising in the solving of problems of increasing the overall protein resources of our planet. The possibility of using many different organic compounds for growing microorganisms, including petroleum hydrocarbons, natural gas, wastes of various chemical industries, as well as an intensive development of methods for producing biomasses of these microorganisms, poses the problem of their wide utilization in solving the problem of the increasing protein shortage in the world.

Here, we must stress the rapid pace and considerable progress in the development of the studies on the problem of proteins of unicellular organisms in the USSR. In the Soviet Union, large-scale investigations are carried out on the above-mentioned potential sources of microbiological proteins, including yeasts, bacteria, and algae. The progress of Soviet specialists was particularly significant in the area of studies on yeasts develing on hydrocarbons.

The Soviet Union is the first country to organize a large-scale production of nutrient yeasts on the basis of petroleum hydrocarbons. At the present time, the Soviet Union is building large plants for the production of protein concentrates of microbic origin.

The significance of these new means of producing proteins cannot be overemphasized. Calculations show that only 2 percent of the presently available petroleum is sufficient for the production of 25-30 million tons of yeast proteins capable of feeding 2 billion people. It is natural that the introduction of the fundamentally new method of obtaining protein substances and the discussion of the possibility of using the proteins of unicellular organisms as nutrient products for people and animals will require thorough medico-biological studies.

This book summarizes the results of the first stage of medico-biological studies on hydrocarbon yeasts which were carried out, chiefly, at the Institute of Nutrition of the USSR Academy of Medical Sciences.

The investigations reported in this book cover a relatively long period of time: from 1964 to 1970. Naturally, in the course of these studies both the general ideas of the researchers of BVK (protein-vitamin concentrates) and the research methods were improved gradually. Thus, the papers published here reflect, to a certain degree, the evolution of the investigation process.

The introductory part of the book treats the substantiation of the statement of the problem and the selection of the study methods.

The second section presents a series of experimental studies on the biological value and harmlessness of natural (BVK) and extracted (biological oil-seed meal) yeasts grown on media containing petroleum n-alkanes as a source of energy.

The third section treats the biological value and harmlessness of animal husbandry products obtained as a result of using BVK in feeding agricultural animals.

The fourth section describes the results of control observations on groups of volunteers from the members of the Institute of Nutrition of the USSR Academy of Medical Sciences and the All-Union Scientific Research Institute of Protein Synthesis of the Glavmikrobioprom [expansion unknown] who were receiving meat products in their rations from animals fed with the addition of BVK regularly for many months.

The last part of the book gives a brief analysis of the obtained results and an attempt at generalizations and conclusions regarding the possibility of practical utilization of BVK in animal farming. On the whole, the results of the medico-biological studies on the problem of BVK have served as an adequate basis for important decisions on the use of this new and very promising source of nutrient yeast for animal husbandry.

The accumulated data will, undoubtedly, serve the purpose of further development of investigations on the problem of rational utilization of proteins of various species of unicellular organisms directly in human diets.

In conclusion, we would like to mention that, in spite of a large number of authors, the book is of a monographic nature because of its single plan of methodological approaches and the consistency of presentation. In essence, the materials presented in this book are the first broad generalization of the results of medico-biological studies on this problem, and they can serve as an important guide for a basic evaluation of the prospects for the development of a new branch of technological microbiology -- the production of protein products on the basis of microbiological synthesis.

RESULTS OF MEDICO-BIOLOGICAL TESTS ON HYDROCARBON YEASTS

[Article by A. A. Pokrovskiy, Institute of Nutrition, USSR Academy of Medical Sciences, Moscow; Moscow, Mediko-Biologicheskiye Issledovaniya Uglevodorodnykh Drozhzhey, Russian, 1972 pp 449-463]

This book summarizes the results of six years of medico-biological studies which were conducted by the Institute of Nutrition, USSR Academy of Medical Sciences, (from 1964 to 1970) for evaluating the quality of yeasts grown on a base of the paraffin fraction of petroleum whose dry biomass was called BVK [protein-vitamin concentrate]. This book is somewhat fragmentary in nature and has been written by a large group of authors. Nevertheless, it is based on one general plan and has a single purpose. The book treats two most important problems which substantially determine the possibility of the development of a new branch of microbiological industry: firstly, the establishment of the biological value and harmlessness of hydrocarbon yeasts and, secondly, the determination of their complete harmlessness when used in animal husbandry.

The great theoretical and practical significance of this new source of protein stems, first of all, from the use of a new energy source, petroleum hydrocarbons, for the biosynthesis of protein and other nutritional substances. Petroleum hydrocarbons have never been used before for this purpose in the process of industrial biosynthesis. In connection with the huge reserves of petroleum that have already been discovered on the earth, the possibilities of its utilization for increasing the protein resources of our country have, naturally, attracted the attention not only of the representatives of the oil industry, but also of many scientific research laboratories in the world.

A very important step was the interpretation of the biochemical mechanisms of the first stages of the utilization of the energy of paraffins by yeasts and the discovery of alkane dehydrogenase, a key enzyme which makes it possible for microorganisms to use the energy of hydrocarbons of normal series.

Considerable progress in the development of the production technology of new kinds of yeast in our country has created real prerequisites for organizing their large-scale production. Scientists working in the field of animal husbandry, as a result of their long-range investigations conducted simultaneously with medico-biological studies, gave identical answers regarding the possibility of using these yeasts as sources of additional proteins in the nutrition of some species of agricultural animals. At the same time, the problem of the possibility of a wide use of hydrocarbon yeasts in animal husbandry has been left open.

The fundamentally new product, its use in industry as the main energy source of the paraffin fraction of petroleum, and the presence of residual hydrocarbons in it, as well as the possibility of accumulation of unusual products of their transformation, made it necessary to subject BVK to detailed medical studies directed, first of all, toward revealing the possibility of negative biological effects. For this, not only the usual methods were used for revealing an acute chronic toxicity and cancerogenic, mutagenic, and teratogenic effects, but also scrupulous studies were made on the effect of BVK on the state of the basic physiological systems of the organism, the progress of metabolic process, and the state of enzymic constellation, as well as on the routes of transformation of residual hydrocarbon.

The medico-biological studies were done on the basis of a single complex plan and unified schemes and methods of study approved by the Ministry of Health.

Studies of BVK were conducted on tens of thousands of experimental animals, including several populations of white rats, dogs, rabbits, guinea pigs, and monkeys.

Various specimens of hydrocarbon yeasts were studied. The tests revealed a clear tendency toward improvement in their biological qualities as the technological process was improved. For example, during the first stages of work, yeast grown on petroleum paraffins with a high content (up to 2 percent) of residual hydrocarbons was used. The experimental rats which received such

yeast had sufficiently marked pathological disturbances, particularly in the liver and the kidneys, which were beyond the limits of changes resulting from deficiencies of individual irreplaceable amino acids.

Subsequent studies were carried out with improved specimens of yeasts whose content of residual hydrocarbons did not exceed 0.6 percent, then 0.5 percent, and finally, 0.1 percent. It was established that the proteins of hydrocarbon yeasts were assimilated by animals quite satisfactorily at a definite quota included in their rations and ensured normal growth and development both in experimental and agricultural animals.

In metabolic tests, the processes of assimilating protein of hydrocarbon yeast were studied in detail, particularly the indexes of nitrogen balance, digestibility, assimilability of protein, and others.

In numerous series with replacement of various amounts of protein in food rations with yeast protein, the biological properties of BVK proteins were compared with known rich proteins (casein -- a protein complex of rich vivarium rations), as well as proteins of other kinds of nutrient yeasts. As a result of this, a fundamentally important conclusion was made that the best specimens of hydrocarbon yeasts were not any inferior to other kinds of nutrient yeasts, such as sulfite and hydrolytic yeasts, in the indexes of their effect on the development of growing animals.

At the same time, it was shown that in the cases when yeasts were the only source of protein, regardless of the content of residual hydrocarbons in them, the experimental animals developed considerable disturbances in their organs which were characteristic chiefly of a deficiency in sulfur-containing amino acids. The degree of these disturbances decreased when the percentage of the yeast in the rations was reduced or when calculated amounts of amino acid (methionine) limiting the biological values of the yeast were added. Then, the degree of balance of the amino acid composition of the ration as a whole improved substantially.

When hydrocarbon yeasts were used in amounts not exceeding 25 percent (on conversion to protein content) these changes were minimal, and when 10-15 percent of yeast was added, no changes were apparent in any of the cases.

Thus, hydrocarbon yeasts corresponding to definite conditions can be evaluated as a quite satisfactory source of additional nutrient protein.

Special experiments were conducted in order to study, in detail, the fate of the components of the lipid fraction of hydrocarbon yeasts. Its fatty acid composition, as well as the phospholipid and sterid components, were studied. It was established that their lipid fraction contained a relatively large amount of fatty acids with odd numbers of carbon atoms (ZhKN) unusual for natural foodstuffs. It was also established that these acids are also capable of being accumulated by the tissues of experimental

and agricultural animals; the degree of ZhKN accumulation was higher when the yeast quota in the food rations and the lipid content in them were greater.

Due to this, the fatty acid composition of the triglyceride fraction could change substantially in experimental animals, and a considerable percentage of ZhKN accumulated in the fat, particularly in the internal fat.

Taking into consideration the fundamental importance of determining the possibility of transition of hydrocarbon into animal tissues and the fact that this problem had not been studied to any extent, special attention was given to developing methods for the determination of residual hydrocarbons in foodstuffs. It was shown that the methods of extracting hydrocarbons with hexane which had been accepted as standard for analyzing hydrocarbon yeasts were completely unsuitable for analyzing foodstuffs. It was also found that the method of distilling hydrocarbons with phenol developed by a group of researchers in the Institute of Nutrition of the USSR Academy of Medical Sciences was practically unsuitable for this purpose because of its low sensitivity. Satisfactory results were obtained on the basis of extracting foodstuffs with a mixture of chloroform with methanol (2:1), two-stage extraction with hexane, subsequent separation of the extract by means of chromatography in a thin layer of silica gel, and, further, by means of a gasochromatographic analysis of hydrocarbon components.

These methods provided a basis for revealing the fact of the transition of residual hydrocarbon yeasts into the tissues of organisms of experimental and agricultural animals and for the study of the dynamics of their elimination from the organisms. It was established that the maximum accumulation and the longest retention of hydrocarbons in the organisms of animals are observed in the fat tissues and, particularly, in the so-called internal fat.

A special series of experiments was intended for studying hydrocarbon metabolism by using carbon-labeled octadecane which constitutes about 15 percent of all paraffins contained in yeasts. It was shown that this hydrocarbon was rapidly absorbed and distributed in the tissues of experimental animals when it was introduced into their gastrointestinal tracts. When octadecane was introduced once in a tracing dose, considerable quantities of it were detected in the tissues during the first 24 hours. By the 15th day, its concentration dropped sharply, and after two months the radioactive labeling was found only as traces, chiefly, in the fat tissues. At the same time, it was established that octadecane was gradually metabolizing in the organism and the radioactive labels moved to the fraction of fatty acids. Thus, the concept of the extreme stability of hydrocarbons in the tissues of the organism must be supplemented with the idea of their possible oxidation and transformation into corresponding fatty acids.

The above-mentioned data on the accumulation of lipids and hydrocarbons in animal tissues became a basis for the decision of the Board of the USSR Ministry of Health to allow the use of BVK in meat husbandry (pigs, horned cattle, broilers) only under the condition of the maximum permissible content of residual hydrocarbons in the yeast (0.1 percent) and the necessity of excluding BVK from the rations of agricultural animals and fowl at a specified time before they are slaughtered. Let us note that this conclusion is, to a considerable degree, in agreement with later publications issued by the research laboratories of the "British Petroleum Company," where agricultural animals were fed with yeast in which the residual amount of hydrocarbons was also less than 0.1 percent (Lovellin, Shekledi).

Special studies dealt with the development of methods and attempts to detect the representatives of the aflatoxin family in the composition of hydrocarbon yeasts. As is known, they are quite widespread metabolites of a number of microscopic fungi possessing a high degree of hepatotropic toxicity and cancerogenicity. By applying the extracting of yeasts and animal tissues with Folch's mixture with subsequent thin-layered chromatography and a study of the chromatograms by the methods of luminescence and spectroscopic analyses made it possible to make a reliable conclusion that there were no aflatoxins in the submitted yeast specimens. At the same time, when some specimens of yeast were studied by these methods, the chromatograms revealed the

presence of some substances with a characteristic yellow-green fluorescence which must be studied further.

In the medico-biological studies, attention was concentrated, chiefly, on studying foodstuffs obtained from agricultural animals which received BVK in their food. A wide spectrum of foodstuffs were studied: flesh of pigs, subcutaneous and internal fat, various kinds of subproducts, flesh of broilers, milk, butter, cottage cheese, chicken eggs, and others.

Detailed studies of the hygienic, physicochemical, and organoleptic properties did not reveal any positive changes in these products. Evaluating them by a number of tasters did not reveal any differences from the corresponding control products. For example, the meat of the experimental animal was sufficiently tender, aromatic, and tasty. Neither the meat itself nor the boullions obtained on its basis had any unusual odors or tastes. Chemical studies on the composition of meat products obtained from agricultural animals fed according to the recommendations of the Board of Ministry of Health did not reveal any positive changes in their chemical composition.

Long-range experiments on the effects of these meat products (including subproducts) on the development of several generations of rats did not reveal any deviations in their behavior and in the dynamics of their growth and development, as well as in the state of their protein, lipid, and carbon matabolism, and the activity of the studied enzymic systems in the condition of

a number of hematological, morphological, and histochemical indexes. The definitely positive results obtained in the detailed experimental studies of meat products made it possible to conduct large-scale control observations of the effects of regular consumption of experimental meat products on large groups of volunteers from among the members of the Institute of Nutrition of the USSR Academy of Medical Sciences. The observations were carried out twice in the course of six months on groups of over 100 persons. These studies confirmed that these products were sufficiently tasty and highly nutritious, and that there were no unfavorable effects resulting from their consumption. These conclusions are based on varied and systematic clinical, hematological and biochemical studies on the above groups of volunteers.

Particularly thorough studies were carried out on assessing the biological value and harmlessness of milk, dairy products and eggs from cows and chickens fed with the addition of BVK. The point is that the interruption in feeding with BVK in order to free the organisms of the animals from unusual substances (paraffins, ZhKN) does not serve its goal in the case of lactation of the cows or the production of eggs by laying hens.

Long-range studies of these products yielded very favorable results with respect to the growth and development of the experimental animals. No differences were detected between the experimental and control groups of rats, as well as in the morphological and hematological indexes, enzymic activity in the

tissues of liver and blood, basic indexes of protein and hydrocarbon metabolism, and so on. At the same time, tendencies toward an increase of β-lipiproteid fraction, cholesterin and general lipids of the blood serum were revealed during later observation periods (6-7 months) in animals receiving experimental products. The observed changes in some indexes of lipid metabolism resulting from the consumption of dairy products and eggs, since these types of products are used daily and widely by all strata of the population in their nutrition, particularly by children and elderly people, make it necessary to have a more careful approach to the problem of whether or not it is expedient to use BVK as an addition to the food of lactating cows and laying hens. This conclusion is substantiated even more by the fact that there are widespread disturbances in lipid metabolism among middle-aged and elderly persons.

Summing up the results of medico-biological studies, it should be mentioned that hydrocarbon yeasts may be considered as a very promising source of additional proteins. Many problems which existed at first in this connection have now been eliminated, and representatives of various branches of biology have arrived at the same conclusions.

The most important results of medico-biological studies on the problem of BVK may be presented briefly in the form of answers to the questions which were posed to the researchers of the USSR Ministry of Health.

Quest
n: Are cancerogenic substances present in the com-
position of
drocarbon yeasts?

Answe
If the raw materials are carefully selected, par-
ticularly if
ydrocarbons of the cyclic series are eliminated from
the paraffi
raction, there are positively no cancerogenic or
leukosogenic
ffects from hydrocarbon yeasts. Also, no marked
cocancerogen
effect of these yeasts has been detected.

Quest
n: Do hydrocarbon yeasts have a teratogenic effect?

Answe
No teratogenic effects were ever found in studies
on experimen
l animals or in large-scale agricultural experiments.

Quest
n: Do hydrocarbon yeasts have a mutagenic effect?

Answe
No mutagenic effects were revealed in using BVK.

Quest
n: What is the biological value of hydrocarbon
yeasts and w
t are the indexes of nitrogen retention in using
these yeasts
n feeding various species of animals?

Answe
The results of experiments on many species of ex-
perimental
agricultural animals, including monkeys, make it
possible to
ve an identical answer: the protein components of
these yeasts
re assimilated quite satisfactorily. The retention
of nitrogen
in agreement with the amino acid composition of
the protein
f a given kind of yeasts. A comparatively low con-
tent of meth
nine limits, to a certain degree, their biolog-
ical value
is the primary reason to limit the amount of yeasts
introduced
o the rations or to enrich them with a calculated
amount of me
ionine.

Question: Have definite differences been revealed between the biological value of BVK and other kinds of nutrient yeasts?

Answer: When experimental animals are fed with the addition of hydrocarbon yeasts according to the medical requirements stated in the resolutions of the Board of the USSR Ministry of Health of 1968, their effect on the development of all species of the studied animals does not differ in any respect from other types of nutrient yeasts.

Question: Are there any changes in the quality of the products obtained from cattle fed with the addition of hydrocarbon yeasts?

Answer: No definite changes have been detected in the quality of meat products when studies were carried out on a wide spectrum of their hygienic, physiocochemical and organoleptic properties.

Question: Have the limitations in the use of hydrocarbon yeasts for cattle feeding been substantiated?

Answer: Yes, they have been substantiated. In calculating rational doses of BVK (with respect to the protein in the rations), it is not expedient to increase its amount above 10-15 percent. When the yeast dose is increased above 25-30 percent, there are definite deviations from the normal development of experimental animals (some lag in gaining weight, sometimes fat infiltration of liver, and so on).

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uestion: What are the reasons for these limitations which
dical workers?

nswer: Apart from the above-mentioned nonoptimal amino
po ition, the following facts make it necessary to exer-
rtness:

he presence of a certain amount of residual petroleum
bons in the composition of hydrocarbon yeasts; it should
onsidered that residual petroleum hydrocarbons are iden-
hydrocarbons of some plant products which are mostly of
ecular weight and are, chiefly, contained in vegetable
26; C_{34});

he presence of rather uncommon lipids in paraffin yeasts,
arly, a relatively high content of fatty acids with odd
f carbon atoms, composition of sterols unusual for animal
and so on;

n unusually high content of purine and pyrimidine bases
easts, which, however, is characteristic of the absolute
of microorganisms and which is of utmost importance in
mpts of using them in diets of human beings.

uestion: Can normal paraffins be absorbed in the gastro-
al tract?

nswer: Yes, they can. Studies with direct determination
fins in yeasts and tissues of experimental animals by the
of gas chromatography, as well as experiments with paraf-
eled for carbon, made it possible to reveal their ability

to penetrate rapidly through the intestinal mucosa and be retained in the tissues of the organism.

Question: In what tissues does maximum accumulation of residual hydrocarbons take place and where are they retained for long periods of time?

Answer: These tissues are the subcutaneous and, particularly, internal fat. It is here that hydrocarbons accumulate the most and are ratained for comparatively long periods of time. After these facts had been established, it became possible to suggest methods for effective control over the quality of meat products obtained from animals fed with the addition of hydrocarbon yeasts.

Question: Can fatty acids with odd numbers of carbon atoms accumulate in the organism tissues?

Answer: Yes, they can. It has been proven that some changes in the fatty acid composition of the organism tissues are observed when considerable amounts of BVK are used in the diets of laboratory and agricultural animals.

Question: In what tissues the accumulation of these acids is particularly expressed?

Answer: The largest amounts of fatty acids with odd numbers of carbon atoms were found in the internal fat, and short-term observations also revealed them in the liver.

Question: Can residual hydrocarbons and fatty acids with odd numbers of carbon atoms be metabolized in the organism of animals?

Answer: Yes, they can. According to the available data, fatty acids with odd numbers of carbon atoms are subjected to sufficiently intensive processes of biological oxidation. However, some fermentation stages of this process differ from the transformation processes of fatty acids with even numbers of carbon atoms which are usual for the organism. As for n-alkanes, our experiments with labeled octadecane yielded convincing proofs of the possibility of a gradual transformation of alkanes into the corresponding fatty acids.

Question: Does BVK contain substances of the furocumarine series of the type of aflatoxins?

Answer: The BVK specimens submitted for study were completely free from such substances.

Such were the results of the medico-biological studies which, undoubtedly, characterize the new type of yeasts as an interesting and promising source of additional proteins and, to a considerable degree, map out the routes for their effective and safe application in agricultural production.

The results of these investigations, together with the published data, can make it possible to give an unambiguous answer to the alternative question: which course of study should be preferred in the immediate future for the investigation of single-cell organisms -- a direct one or through an animal organism.

In comparing the peculiarities of the production of hydrocarbon yeasts, it is necessary to consider the fundamental differences

both in the source used for the biosynthesis of energy, and in the food chains connecting them with man, on the one hand, and proteins in the ordinary agricultural production, on the other (diagram).

Key: 1) Man
2) Products of animal origin (milk, meat, eggs)
3) Agricultural animals
4) Solar energy
5) Plant biomass
6) Biomass of yeast cells
7) CO_2 of air
8) Soil substances, fertilizers
9) Nutrient medium (hydrocarbons + $+ NH_4^+ +$ mineral salts)

In one instance, the role of an intermediate link in the chain of the assimilation of food substances from the environment is played by multicellular plant organisms equipped with a complex mechanism of selective sorption of only those substances which are necessary for the organism and having a considerably more expressed, genetics-dependent stability of their chemical composition. In another instance, the intermediate link in the

food chain is represented by rapidly developing cultures of unicellular organisms which are much more capable of absorbing foreign substances and admixtures from the nutrient medium. In this case, the introduction of petroleum hydrocarbons into nutrient media and the possibility of their accumulation in the cellular biomass caused particular concern. However, apart from hydrocarbons, in evaluating the potential harmfullness of hydrocarbon yeasts, it is also necessary to consider the possibility of the presence of other substances unusual for natural foodstuffs in them, for example, unusual components of the lipid fraction (sterols, ZhKN, and the like), unusual amino acids (for example, diaminopimelic acid, D-optical isomers of amino acids, and so on), amino sugars and peptides, particularly in the composition of the cellular membrane, an extremely high content of nucleic acids, biologically active compounds of the type of mycotoxins and antibiotics, and others so far unknown toxic components. All these facts, as well as a considerable changeability of the properties of microorganisms and the insufficient understanding of the laws of this changeability in the process of the mass production of the biomass, do not leave any doubt about prematureness and a considerable risk of a wide introduction of proteins of microbiological origin directly into the diet of human beings at the present time.

In making prognoses for the means of solving the food problem for the next decades, it should be expected that natural

productivity of the biosphere based on the accumulation of solar energy, rationally adjusted by the methods of agricultural production and reinforced by the achievements of the scientific and technical progress, first of all, by chemistry and microbiology will remain the main source of nutrition for the population of of our planet. This means that the main forces of science must not be diverted from the agricultural production, but to concentrate on how to make it more profitable and productive, how to utilize solar energy to the fullest, and how to increase the indexes of food utilization of the total productivity of the biosphere. We are deeply convinced that it is from these positions that we should solve the problem where to use proteins of single-cell organisms, in general, and hydrocarbon yeasts, in particular, in the national economy in the near future. Moreover, we should also remember that the population has a definite psychological prejudice against various substitutes in the sphere of nutrition and has an attachment to traditional foodstuffs to which they are accustomed.

Thus, proteins of hydrocarbon yeasts must find application, first of all, in the sphere of feeding agricultural animals. However, even in this case, protein-vitamin concentrates based on these yeasts must satisfy sufficiently strict requirements which would fully guarantee their safe use in the national economy.

We shall attempt to summarize the requirements for the quality of hydrocarbon yeasts based on medico-biological studies.

Most of them can, evidently be achieved under the conditions of a large-scale production. The list of preliminary requirements for the quality of BVK is given below.

Indexes of Chemical Studies

	Content
Moisture	Not over 10%
Total Nitrogen	Not less than 8%
Content of	
protein (N x 6.25)	Not less than 48%
nucleic acid	Not over 8%
lipids	" " 5%
residual hydrocarbons	" " 0.1%
polycyclic hydrocarbons	0.000
Lead	Not over 5 mg/kg
Arsenic	" " 5 mg/kg
Aflatoxins	0.000
Indexes of microbiological studies. Content of microorganisms:	
pathogenic	00
live yeast cells	00
nonpathogenic	100,000 per 1 g

Indexes of Biological Studies

The most important requirement for the biological qualities of nutrient yeasts is the absence of any kind of toxicity when they are used, including acute or chronic toxicity, as well

as the absence of cancerogenic, leukosogenic, teratogenic, and mutagenic effects established on a number of biological species.

An even more necessary test of the safety of using hydrocarbon yeast in feeding agricultural animals is the evaluation of the quality of foodstuffs obtained from them. The most important requirement in this case is the invariability of the chemical composition of foodstuffs and preservation of high organoleptic properties, and good technological and marketing conditions.

These products, when used regularly, must not have any negative effects on metabolism and, in particular, facilitate the acceleration of the development of atherogenic processes in aging organisms.

The results of medico-biological studies described in this book make it possible to assert that protein-vitamin concentrates obtained in our country by utilizing the paraffin fraction of petroleum even now satisfy, to a considerable degree, the above-mentioned requirements. Also, it can be stated with assurance that technological processes of the BVK production are being improved constantly. One of the important goals of these improvements is to improve the quality of BVK, to lower the content of residual hydrocarbons in them, and to improve the stability of the processes and conditions of yearly production, which, in the final analysis, must lead to a higher guarantee of complete safety of their use in animal husbandry.

This conclusion is based on the detailed study of the dynamics of the accumulation of hydrocarbons with artificial introduction of n-paraffins in various quantities into BVK.

It was shown that, on the average, 1-2 percent of hydrocarbons contained in rations can be retained in the fat tissue. The processes of hydrocarbon accumulation progress slower during the first half of the year and more intensively during the second half of the year.

Thus, the fat tissue may be defined as a kind of biological trap accumulating hydrocarbons which get into the organism from the environment. We were able to confirm this later in our studies at the agricultural animal fat depots.

Studies on the processes of hydrocarbon accumulation in pigs revealed their insignificant amounts in the subcutaneous and internal fat only when the content of n-paraffins in BVK was brought to 0.5 percent.

The above data ensure full prevention of the retention of minimal amounts of hydrocarbon in the organisms of animals if the recommendations of the Board of the USSR Ministry of Health are followed in animal husbandry.

Thus, the utilization of hydrocarbon yeasts for the development of animal farming may be the first great stride in the development of the branch of microbiological industry producing high protein products. Of course, it is also necessary to consider the economic aspect of the problem determining the expediency of producing nutrient hydrocarbon yeasts. However, the

degree of economic expediency is determined by the concrete conditions in each country, the availability of raw materials for their production, costs of other sources of nutrient proteins, and so on. Evidently, the profitability of their production is beyond any doubt to the majority of European countries which have to import fish and soy bean meal for animal food. At the same time, in the U.S.A. the availability of considerable amounts of inexpensive soy bean meal does not justify the production of protein-vitamin concentrates on the basis of hydrocarbon yeasts. As a result of this, specialists of the U.S.A. are stressing the development of studies on the use of microbiological proteins directly in human diets.

We do not share this point of view and are not considering the introduction of proteins of unicellular organisms into human diets in the near future. In our opinion, this concept is connected with a considerable risk. However, scientific investigations in this direction appear to be expedient. It should be kept in mind that the biomass of unicellular organisms under certain conditions may become a necessary source for the production of additional nutrient substances. For this reason, the reserves of our country must also include methods for industrial processing of unicellular organisms for foodstuffs.

The medico-biological investigations which have been carried out, as well as the familiarization with the published data, make it possible to formulate some preliminary requirements for

protein products produced from unicellular organisms which are intended for food. It should be stressed that these considerations are strictly preliminary. However, they, obviously, must be taken into consideration to some degree by the scientists working in this area.

In cases when it is intended to use proteins of unicellular organisms not for flavoring the food or additions to it, but as a significant source of nutrient protein, these products cannot be presented in the form of an unprocessed dry or moist biomass of microorganisms, regardless of what structure or taste is given to it. According to the available information, all attempts to use washed biomass of various microorganisms in the human diet usually produced definite complications connected with the presence in these preparations of high concentrations of nucleic acids, unusual lipids, including sterols and ZhKN, biogenic amines, unusual amino acids and peptides, amino sugars and mucopolysaccharides, substances having a specific antigenic and allergy-producing effect, and, finally, with the presence in some of them of highly toxic substances specific for a given species or even a strain of the microorganisms.

All this compels us to conclude that when attempts are made to use the proteins of unicellular organisms as food, the biomass of the microorganisms must be subjected to a rigid technological processing in order to isolate protein substances which are purified as much as possible. Some conditions for this

process can be outlined even now. They should include, first of all, the elimination of excessive amounts of nucleic acids. Evidently, the maximum level of nucleic acid content in the isolated protein substances must not exceed 1-2 percent; secondly, maximum levels must be established for unusual biogenic amines, amino acids of lipids and so on, for which it is still impossible to establish any concrete values. It should be stressed that, evidently, a considerable part of these substances can be removed with the structures of cellular membranes which is technologically possible and expedient. Thirdly, special attention must be given to an almost total elimination of substances having a specific antigenic and allergy-producing effect; fourthly, close attention must be given to a total absence of the possibility of synthesis by microorganisms of toxic substances of the type of aflatoxins and other substances producing cancerogenic, teratogenic and mutogenic effects; fifthly, sterility of the preparations, that is, the absence of live producer cells in them; sixthly, a total absence of pathogenic microorganisms; seventhly, the establishment of norms for a possible contamination by nonpathogenic flora. Of course, the above-mentioned requirements for animal feed are fully applicable to the nutrient protein preparations.

Summing up the work done to date, we must stress that its significance is connected with the scientific and technical predictions regarding the ever increasing role of the microbiological industry in the life of human society. There is no doubt

that mankind is entering a new era of microorganism utilization. Until recently, the attention to the microorganisms was attracted because they were potential causative agents of diseases. Since the moment of the discovery of antibiotics, microorganisms became recognized as producers of powerful medicinal substances, and this specialized branch of the microbiological industry has developed considerably.

At the present time, we are on the threshold of an extensive introduction of microorganisms as a powerful productive force into various branches of the national economy which may prove to be extremely effective. One of the promising directions in the production of unicellular organisms is their utilization as unusually effective producers of protein substances. Evidently, humanity must be sufficiently prepared for this possibility in order to avoid significant errors and dangers connected with making decisions insufficiently substantiated medically. The role of medico-biological studies in this case increases greatly.

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