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TRANSLATIONS FROM GIGIYENA I SANITARIYA (HYGIENE AND SANITATION)

USSR

No. 10, 1962

Following is a translation of five articles in the Russian-language periodical Gigiyena i sanitariya (Hygiene and Sanitation), No. 10, Moscow, 1962. Complete bibliographic information accompanies each article.

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THE USE OF CULTURING AND COAGULATION FOR THE
DETECTION OF PATHOGENIC MICROBES IN WATER

Following is a translation of an article by Junior Scientific Worker Ye.N. Milvayeva and Candidate of Medical Sciences G.M. Fisher from the Kuybyshev Scientific Research Institute of Epidemiology, Microbiology, and Hygiene in the Russian-language periodical Gigiyena i Sanitariya (Hygiene and Sanitation), No 10, Moscow, 1962, pages 57-58. The article was submitted for publication on 27 September 1961.

The existing methods of detecting pathogenic microbes in water are rather complex, laborious, and require special apparatus (centrifuge, vacuum pumps, bacterial filters, etc.) or coagulating substances (green vitriol, ferric chloride, alum, etc.).

In our work we employ a simpler method which was developed by one of the authors (G.M. Fisher) which consists of the following. 450 milliliters of water are poured into a sterile dish. Chlorinated water is
B.2) is added to the sample. Thus the concentration of peptone in the test water becomes 1%. The sample is then placed in a thermostat at a temperature of 37

degrees Centigrade. After three hours 2.5 milliliters of a 10% solution of potash alum is added to the water. During the time the water is in the thermostat coagulation occurs and a flocculent precipitate is formed on the bottom of the dish. It should be noted that after 15 to 20 minutes large flakes of the coagulant rise to the surface and therefore the mixture must be agitated. After an hour the dish is removed from the thermostat and the clear part of the liquid is poured off so that 100 to 200 milliliters of the liquid with the precipitate remains. The remaining liquid is filtered through a sterile piece of filter paper. The precipitate which is obtained in the form of a pasty mass is removed from the filter paper with a glass rod and is transferred to five Petri dishes with Ploskirev's culture medium and is pulverized with a spatula. During the summertime when the water temperature is high (18-20 degrees Centigrade), the culturing should be shortened to one hour. It was established experimentally that the detection of pathogenic microbes using the indicated method is successful for concentrations of 100 microbe bodies per liter of water.

The indicated method was used to investigate 199 samples from different water sources. In 9 samples (4.52%), the pathogens of dysentery and paratyphoid fever were isolated in a pure culture (see the Table).

Place where sample was obtained	No of samples tested	Coli-titer	No of positive samples	Types of pathogenic microbes isolated
River tap water, not purified /See note/	66	0.01-11.11	2	Flexner's and Newcastle's dysentery bacilli
Purified tap water /See note/	18	22.2-111.1	-	-
Water from the Volga	45	0.01-11.11	2	Flexner's and Newcastle's dysentery bacilli
Water from the Samara	9	0.0005-0.006	1	Typhoid bacteria
Water from the Sok	33	0.46-111.1	-	-
Water from tubular wells	23	3.6-250	3	Paratyphoid B bacteria, Flexner's and Newcastle's dysentery bacilli
sewer water	2	-	1	Typhoid bacteria

Total....199 - 9 -

/Note/: There was no standard water

The cultures which were obtained had the following cultural properties. Three strains of Flexner's dysentery were biochemically typical in their action and gave a positive reaction to agglutination with Flexner's dysentery serum but were phagoresistant. One of the strains gave a positive result with Flexner's A-type serum. Three Newcastle strains had all the typical biochemical and serological properties. Two strains of typhoid were typical and gave a reaction to agglutination with Vi and O serums and were able to undergo phagolysis. The isolated strain of paratyphoid B, besides the typical biochemical properties, gave a positive reaction with monoreceptor serums O antigen (I, IV, V) and N antigen (b,1,2).

In addition, 8 atypical cultures were isolated, the study of which in 6 months indicated that these cultures were para-intestinal bacilli.

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THE USE OF FLUORESCENT ANTIBODIES FOR THE
ACCELERATED DETECTION OF DYSENTERY BACTERIA

Following is a translation of an article by Candidate of Medical Sciences S.I. D'yakov, R.V. Kasatkina, Candidate of Medical Sciences V.M. Nikitin, and Z.V. Pestryakova from the Department of Microbiology of the Military Medicine Order of Lenin Academy (meni S.M. Kirov and the Sanitary Epidemiological Station (Leningrad) in the Russian-language periodical Gigiyena i Sanitariya (Hygiene and Sanitation), No 10, Moscow, 1962, pages 59-62. The article was submitted for publication on 29 January 1962.7

The existing methods of detecting pathogenic and sanitary-indicator microorganisms in milk, as in many other rapidly spoiling products, are long; therefore, the development of rapid and dependable methods of indicating pathogenic microbes in food products is an important task of sanitary bacteriology. In the present article data are given on the use of the immuno-fluorescent method for the rapid detection of dysentery pathogens in milk.

We prepared some extremely thin smears of milk. After drying the preparation we fixed it with Nikiforov's mixture for 10 to 15 minutes. In order to eliminate the background fluorescence, the smears were

given an additional treatment with benzene for 2 to 5 minutes.

In the work we used fluorescent conjugates of adsorbed Flexner's dysentery serum and its gamma globulin fraction which were marked by fluorescein isocyanate and fluorescein isothiocyanate (the fluorochromes were kindly presented to us by G.I. Mikhaylov to whom we are deeply grateful). For control staining we employed fluorescent conjugates of both normal and heterological (tularemia and typhoid) rabbit serums and their gamma globulin fractions. As an additional control we used the treatment with dysentery conjugates of preparations of samples of milk which knowingly did not contain dysentery bacteria. The preparations were stained for 10 to 20 minutes at 37 degrees Centigrade in a humid chamber. The smears were washed with a stream of tap water for 2 to 3 minutes; after drying they were examined under an ML-1 luminescent microscope using SZS-7, FS-1, BS-8, and T-2N light filters. For the study of the general microflora of the milk and its quantitative evaluation, the smears were stained with a 1:1,000 solution of auramine for 1 to 2 minutes. After staining they were dipped in 70% alcohol for several seconds and then were carefully washed with water.

The use of the fluorescent antibodies method for the accelerated detection of dysentery bacteria in milk can satisfy the practical requirements in three basic respects: rapidity in obtaining an answer, sensitivity, and specificity. As a rule, 30 to 45 minutes were sufficient for the preparation, staining, and microscopic examination of the preparations. In

order to determine the sensitivity of the method, 5 to 10 milliliters of bottled milk were infected with various doses of Flexner's (Type O) dysentery bacteria. After 30 minutes of contact the samples were carefully mixed and smears were prepared from them which were stained with fluorescent conjugates and a solution of auramine. The smears which were stained with auramine showed a large amount of brightly fluorescent yeast cells, bacilliform and spherical cells, etc. In contrast to this, the preparations which had been treated with fluorescent dysentery conjugates, depending on the concentration of dysentery bacteria which were introduced into the sample, showed the presence of various amounts of bacilliform microbe cells which were intensely fluorescent with a yellow-green light and which had even more intensely fluorescent edges. The results obtained are given in the table.

Summary indices of the sensitivity of the method of fluorescent antibodies in detecting dysentery bacteria in artificially infected samples of milk

Concentration of dysentery bacteria in 1 ml of test milk	Quantity of fluorescent cells in the field of vision											
	Centrifuging			Flocculation				Centrifuging in Margolin's position			after 6 hours followed by centrifuging	
	creamy film	liquid above the precipitate	precipitate	in 20 minutes	in 2 hours	in 24 hours	after centrifuging	in 5 hours	in 24 hours	in 5 hours	in 24 hours	after 6 hours followed by centrifuging
100,000,000	trace	0-1	trace	trace	trace	trace	trace	up to 5-10	10-20	trace	trace	trace
10,000,000	10-20	-	trace	3-10	trace	trace	trace	1-3	10-20	trace	trace	trace
1,000,000	up to 10	-	up to 20	0-2	up to 10	up to 20	up to 10	0-1	up to 10	trace	up to 10	trace
100,000	1-2	-	1-5	-	1-5	up to 10	up to 10	-	up to 10	trace	up to 10	10-20
10,000	0-1	-	1-2	-	0-1	2-3	2-3	-	up to 3-5	trace	up to 3-5	up to 10
1,000	0-1	-	1	-	1-2	1-2	1-2	-	up to 3-5	trace	up to 3-5	1-2-3
100	-	-	-	-	-	-	-	-	-	-	-	-

In order to increase the sensitivity of the fluorescent antibodies method we employed methods of concentrating the bacteria in the milk: centrifuging, flotation, and brief culturing in nutrient media, etc. The infected samples of milk were centrifuged in volumes of 5 to 10 milliliters at 2,500 to 3,500 revolutions per minute for 5 to 60 minutes. The smears were made from the upper creamy film, the clear liquid above the precipitate, and the whitish precipitate. For flotation, 0.3 to 0.5 milliliters of xylol were added to 9 milliliters of a test sample of milk, after which the test tubes with rubber stoppers were agitated for 10 to 15 minutes in an agitating apparatus. For culturing, 0.2 to 1 milliliter volumes of milk were seeded in a semi-liquid medium of Floskirev's medium, Martin's bouillon and mannitol (0.5%). Muller's fluid and the Kessler-Svenarton medium were also tested. The remaining milk in a volume of 5 milliliters was put in a thermostat at 37 degrees Centigrade. Smears were prepared after 2, 4, 6, 12, and 24 hours of incubation in the thermostat. The summary indices of the tested methods of increasing the sensitivity are given in the table.

The indices which were obtained indicate that the employment of additional steps which make it possible to concentrate the microbe cells increases the sensitivity of the fluorescent antibodies method to the extent of obtaining positive results for the presence of from 1,000 to 10,000 microbe cells of dysentery bacteria in 1 milliliter of tested milk.

The matter of the specificity of any method of

diagnosis in the final analysis actually determines its practical value. First of all we studied the intensity of the specific staining of the dysentery bacteria in relation to their being in artificially contaminated milk. With this aim samples of raw bottled milk were infected with 50 million microbe bodies per milliliter and were kept for 10 days at 2, 4, 18-22, and 37 degrees Centigrade. Smears were prepared daily. At the same time we conducted seedings in differential-diagnostic and elective batches of Endo's and Ploskirev's cultural mediums. The results of the tests showed that the cells of the dysentery bacteria over the entire observation period did not lose their ability to be stained specifically by fluorescent antibodies; however, the intensity of the luminescence of the cells and the quantity of fluorescent cells in the field of view of the microscope were lowered somewhat. It is interesting to note that beginning from the third day of the incubation of samples of infected milk in the thermostat at 37 degrees Centigrade and at room temperature, the number of colonies of dysentery bacteria which grew in the dishes gradually decreased and, beginning with the eighth day, dysentery microbes were not sown at all. The positive finding in the indicated samples of fluorescent cells demonstrates that the fluorescent-immunological method exposed the unviable cells of dysentery bacteria.

Our basic attention in studying the specificity of the fluorescent antibodies method was directed at investigating commercially-produced milk. In all we investigated 181 samples of such milk. Upon receiving

the samples at the laboratory we immediately prepared smears directly from the milk, from the creamy film (after 5 to 10 minutes of centrifuging 5 to 10 milliliters of milk at 3,000 revolutions per minute), and also from the precipitate after 6 hours of culturing in Martin's bouillon and centrifuging the seedings. The preparation was carefully examined for three minutes under a fluorescent microscope. In recording the data we devoted our attention to the morphology of the fluorescent formations, the intensity and color of their fluorescence, the nature of the staining, and the quantity of fluorescent cells in the field of vision or in the preparation. In evaluating the fluorescent images the results were compared with the data from the control preparations which were treated with normal and heterogeneous fluorescent serums. Parallel with this, each sample of milk was tested bacteriologically for coli titer, microbe number, and also the presence of dysentery and typhoid-paratyphoid bacteria. In addition, the colonies which grew in dense culture mediums were tested ^{on} Bacillus coli which had been positively agglutinated by Flexner's dysentery bacteria.

The results of the observations which were conducted were as follows. In 99 samples (54.7%) not a single fluorescent cell was discovered in the luminous microscopy of all three series of samples. Bacteriological tests for the presence of dysentery bacteria also gave negative results. In five samples strains of Bacillus coli which were agglutinated by Flexner's dysentery serum were isolated. In 9 samples (5%) intensively fluorescent cells with a characteristic

staining structure were detected after treatment of the preparations with normal fluorescent conjugates.

In 23 samples of milk (12.7%) fluorescence of the microbe cells was observed when the smears had been treated with both dysentery and normal fluorescent serums. This indicates that the observed fluorescence is a non-specific staining of the microbe cells. With a bacteriological test one strain of Morgan's bacillus and 6 strains of *Bacillus coli* which were agglutinated by Flexner's serum were isolated. Thus, summarizing the data from the testing of 131 samples of milk (72.4%), we can consider that they all belonged to the negative group. According to the data from lumin-
escent microscopy, 28 samples of milk (15.4%) should be listed under the weakly positive group. All these samples were characterized by the fact that upon staining with dysentery serum, only individual specifically fluorescent cells were detected (1 to 4 for all of the smears). From these smears, 7 strains of *Bacillus coli* were isolated which were agglutinated by dysentery serum. Dysentery bacteria were not discovered in these samples. With a weakly positive response in a given series of samples we oriented upon the individual nature of the findings of fluorescent cells in the entire preparation and on the absence of an increase in the number of fluorescent cells in the preparations which were prepared from Martin's bouillon after 6 hours of culturing.

Finally, the remaining 22 samples of milk (12.2%), according to the data of the fluorescent antibodies method, can be assigned to the group with a

clearly positive response. However, it should be emphasized that not in a single case was it possible to detect dysentery bacteria in these samples using a bacteriological test. In 21 samples of milk we isolated strains of *Bacillus coli* which contained antigens which were common with Flexner's dysentery bacteria.

Thus, despite the negative results of the bacteriological testing of 181 samples of commercial milk, 22 of the samples, according to the luminescence analysis data, were positive. Obviously further research is required in order to evaluate the positive results of the fluorescent method.

Of some interest is the material from the detailed study of 62 parastrains which we isolated from the milk. After the isolation of the pure cultures and their brief laboratory storage, we noticed a sharp lowering of both the ability of the cultures to be agglutinated by Flexner's dysentery serum and the intensity of the luminescence of the microbe cells which had been stained by fluorescent dysentery conjugates. Thus after 2 or 3 resowings the microbe cells of 46 parastrains fluoresced to ++ and only individual cells of some of these strains gave a brighter fluorescence of up to +++. Only 4 strains fluoresced sufficiently brightly (+++), while individual cells fluoresced up to ++++; 12 strains completely lost the ability to fluoresce. Most of the strains were characterized by a diffused luminescence of the entire body of the microbe cell of a weak or average intensity (+ and ++). As a rule, brightly fluorescent edges were absent in all parastrains, although there were four exceptions.

Only some small grain-like sectors on the surfaces of some microbe cells fluoresced more brightly, sometimes creating the impression of bipolar luminescence. There were particularly sharp manifestations of uneven staining of the cells in the preparations.

The peculiarities which were noted in the fluorescent staining of the cells of parastrains in pure cultures made it possible to distinguish with sufficient accuracy between the majority of them and Flexner's dysentery bacteria.

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ON CASES OF BOTULISM DUE TO HOME-MADE
CANNED FRUITS AND VEGETABLES

Following is a translation of an article by Candidate of Medical Sciences Ye.S. Krasnitskaya from the Main Sanitary Epidemiological Directorate of the Ministry of Public Health of the RSFSR in the Russian-language periodical Gigiyena i Sanitariya (Hygiene and Sanitation), No 10, Moscow, 1962, pages 73-74. The article was submitted for publication on 10 January 1962.

In our country the occurrence of botulism as a result of using the food products of the enterprises of the canning industry, because of the considerable sanitation measures which are employed at these enterprises, has almost been completely eliminated.

The individual outbreaks of botulism which are still recorded occur, as a rule, in ordinary life, are of a group or more often of a family nature, and in the main are the result of the consumption of food products prepared by an individual.

The study of the epidemiology of botulism in the RSFSR indicates that the main source of this ailment is fish products. In rare cases the outbreak of botulism was connected with the consumption of meat products.

With respect to vegetables and fruits, there was not a single case noted for a number of decades where the source of botulism was vegetable or fruit products.

Two cases of botulism which have occurred as a result of eating home-canned fruits and vegetables are therefore of great epidemiological interest.

In the first case three persons became ill (two adults and one child); the typical clinical aspects of botulism were present. The individuals experienced difficulty in breathing, a choking feeling, aphonia, diplopia, ptosis, and sharp adynamia which was accompanied by paresis of the legs; one person experienced vomiting; the stool was normal for all individuals. Death came at 19, 24, and 40 hours from the onset of the disease.

Epidemiological investigation indicated that the illness was connected with the consumption of an apricot compote which was prepared at home using ordinary home pasteurization (putting the glass jar with the compote into a kettle of boiling water).

The incubation period lasted from 5 to 33 hours. A laboratory test conducted at the Rostov Institute of Epidemiology, Microbiology, and Hygiene confirmed the diagnosis of botulism. *Botulinus bacilli* were isolated from the mesenteric gland, the liver, the brain and spinal cord, and the contents of the stomach of the cadaver. A biological test on mice with an extract from the compote was positive. After this the same strain was detected in sections from the lymph gland of the dead mouse. A biological test on mice using heated extracts from sections of the internal organs of the cadavers also gave a positive result.

The type of pathogen was not established exactly; The isolated strain was not agglutinated by diagnostic

serums types A and B.

The second case of botulism occurred as a result of eating home-canned wild onions (*Allium ursinum*). Two members of one family aged 48 and 22 became ill. The first symptoms of the disease appeared 8 to 20 hours after eating the wild onions and consisted of dizziness, diplopia, dryness in the mouth, and thirstiness. The persons began to vomit and experience spasms. Within a day their condition grew worse and they were hospitalized as a result. In the infirmary they experienced a worsening of their vision, ptosis, difficulty in swallowing, aphony, and acute weakness.

Examination indicated cyanosis of the skin, a dry tongue covered with a fine coating, enlarged pupils, and a normal stool; the temperature did not increase. Despite the steps which were taken (gastric lavage, administration of polyvalent ABE antitoxin serum), the persons died on the second and third days after the onset of the disease.

Upon opening the cadavers it was noted that there was polyemia of the internal organs; microcellular bleeding was observed on the apexes of the folds of the small intestine.

The epidemiological investigation revealed that fresh wild onions had been purchased at the market one and a half months prior to the outbreak of the disease and had been canned in half-liter glass jars. The canning of the wild onions was conducted in the following manner: boiling water was poured over the wild onions; they were placed in jars; and a vinegar solution was added. After this the jars were closed. The wild

onions were stored in a bathroom at a comparatively high temperature. Five jars of canned wild onions remained on hand at the time of the outbreak of the disease; four of them were infected.

The remaining preserves and the organs of the cadavers were subjected to a bacteriological investigation.

From the contents of one jar and the cadaver material, the pathogen of botulism was isolated (the isolated culture was not typed). By a positive biological test on mice it was proven that botulinus toxin was present in the material being tested. It is to be presumed that in both cases the raw food was not fresh and was covered with soil. The preserving was inadequate, and the long storage of the jars of wild onions under unfavorable temperature conditions facilitated the accumulation of the toxin.

It should be noted that in connection with the considerable development of fruit and vegetable growing in our country, the preserving of fruits and vegetables in the homes is becoming more and more significant.

The improper preparation of preserves, as can be seen from the cases which have been described, can lead to tragic consequences. Therefore, in order to prevent this fatally dangerous disease among the population, it is necessary to perform educational work and to propagandize the proper methods of preserving food. In order to increase the quality and safety of canned fruits, they should be prepared immediately after they have been gathered, because they spoil quickly when stored for a long time, especially in a

warm place. Over-ripe fruits are not suitable for canning because they not only quickly become too soft but, more importantly, because they are more difficult to wash and because pieces of dirt can stick in the wrinkles. Before canning fruit, it should be carefully washed with potable water. In order to destroy the microflora and achieve complete sterility in the jars, it is necessary to maintain an appropriate temperature for a certain length of time. For example, the sterilization in water of compotes which have been placed in liter jars should last for 20 minutes at a temperature of 75 degrees Centigrade. If the temperature of the water is higher, the sterilization time can be shortened accordingly (at 80 degrees -- 15 minutes; at 85 degrees -- 10 minutes; at 90 degrees -- 6 minutes; at 95 degrees -- 3 minutes). After cooling, the tightness of the seal on the jars should be checked.

Before storing the jars they should be wiped dry. It is recommended that the preserves be stored in a cool, dry place which is protected from the sun. The temperature of the place should not exceed 15 degrees centigrade.

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SANITARY AND BACTERIOLOGICAL METHODS AND
THE SOLUTION OF PRACTICAL SANITARY PROBLEMS

Following is a translation of an article by Professor L.I. Mats from the Institute of General and Communal Hygiene named A.N. Sytin of the Academy of Medical Sciences of the USSR in the Russian-language periodical Gigiyena i Sanitariya (Hygiene and Sanitation), No 10, Moscow, 1962, pages 94-100. The article was submitted for publication on 28 February 1962.7

In recent years there have been a number of articles in the magazine Gigiyena i Sanitariya on methodological questions of sanitary microbiology (1960-1961). The magazine has properly designated these methods for discussion, because many of them, without any need, require laborious efforts from the practical laboratories.

A substantial place in sanitary microbiology, as in science, is given to the development of methods of evaluating the sanitary-hygienic state of objects in the external environment and to the indication of pathogens in the same environment.

It is obvious that in solving their basic problems the scientific workers in the field of sanitary microbiology can employ any method which will enable them to better solve the given problem. However, the methods

which are proposed for wide usage in sanitary-epidemiological laboratories for evaluating the sanitary-hygienic and epidemiological condition of objects in the external environment should be very simple. The purpose of sanitary analysis, to include the microbiological, is to place in the hands of the sanitary doctor objective data on the sanitary-hygienic condition of an object in order for him quickly to understand the sanitary situation and to conduct the appropriate preventative, sanitary, and other measures.

The early detection of a pathogen in the external environment is an important link in the struggle to lower the incidence of infectious diseases.

The analysis and the method of conducting it should be very precise, rapid to perform, suitable for any situation, and economical in cost. From the point of view of the sanitary-microbiological and epidemiological characteristics of objects of the external environment, it is important to investigate the greater part of the specimens using simple methods rather than to investigate chance specimens using more complex methods, because the concentration of sanitary-indicator microorganisms and pathogens of infectious diseases and even their accumulation in specimens in the external environment are comparatively small.

The microbiological methods of indicating pathogenic organisms which are ordinarily employed require a long time to be performed. The small concentration of pathogenic microorganisms in the external environment and the abundance of saprophytes and to a considerable degree of antagonists makes it difficult

to detect pathogenic microorganisms in the air, water, and soil. As a consequence of this, in the practice of sanitary microbiology the direct determination of pathogenic bacteria is rather frequently replaced by the search for so-called sanitary-indicator microorganisms (colibacilli, streptococci, staphylococci, bacteriophages, etc.).

The sanitary-indicator microorganisms (for example, varieties of colibacilli) are generally sufficiently delicate indicators of the sanitary-hygienic condition and especially of the processes of atrophy of bacteria in the external environment (water, soil, food products, etc.). However, they cannot replace or search for pathogenic microorganisms as an epidemiological evaluation of the external environment and not all of the proposed methods and sanitary-indicator microorganisms are of equal value in the sanitary evaluation of specimens from the external environment.

It should be noted that the determination of sanitary-indicator microorganisms in the air gives answers which are very tardy for preventative and positive sanitary measures. All attempts to obtain general indicators of the sanitary-hygienic condition of the air using bacteriological test data have borne a chance character and the indicators themselves have a very relative significance.

The sanitary-indicator value of determining the total number of bacteria and strepto-staphylococci as indicators of the sanitary condition of the air of living quarters is very small. The determination of the strepto-staphylococci under practical conditions

in laboratories of sanitary-epidemiological stations is rather complex, cumbersome, and long. The investigation of the sanitary-indicator value of streptococci in the air does not have the same meaning as does the investigation of the sanitary-indicator value of the colibacillus in water. In testing water for the presence of the colibacillus one has in mind not only the indirect determination of the epidemiological state of the reservoir but also the hygienic study of the dynamics of the processes of self-purification and the inhibition of the bacteria in the water of the reservoir which is very important in evaluating the sanitary state of the latter.

The considerable mobility of the aeroplankton means that the analysis establishes the state of the microflora of the atmospheric environment only at one certain point, whereas this state of the microflora changes rapidly.

Bacteriological analysis gives a high percentage of deviations, a tardy answer, is expensive, and using it, it is not possible to obtain firm data for accurate conclusions. This is understood by the researchers who propose various norms for the content of microorganisms in the air.

The norms are given only for unventilated living quarters. However, unventilated living quarters are impermissible from a sanitary-hygienic point of view and should be so considered without a bacteriological test.

At the Fourteenth All-Union Congress of Hygienists and Sanitary Doctors in 1961, Professor V.A. Ryazanov,

a corresponding member of the Academy of Medical Sciences of the USSR, properly pointed out that the study of the bacteriological contamination of the air does not justify itself in evaluating the sanitary-hygienic state of the air.

In ordinary practice the analysis of the aeroplankton should be used only in special cases (in accordance with the order of the Ministry of Public Health of the USSR during the systematic observation of maternity homes, surgical operations, the production of bacterial preparations and sterile medicines, etc.) and in accordance with epidemiological indications. Only the direct immediate determination of pathogenic microorganisms in the air is of definite importance.

In the field of theoretical aeromicrobiology the first problem which comes to mind is the development of methods for the direct and rapid indication of pathogenic bacteria and viruses, especially the flu virus and other adenoviruses and Rickettsia (*Rickettsia burneti*, Q fever).

The Soviet standard establishes the determination of all varieties of the *Bacillus coli* as an indicator of the sanitary-hygienic state of the soil, water, and food products.

There is a considerable number of varieties of the *Bacillus coli*, because various representatives of this group of bacteria differ with respect to the assimilation of different sources of carbon and nitrogen. The *Bacillus coli* has a polyantigenal structure. A considerable number of serological varieties are encountered. All the biochemical and serological varie-

ties of the *Bacillus coli* under certain conditions can cause various pathological occurrences (infections, food infections and intoxications, etc.) in people, especially in children and in a weakened organism; these pathological phenomena depend on the immunobiological state of the macroorganism.

Professor V.M. Zhdanov, an active member of the Academy of Medical Sciences of the USSR, spoke at the Fourteenth All-Union Congress of Hygienists and Sanitary Doctors and pointed out the great importance of the immunological state of the organism when subjected to the pathological processes caused by the *Bacillus coli*.

Under ordinary conditions the *Bacillus coli* is a saprophyte because throughout the long course of history people in general developed immunity to this microbe.

The study of the dynamics of the existence of the *Bacillus coli* in a water reservoir is a good indication of the sanitary state of the water source and a check on the quality of the purification of drinking water. Numerous investigations of different reservoirs in our country which were conducted in the 1930's under the direction of the Central Institute of Communal Hygiene (Professor S.N. Stroganov) showed that the coli titer was not less than 0.3 at points where there was completed self-purification of the reservoir.

The considerable research performed in the Department of Communal Hygiene of the First Moscow Medical Institute (corresponding member of the Academy of Medical Sciences of the USSR, S.N. Cherkinskiy) demonstrated that a coli index for drinking water of not greater than 3, which was accepted by the Soviet-

All-Union State Standard 2874-54 as the norm indicator of the quality of water with respect to bacteriological can be considered as a very dependable indicator of the effectiveness of the purification of water in cases where it has been contaminated with the pathogens of intestinal infections and also of brucellosis and leptospirosis.

It is generally known that the beginning of the decline in the incidence of typhoid fever in inhabited points of Europe and the USA coincided in time with the conduct of measures to prevent the contamination of the soil by waste (planning and organization of public services, purification, sewer systems, etc.) and with the supplying of good-quality drinking water (with a *Bacillus coli* content of 100 or less per liter of treated water) to the population. After the implementation of these measures the death rate from typhoid fever in England, which had been 475 persons out of one million up to the 1890's, began to drop in the middle of the 1890's when they began to provide public services in the towns and to supply good quality water. By 1910 the mortality rate had dropped to 50 persons out of one million. This indicates the great importance of bacteriological analysis in checking on the operation of various water-purifying and disinfecting (chlorinating) units for water systems. In such cases bacteriological analysis is a dependable indicator which makes it possible to judge the degree of purification and disinfection. The slightest disruption of the operation of purification and disinfection equipment has an immediate effect on the quantitative and

qualitative microbe composition of the water being treated.

It is not necessary to complicate the determination of fecal contamination by additional research into other fecal microbes: the fecal streptococci and *Clostridium Welchii* (*Bac. perfringens Welchii*). The group of streptococci is found rather widely in the external environment; for a precise identification of the fecal streptococcus it is necessary to study deeply the biochemical and serological characteristics of the microbe, which is a substantial delaying factor.

There is a significant difference between the characteristics of the *Bacillus coli* and the *Clostridium Welchii*. In the case of fresh contamination, objects in the external environment simultaneously receive bacteria of the groups *Bacillus coli* and *Clostridium Welchii*. The *Bacillus coli* does not form a spore and perishes rather quickly in the external environment; therefore, its presence can indicate more or less fresh contamination. The *Clostridium Welchii* forms spores and is preserved for a long time in an object of the external environment. As a consequence, relatively recent contaminations of objects of the external environment can contain *Clostridium Welchii* but may also be free of the *Bacillus coli*.

M.I. Tarkov (Moldavian Institute of Epidemiology, Microbiology, and Hygiene, 1951) presented convincing data on the possibility of the multiplication of the *Clostridium Welchii* in the soil under certain conditions. The question arises of the necessity of reexamining the possibility of considering the *Clostridium Welchii* as

an indicator of even old fecal contamination.

Professor G.P. Kalina (1960) with some basis criticized the so-called temperature test, i.e., the capacity of a variety of the *Bacillus coli* for gas formation at 43 degrees Centigrade in liquid media with mannitol or glucose; this test is the basic sign of the sanitary-indicator value of the given variety of *Bacillus coli*.

I.S. Minkevich considered that this is the most characteristic sign indicating the origin of the microbes from the feces of man or warm-blooded animals. The *Bacillus coli* in cold-blooded animals does not ferment glucose or mannitol in liquid media at this temperature. But G.L. Zmeyev (1944) and I.S. Minkevich himself (1949) considered that for the southern regions of the Soviet Union (Central Asia) this test is not an indicator for the *Bacillus coli* from the feces of man or warm-blooded animals because the *Bacillus coli* in cold-blooded animals of the South of the USSR is able to ferment glucose and mannitol at 43 degrees Centigrade.

The growth of large inhabited points and the considerable contamination of adjacent rivers by them doubtlessly is causing sharp changes in the composition of the varieties of *Bacillus coli* in the feces of both man and of warm and cold-blooded animals. Further research in this direction is required.

Let us say a few words about the two-phase method of testing household-drinking water for the *Bacillus coli* as recommended by the All-Union State Standard 5216(56). Up to the present time this method has

encountered (without any basis) objections from a number of sanitary bacteriologists. At the same time the two-phase method of detecting the *Bacillus coli* in drinking water is considerably more advanced than the old three-phase method because it shortens the time for the analysis to 24 hours, is easy to perform, especially under field conditions, can be done successfully by a laboratory technician with a secondary medical or biological education, and is no less accurate than the three-phase method. In a small number of cases the two-phase method can sometimes give a small increase in the number of *Bacillus coli* at the expense of other gram negative bacteria such as the cloaca bacillus, *Proteus*, *Bacillus pyocyaneus*, and other bacilli. However, this does not interfere particularly with sanitary supervision because it only leads to somewhat higher demands in processing household-drinking water.

Different authors have established that bacteriophages are detected regularly in drain water from an open reservoir in the area of an inhabited point.

M.N. Fisher and his coworkers (1955) note the relationship between the content of dysentery bacteriophages in the water, its coli titer, and the isolation of dysentery haptene from it. As a result of their research, I.I. Goriyenko and K.F. Goncharova (1961) established a certain parallelism between the frequency of finding a bacteriophage in the water, the coli titer, and the general intake of bacteria. The authors note that most of the secreted phages possessed a strict species specificity but with a broad range of action with a species. Some of these phages were not strictly speci-

ric, possessed polyvalent characteristics, and dissolved some species of bacteria. However, despite the considerable number of works on this problem, tests of objects of the external environment for the presence of phages has not been accepted in the practice of sanitary-bacteriological research.

Detecting phages does not have any advantages in comparison with the titer of *Bacillus coli*; and the conduct of the analysis is considerably more complex than the widely proven detection of the *Bacillus coli*. In epidemiological practice the detection of phages cannot give definite results because of the above-mentioned polyvalence of various phages. In recent times direct and specific determination of various pathogens of infectious diseases in objects of the external environment by determining the increase of the titer of a specific phage has been introduced widely in epidemiology (V.D. Timakov and D.M. Gol'dfarb, 1960).

For the reaction of the increase of the titer of the phage it is necessary to have phages with a rigidly fixed range of action and with known characteristics.

The occurrence of various saprophytes, including the *Bacillus coli*, in food products and all kinds of washings for which there are no corresponding standards on bacterial data (except preserves) cannot serve as the basis for their rejection because the spoiling of the products depends not only on the quality of the saprophytes but also on their quantity. The progressive spoiling of a product is established by simple organo-

leptic determinations. The analysis of individual products sent to a laboratory by sanitary control with a request only to determine the quantity of microbes and the titer of the *Bacillus coli* usually does not give any basis for establishing whether or not the products are fit for consumption. For this it is necessary, in addition to the bacteriological analysis, to make organoleptic and chemical tests of the food product. And one organoleptic determination can often serve as sufficient basis for condemning products.

The content of the various saprophytes, including the *Bacillus coli* in considerable quantities, can serve as only a relative indicator of the quality of the product and, in particular, of the sanitary conditions of the preparation, transportation, and storage of the product; therefore, prophylactic bacteriological analysis of a food product should be conducted when there is constant observation of the production processes, as in a bakery, food plant, etc.

The so-called mixtures and dairy products intended for children are subject to especially careful bacteriological analysis. For these products which undergo special thermal processing or are prepared with special fermenting cultures, the determination of the *Bacillus coli* and of the saprophytes gives the sanitary doctor the data for a sanitary evaluation of the product.

The discovery in food products of pathogenic bacteria, including the pathogens of food infections and intoxication, and also of viruses serves as a direct indicator for forbidding the use of the product as food

without appropriate processing as determined for each separate case.

In performing prophylactic measures it is sufficient to determine only the species of the pathogen. When further study is necessary, the determination of the antigenic structure and the serological type and phage type of the isolated pathogen, including the Salmonella, should be conducted only at well-equipped oblast and republic sanitary-epidemiological stations or even at institutes.

If the practical laboratories of the sanitary-epidemiological stations are freed of this laborious and inappropriate and sometimes even useless work, they will be able to discharge their direct responsibilities more effectively.

A task for theoretical sanitary microbiology is to develop accelerated methods for detecting pathogenic microorganisms in the external environment. The development of the detection of enteroviruses (poliomyelitis virus, coxa virus, in soil, water, and food products is of particular interest. This has been studied only slightly.

Modern achievements in the fields of physics (optics) and biology (preparation of elective media, the serology and utilization of antibiotics, antagonists, and methods of concentrating bacteria) have opened sufficiently broad perspectives for the sanitary microbiologists for developing direct and accelerated methods of detecting pathogenic microorganisms in the external environment.

In recent years the identification of bacteria

using microscopy has broadened considerably, especially with respect to ultraviolet and infrared radiation.

Microscopic objects, including microorganisms, absorb and scatter light waves of a certain wave length, i.e., they possess definite absorptive and diffusing capabilities with respect to light waves of a certain wave length. By selecting the appropriate light filter it is possible to measure the absorptive or diffusing capability of microorganisms with respect to light waves.

The absorption and diffusion spectra for light are peculiar for each species of microorganism and can be used for determining the species and the quantity. By linking for the microscopic study of objects the appropriate light filters, photosensitive elements and electronic photoenlargers and also an oscillograph or television apparatus, it is possible to obtain an image of microorganisms (their morphology, quantity, etc.) on the screen of the oscillograph or television set which is visible to the naked eye (Kidle, 1956; Basis, Marcel, 1959).

In the USSR A.Ye. Mikirov (1957) has been working on the detection of microparticles in precipitation and clouds and in the solid and liquid fractions of aerosols through the study of their optical characteristics on the basis of studying their spectrum.

By selecting the appropriate light filters, A.Ye. Mikirov considers it possible to make a rapid determination and calculation of microorganisms in the external environment. According to the data of A.Ye. Mikirov, L.I. Mats, and Ye.Yu. Lebedeva, it is possible

using a gaseous light filter to detect 8% of the yeast cells which are dispersed in the air.

The analysis of the problems of employing the optical properties of microorganisms together with microscopy, photography, and television for the rapid indication of microorganisms in the external environment (air, water, etc.) offers extraordinary possibilities.

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CHRONICLE AND INFORMATION

Following is a translation of an unsigned article in the Russian-language periodical Gigiyena i Sanitariya (Hygiene and Sanitation), No 10, Moscow, 1962, pages 122-124.7

During April 1962 a plenum was held of the Scientific Council of the Ministry of Public Health of the Ukrainian SSR. The hygienists of the Ukraine took an active part in the work of the plenum. Professor D.F. Chebotarev, the chairman of the Scientific Medical Council and a corresponding member of the Academy of Medical Sciences of the USSR, gave a talk on planning scientific research work. Those who spoke at the sessions expressed the wish that the communication of the institutes with the central problem commissions be accomplished only through the republic commissions.

The chairman of the Kiev Hygienic Society and corresponding member of the Academy of Medical Sciences of the USSR, G.Kh. Shakhbyazan, spoke at the plenum. He said that in the recommendations on planning in the field of hygienic problems, experimental-theoretical research and conclusions should be reflected to a great degree. Such research could be conducted successfully first of all in the departments of medical schools where cadres of highly-qualified hygienists are concentrated (of the 22 hygienists of the republic who are

doctors of sciences, 19 work in the hygienic departments of medical schools); however, the conditions for this have not yet been established. Scientific laboratories have not yet been developed fully in the hygienic departments of medical schools; sufficient funds have not been appropriated for acquiring modern equipment; and there are few scientific assistants. Professor G.Kh. Shakhbazyan considers that the appropriate utilization of the highly-qualified cadres of hygienists of the medical schools of the Ukraine is a very real task.

Doctor of medical sciences and chairman of the problem commission I.I. Medved' spoke in detail on the tasks and progress in fulfilling the scientific plan on the problem of "Industrial hygiene in the leading branches of industry and agriculture."

The proposals which were introduced by the hygienists were reflected in the resolution of the plenum.

In Moscow from 27 to 29 June 1962, the Institute of Industrial Hygiene and Occupational Diseases of the Academy of Medical Sciences of the USSR conducted the First All-Union Symposium on Actual Problems of Occupational Dermatology. During the four sessions of the symposium over 40 scientific reports were given on the organization of the struggle against occupational dermatosis, on the pathogenesis of occupational dermatosis, on the etiology of occupational dermatosis, and on the prophylaxis of occupational dermatosis. Representatives from Moscow, Astrakhan, Ashkhabad, Vladimir, Volgograd, Voronezh, Gorkiy, Dzerzhinsk,

Donetsk, Izhevsk, Karaganda, Kaunas, Kemerovo, Kiev, Krasnodar, Leningrad, Minsk, Novorossiisk, Odessa, Omsk, Podolsk, Rязan, Serpukhov, Tbilisi, Tashkent, Ufa, Kharkov, and Yaroslavl spoke.

The Moscow City Sanitary-Epidemiological Station and the section of the organization for public health of the Moscow branch of the All-Russian Scientific Medical Society of Hygienists and Sanitary Doctors on 10 July 1962 conducted the first scientific-practical conference on problems of the organization of sanitary-epidemiological work. At the conference reports were given on the following themes: new forms of the organization of the sanitary-epidemiological service of the population of Moscow, the organization of the work of the sanitary-epidemiological station in lowering the incidence of intestinal infections, the experience from the work of the public council of the sanitary-epidemiological station of the Timiryazevskiy Rayon of Moscow, the transfer of some functions of the sanitary-epidemiological station to the public, experience in organizing prophylactic work in a doctor's sector, ways of improving the organizational forms of the sanitary service of the workers of industrial enterprises, experience in organizing periodic medical examinations of workers at industrial enterprises and their significance to public health, the role of a sanitary description of an area in the long-range and current planning of sanitary-epidemiological work, and the basic figures on the natural movement of the population of the rayons of Moscow during 1961.

At the Leningrad Sanitary-Hygienic Medical Institute there was a reader's conference on the magazine Gigiyena i Sanitariya for 1961. Professor A.Ye. Gutkin, a member of the editorial council of the magazine gave a talk on its activities. Professor A.I. Shtreys, Professor V.I. Bashenin, and secretary of the hygienic society K.K. Bogolyubov participated in the review discussions. The speaker and those who took part in the discussions noted the positive aspects of the journal: the increase in the number of experimental works which were published, the organization of new sections of the journal, its pertinence and interest, etc. At the same time it was noted that there are still too few articles on practical sanitary doctors and articles of practical significance. The material is still held by the editors for a long time before it is published (on an average of nine months); few articles are published on the hygiene of children and adolescents. No articles were published on epidemiology, even from a social-hygienic point of view. Almost no articles were published on rural hygiene. The work of the Fourteenth All-Union Congress of Hygienists and Sanitary Doctors was weakly reflected in the magazine. Desires were expressed to see an expansion of the section for letters examining hygienic principles in theory and in practice, to describe the activities of the sanitary organs and to cite their experiences, to include more review articles on various branches of hygiene of both a descriptive and a critical nature, and to devote attention to problems of teaching in sanitary-hygienic faculties and to material on the

activities of local branches of the hygienic society. A desire was also expressed to the hygienists and sanitary doctors of Leningrad for them to send more articles and informational material to the magazine.

The Ministry of Public Health of the RSFSR issued "Methodological Instructions on Conducting Preliminary and Periodical Medical Examinations of Workers in Certain Petroleum-Chemical Production Processes" as No OS-5-443, as approved on 9 February 1962. The instructions were prepared by senior scientific personnel of the Moscow Scientific Research Institute of Hygiene imeni F.F. Erisman, namely by A.M. Vyalov, Yu.L. Yegoriv, and M.I. Fongaus. The instructions give the sanitary characteristics of technological processes and of sanitary working conditions, a short toxicological description of the basic substances encountered by workers in the production of phenol, acetone, synthetic aliphatic acids and higher aliphatic alcohols, and also the clinical aspects of the treatment and prophylaxis of occupational diseases of workers in the production of phenol and acetone, synthetic aliphatic acids and higher aliphatic alcohols. The instructions were issued in 500 copies and were sent to the therapeutic-prophylactic and sanitary-hygienic institutions of the autonomous republics, krais and oblasts of the RSFSR.

At the Institute of Hygiene of Children and Adolescents of the Academy of Medical Sciences of the USSR a seminar, which was called by the Ministry of Public Health of the USSR, was held on 15-16 March 1962.

It was for school sanitary doctors of republic and oblast sanitary-epidemiological stations and was on the organization of preventative sanitary observation of children, pre-school facilities, and schools. There were 86 doctors present from 14 union republics and 49 oblasts and krais of the Russian Federation. Lectures were given at the seminar on new principles of planning children's pre-school facilities and schools, on new plans for the norms and technical conditions of planning general schools, international schools, and children's pre-school facilities, on the tasks of sanitary observation in connection with the new polytechnical aspects of schools and the appearance of children's pre-school institutions and of schools of a new type. Those present at the seminar became acquainted with new types and experimental projects for different types of schools and children's pre-school facilities and also examined a display of new equipment for children's pre-school institutions at the Giproprosv State Institute for Planning. The participants in the seminar exchanged experiences and posed a series of questions for the State Sanitary Inspection of the USSR which are to be considered in developing sanitary regulations.

In June 1962, the Ufa Scientific Research Institute for Hygiene and Occupational Diseases conducted a seminar for industrial sanitary doctors of sanitary-epidemiological stations and doctors of medical-sanitary units and health centers of the RSFSR on questions of industrial hygiene, occupational pathology, and industrial toxicology in the petroleum and petroleum-chemical industries. Participants in the activities

of the seminar included representatives from the Bashkir, Tatar, and Chechen-Ingush Autonomous SSR's, Krasnodar Kray, Kuybyshev, Leningrad, Omsk, Perm, and Ryazan Oblasts, and the city of Gur'yev of the Kazakh SSR.

The State Sanitary Inspection of the Ukrainian SSR during 1961 and 1962 approved ten methodological letters which were prepared by the Kiev Institute of Industrial Hygiene and Occupational Diseases. These methodological letters and instructions cover actual problems of industrial hygiene and occupational pathology. The following methodological letters and instructions were issued: 1) "Methods of determining the activity of cholinesterase of blood (a sensitive indicator of the action of phosphoro-organic insecticides on the organism)"; 2) "Methodological letter on detecting tetraethyl lead in the air by employing silica gel as a sorbent"; 3) "Methodological letter on the employment of solid sorbents for selecting samples of air which contains toxic admixtures"; 4) "Methodological letter on periodic medical examinations for personnel working with radioactive substances and sources of ionizing radiation"; 5) "Methodological instructions on sanitary-dosimetric observation of working conditions when working with sources of ionizing radiation"; 6) "Methodological letter on the prophylaxis of poisoning when employing diene insecticides in agriculture"; 7) "Methodological instructions on sanitary observation of the construction of annular furnaces in brick plants"; and 8) "Methodological letter on the conduct of periodical medical examinations of personnel—

working with DDT."

The Inter-departmental Commission on the Study of the Chemical Composition and Nutritive Value of Food Products of the State Sanitary Inspection of the Ministry of Public Health of the USSR prepared Tablitsy khimicheskogo sostava i pitatel'noy tsennosti pishchevykh produktov (Tables of the Chemical Composition and Nutritive Value of Food Products) which were published by the State Publishing House of Medical Literature in 1961. By order of the Ministry of Public Health of the USSR (Order No 34, dated 20 January 1962) the indicated tables (second edition) were put into effect and the tables of the first edition were announced to be invalid.

The book has four groups of tables: a) the chemical composition and calorificity of the edible part of food products; b) the chemical composition and calorificity of products which have not been rid of waste; c) the chemical composition and calorificity of the assimilated quantities of the edible part of products and of products which have not been rid of waste; and d) calculating tables (based on 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 grams of the products). The tables contain data concerning 802 different food products. Besides the content of proteins, fats, carbohydrates, ash, water, and calorificity, there are also data on the content of mineral elements (potassium, calcium, magnesium, phosphorous, and iron) and vitamins (A, carotene, thiamine, riboflavin, nicotinic acid, and ascorbic acid).

The publicity-publishing plant of the Moscow City Executive Committee (3 Second Kolokovskiy Pereulok, Moscow I-61) produces tapes of lectures, talks, speeches, reports, etc. The tapes can be transmitted by radio in all cities and towns of the Soviet Union and also over the local radio net in industrial enterprises, in clubs, Red Corners, libraries, etc. The cost of preparing an original tape of up to five minutes duration is 180 to 200 rubles; the cost of each copy is two rubles. The plant will send the tapes at the request of the orderer in accordance with the existing postal rates.

From 16 to 23 April 1961, the Angara Scientific Research Institute of Industrial Hygiene and Occupational Diseases conducted an inter-oblast seminar for sanitary doctors and other medical personnel serving industrial enterprises on the topic of matters of hygiene and occupational pathology. Seventy persons -- personnel of the sanitary-epidemiological stations and therapeutic-prophylactic institutions of the Buryat and Yakutsk Autonomous SSR's and Irkutsk and Chita Oblasts -- were present at the seminar. The questions which were discussed included preventative sanitary surveillance in industry, protecting the atmosphere from industrial contamination and the laboratory control of the cleanliness of the air, occupational pathology from the action of lead, manganese, mercury, ethyl gasoline, and carbon monoxide, the vibration factor and dust. Demonstrations were given at the seminar of cases of occupational diseases.

In accordance with the new statute on the work of editorial boards of medical journals which establish^{ed} the periodic change of part of the members of the boards, by order of the Ministry of Public Health of the USSR No 151, dated 2 April 1962, Yu.D. Lebedev, Professor S.Ye. Sovetov, and Professor N.I. Orlov are freed from their duties as members of the editorial board of the journal Gigiyena i Sanitariya. By this same order K.F. Smirnov, Chief of the State Sanitary Inspection of the Ministry of Public Health of the USSR, Professor S.M. Grombakh, Deputy Director of the Institute of Hygiene of Children and Adolescents of the Academy of Medical Sciences of the USSR, and Doctor of Medical Sciences A.V. Bykhovskiy, a member of a branch of the Institute imeni Karpov, were selected to the editorial board of the magazine.

Professors S.Ye. Sovetov and N.I. Orlov were added to the Editorial Council of Gigiyena i Sanitariya.

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