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Quick Diagnosis and Sanitary Expertise of Water Contaminated by Pathogenic Microbes or their Toxins

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A sanitary expert examination of water presents a time consuming and important task performed by health officers together with epidemiologists and bacteriologists.

There are very many methods of detection and identification of microbes or their toxins. In examination of water one must use the methods which offer the most complete and reliable data in the shortest possible time.

Much consideration has been and is still given here and abroad to the problems of a quick diagnosis and identification of pathogenic bacteria in natural medium.

The most promising are the methods of quick diagnosis that are based on the use of polysaccharide-hapten in seroreactions. This substance, as we know, is the only carrier of serological specificity of most pathogenic bacteria.

We, in the Soviet Union, have developed a method of this reaction with 1 (0)-group of human erythrocytes, as reported by...
KRAVCHENKO and SOLOLOV (1). In addition to the positive side, this technique also has a number of important disadvantages. Its basic negative factor is that, being a living biological substrate, human erythrocytes die away rather quickly and are destroyed outside the organism; even a slight hemolysis accompanying this process causes a spontaneous agglutination, which is indistinguishable from the agglutination yielded by specific sera. Sometimes, the same autoagglutination begins because erythrocytes possess their antigenic nature. One can prevent, for some time, a quick destruction of erythrocytes by keeping them in the cold.

The use of banked blood and desiccated stromal erythrocytes reduces the sensitivity of reaction.

A.V. ORLOV (4) proposed to use cholesterol that possesses no natural antigen, but is a component of stromal erythrocytes; it has long since been used in the serological diagnosis of syphilis as an ingredient intensifying the sensitivity of antigens.

The author named his reaction "haptocholic flocculation in vitro". Its essence is the same as that of the reaction of KRAVCHENKO and SOLOLOV. The distinctive feature is the substitution of alive, very unstable and costly adsorptive \((\gamma)^{(0)}\)-group of human erythrocytes by a chemical ingredient (cholesterol) which can be kept in a powder form for years.

We checked the sensitivity and specificity of the reaction of KRAVCHENKO and SOLOLOV using \((\gamma)^{(0)}\)-group of human erythrocytes; we also checked the haptocholic flocculation reaction in vitro proposed by A.V. ORLOV.

To prepare polysaccharide-hapten for use in our experiments,
Table 1

Findings on Sensitivity of haptococholysis: Floculation Reaction in Vitro Compared with those of the KRAVCHENKO-SOLODUC reaction with Pure Haptens of Dysentery Group and with those of the Mixture of Added Coliform Bacteria

<table>
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<tr>
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<th>Initial concentration of microorganisms in 1 ml of investigated fluid (in millions)</th>
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<tr>
<td></td>
<td>Haptocholic floculation in vitro</td>
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<tr>
<td></td>
<td>25</td>
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<tr>
<td>FLEXNER'S dysentery bacteria</td>
<td>--</td>
</tr>
<tr>
<td>SCHMITZ-SCHUTZER'S dysentery bacteria</td>
<td>+</td>
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<tr>
<td>SCHONNE'S dysentery bacteria</td>
<td>++</td>
</tr>
<tr>
<td>Mixture of polysaccharide-haptens of dysentery and coliform bacteria added proportionally</td>
<td>FLEXNER'S dysentery bacteria 1:5</td>
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<td>1:10</td>
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<td>SCHMITZ-SCHUTZER'S dysentery bacteria 1:5</td>
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we obtained FLEXNER'S dysenter bacteria "C", No.3: then SCHMITZ's bacteria, also coliform bacteria, also coliform bacteria. 290.

SCHUTZER'S and SONNE'S dysent (KCHISELKOV) No.1 and 5, as well as SCHMITZ's and SCHUTZER'S dysent bacteria were prepared according to modified methods in water, contained 1 billion of organisms in 1 ml.

Polysaccharide-hapten was prepared according to KCHISELKOV method from bacterial suspensions in water from 25, 50, 100 and 200 million of organisms.

The essence of modification was confined to our use of a smaller quantity of "C" and this expressed itself more favorably in osmotic aspects solutions. The method of preparation has been described in detail by A. SOKOLOV (6) and A.V. ORLOV.

The densification of suspensions was determined according to optimal intestinal standards. The volume of liquid in the assay process took place was 5 ml. Partial reactions also accomplished in 12.5 ml volume for comparison purposes.

Serum diluted 1:10 was used with the titers FLEXNER's polyvalent - 12,800; SCHMITZ-SCHUTZER's - 25,600 and SONNE'S - 6,400.

In all, we performed 48 experiments with pure polysaccharide haptenes of dysentery and 108 experiments with a mixture of added...
Collective experimental results are presented in Table 1.

As we see from Table 1, the haptchocholic-flocculation reaction in vitro was positive in the presence of a 50 million concentration of microorganisms in 1 ml of initial suspension in the case of SCHMITZ-SCHUTZER's dysentery bacteria; also in the presence of 100 millions of FLEXNER'S dysentery bacteria and 200 millions of SONNE'S dysentery bacteria. The experiments with the KRAVCHENKO-SOKOLOV reaction were affirmative in the presence of 25 million concentration of microorganisms in initial suspension of SCHMITZ-SCHUTZER'S dysentery bacteria and with 50 million concentration of FLEXNER'S and SONNE'S dysentery bacteria.

Dissimilar data pertinent to the sensitivity of both reactions with various types of dysentery bacteria are, apparently, influenced by sera of used serum.

The evaluation of the haptchocholic flocculation in vitro was hindered by insufficient homogenization of cholesterol in the physiological solution after centrifugation and in sera during the performance of experiments. In connection with the above, we considered a positive reaction with such concentrations of haptens, which would make quite impossible to confuse a specific and non-specific conglomeration of haptchocholic suspension in vitro during basic experiments and also under control conditions. In the reaction evaluated by us as positive, the agglutination of suspended cho-lesterol particles was clearly visible, also their enlargement and a distinct clarifying of fluid were apparent. We rated such reaction evaluation by two crosses (++) or three crosses (+++) were
indicated in cases in which the agglutination, the enlargement of cholesterol particles and the clarity of fluid were still better expressed. In the presence of massive clottings and complete clarity of fluid, the reaction was rated by four crosses (++++). All reactions with cholesterol were examined behind a black background within 2 to 5 minutes. Reactions after 5 minutes were disregarded.

The KRAVCHENKO-SOKOLOV reaction is more pliant in a sense of evaluation, consequently it offers an opportunity for detection of erythrocytes' agglutination at much lower concentrations of haptens, i.e. when fresh human erythrocytes are used of the I (C)-group and have been washed just 2 or 3 times. Erythrocytes kept in the cold for 4 or 5 days, or at room temperature for one week gave a spontaneous agglutination, which could be prevented by adding erythrocytes at least 4 times.

Thus, we accepted as positive the KRAVCHENKO-SOKOLOV reaction and rated it by two crosses (++), while applying three crosses (+++) to the haptocholic flocculation in vitro.

The addition of heterogeneous antigens to tested substances in proportions of 1:5, 1:10 and 1:15 failed to have a noticeable effect on the sensitivity of both reactions.

The specificity of the haptocholic flocculation reaction in vitro and that of the KRAVCHENKO-SOKOLOV reaction has been confirmed in numerous experiments with the reciprocal agglutination involving heterogeneous and homologous serums.

The number of positive reactions increased in solutions containing 25 to 50 millions of microorganisms, following the in-
crease from 5 to 12.5 ml of the volume of fluid in which the adsorption process took place, while the concentration of microorganisms remained unchanged in 1 ml. The same effect was not observed in mixtures containing much higher concentrations of microorganisms.

The contact photographing helped us in reaction readings and in computation of the reaction intensity during our research; it could also be successfully used in a practical performance similar to the procedure with the diaphragm filters at the waterworks laboratories to determine the indexing of water.

Our verification of the improved reaction method suggested by A.V. Grlov and involving haptocholic flocculation with the use of diaphragm ultrafilters, fully confirmed the findings of the author, in that cholesterol, as a suspended matter, is more stable in suspensions. There is an obvious and greater reaction sensitivity reflected by way of a fuller adsorption of polysaccharide haptens from mixtures.

**Conclusions**

1. Seroreactions with polysaccharide-haptens are sufficiently sensitive and can be fully used in diagnoses of pathogenic bacteria during expert examination of water.

2. In practice, the haptocholic flocculation reaction in vitro is not inferior to the Kravchenko-Sokolov reaction.

3. It is essential to examine much closer the mechanism of the haptocholic flocculation reaction in vitro and to search for such colloidal system, which would give more homogeneous and stable suspensions of cholesterol.
Literature Cited


Summary Printed in English

Reaction of haptocholic flocculation on the glass with polysaccharide hapten of Flexner's dysentery bacteria "C", No.390, Starzer-Sheitz and Conne, put forward by A.V. Orlov, does not practically yield in its sensitivity to Kravchenko-Sokolov's reaction.

The flocculation of cholesterin and erythrocytes charged with polysaccharide hapten set in, as a rule, only under influence of specific sera.