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[Evaluation of the toxic action of prophylactic and therapeutic preparations on cell cultures. III. The detection of toxic properties in medical biological preparations by the degree of cell damage in the L132 continuous cell line].

[Article in Russian]

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The methods of the quality control of medical biological preparations, including tests on animals, do not ensure the complete absence of toxicity in a final product. The use of the method of "subcultures with the introduced preparation" makes it possible to determine the toxicity of both specific and nonspecific components of vaccines and sera from the number of dead and damaged cells. The toxic action of preparations kills and damages the cells at the site of injection, thus inducing the formation of autoantigens whose effect on the body cannot be predicted. Thus thimerosal, commonly used as preservative, has been found not only to render its primary toxic effect, but also capable of changing the properties of cells. This fact suggests that the use of thimerosal for the preservation of medical biological preparations, especially those intended for children, is inadmissible.

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## **EVALUATION OF THE TOXIC ACTION OF PROPHYLACTIC AND THERAPEUTIC PREPARATIONS ON CELL CULTURES**

**PAPER 3: The detection of toxic properties in medical biological preparations by the degree of cell damage in the L-132 continuous cell line.**

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Methods of quality control for medical and biological preparations (MBP) are as diverse as their indications and attributes. But a common requirement in testing all MBP is determination of safety, specificity and activity.

All medications injected into humans must be free of foreign microbial or viral contamination. Detection of sterility and safety is one of the most important steps in MBP quality control. MBP safety is determined on lab animals by changes in their weight or their death after a single MBP injection. According to the World Health Organization's (WHO) statement, there is no single animal or taxid family which is ideal for toxicity and safety testing.

It is well known, that for quality control, toxicity in cell cultures is the first and principal step in determination of possible adverse reactions in humans. Only after these tests is MBP testing performed on animals. This is mandatory, especially for MBP used in pediatrics. This quality control includes not only main chemical/biological component testing but also all ingredients, including additives, stabilizers, etc., because these chemicals may change MBP pharmacokinetics. But according to scientists, even with this accurate testing design, there is no 100% guarantee of no adverse reactions in humans thereafter. In the USSR, many MBP are used in pediatrics (against measles, tetanus, polio, rubella, etc.). These MBP, especially TB-vaccine (BGV) and pertussis, cause long-term allergic reaction in children after vaccination. This reaction is strong not only on repeated vaccine injection but with all MBP, especially live vaccines.

The technique of cell culture may be used for precise quality control. It is known that even the most promising chemotherapeutic antiviral medications are not approved for animal testing if their effective antiviral dose is toxic for cell cultures.

We tested DTP vaccine on the cell line L-132, whose advantages over other continuous cell lines have been studied by us and are described in detail in previous reports. In this research, we used MBP from the Scientific Institutes of Vaccines and Serum in Moscow and Ufa (USSR).

**Methods:** Cell culture with trypan blue for % cell death.

**Results:** We have already reported on the toxicity of DTP-vaccine and its components in our previous publications. In this paper, we report that different types of DTP-vaccine, produced by different or even the same manufacturers, may have different harmful effects on L-132 cell cultures. Cytotoxic titers varied over a dilutional range of 1:32 to 1:2048.

The most toxic effect on cells was caused by components of pertussis antigens and merthiolate solution. Comparing the results of titrating a series of DTP vaccines in cell culture with clinical symptoms showed a correlation between a high degree of cell damage in culture and a strong local reaction in children, above normal limits and characteristic of strong local allergens, thus showing the usefulness of cell cultures assays in MBP testing.

The next series of experiments showed that the same high toxicity (1:2048-1:4096) as DTP vaccine is characteristic of anti-rabies vaccine (Fermi), developed on sheep brain cell culture in which the virus is inactivated with phenol. Anti-rabies vaccine inactivated with ultraviolet radiation instead of phenol damaged cells at a titer of 1:32. Cultured anti-rabies vaccine does not contain brain tissue, phenol or merthiolate; this vaccine does not cause any changes in cell morphology even when undiluted.

Merthiolate in a concentration of 1:10,000, contained in one dose of vaccine, can damage cells in subsequent 2-fold dilutions up to 1:128. It was found that all tested MBP containing this preservative (see table – not included – EK) have in common the ability to damage cells in titers up to 1:128.

Anti-rabies and anti-influenza vaccines with no preservatives are not damaging or have a cytotoxic effect in titers not exceeding 1:2-1:4.

Immunoglobulin evaluation shows that MBP toxicity depends on manufacturing technology. MBP with merthiolate are highly toxic (1:256). Immunoglobulins without preservatives have no toxic effect on cells and their cytotoxic dosage (CTD) is not higher than 1:2 dilution. Because the addition of merthiolate does not improve MBP quality, many manufacturers make immunoglobulins without preservatives.

We have tested cell culture damage by some allergens prepared from bacteria with Ando-Verchikovskiy methodology. All tested series of MBPs had no toxic effect on cell cultures in dosages 10-100 times greater than those used for intradermal injections in humans.

Our research results have shown that studied MBPs may be of different toxicity (high cytotoxic effect (CTE) titer of 1:256-1:4096; medium – 1:32-1:256; low – 1:2-1:4). As our previous studies have shown, preservatives (merthiolate) and absorbent (aluminum hydroxide –Al<sub>2</sub>(OH)<sub>3</sub>) are highly toxic to cell cultures.

It is clear now, that the less of these nonantigenic but toxic substances that are added to serum and vaccine preparations, the less often they would cause unusual reactions in vaccinated children.

In MBP testing for non-specific antigens present in prophylactic vaccines, different cell cultures may be used, like HeLa, Hep-2, MIO, chicken fibroblasts, etc. that any manufacturing and diagnostic lab should have. We recommend using continuous cell lines obtained from human tissues. These cell lines are preferable due to their human specificity for the damage caused by MBPs and their ingredients.

It is obvious that studying toxicity by means of cell damage in vivo is more complicated than using cultured cells. CTE should be determined for every MBP. The continuous cell line method can also accurately and more quickly detect microbial contamination, which was found in some anti-rabies and DTP vaccines.

At present, there are highly effective MBP without toxicity in cultured cells that may be used for prophylaxis and treatment (killed influenza vaccine without preservatives, immunoglobulins without merthiolate, etc.). These medications cause no local reactions after injection as well.

Our experiments show that merthiolate in 1:10,000 titer can not only damage cells in culture but also change their properties. More thorough quality control should be applied if an MBP's CTE is higher than 1:256, and reasons for it other than added preservatives should be sought with more comprehensive techniques.

Currently (1986), a lot of evidence has accumulated confirming merthiolate's high allergenicity. Increased sensitivity to this mercury compound has been frequently noted in medical literature, and deserves particularly close attention. Although there are numerous clinical studies confirming merthiolate's damaging action on humans, MBP preservation with it still continues and is even recommended by WHO. Recent studies showed that merthiolate lowers interferon production by 8-fold.

All of the above show that merthiolate usage for MBP manufacturing is inadmissible, especially in pediatrics. A complicated mixture of antigens and non-antigenic substances may trigger many reactions in the human body after vaccination, and specific correlation between the specific trigger and gotten effect sometimes is difficult to prove. Chemicals and their characteristics may determine the body's reaction to immunoglobulin injections. Vaccines must contain only specific substances, free of ballast. There is no way that cell damage can cause no harmful sequelae in the body. This process is followed by auto-antibody production to antigens of the damaged cells, and in turn, their adverse actions are difficult to predict and determine.

Cell culture techniques may be used to determine toxicity and microbial contamination for inactivated as well as live viral vaccines. In the latter case, vaccine virus should be first inactivated with specific serum.

The current state of the art of molecular biology allows one to predict MBP adverse reactions with the help of cytomorphologic, cytotechnical, karyologic and other methods of study. Some cellular processes which occur in vivo may be studied only by modeling. The results of our experiments demonstrate that continuous cell lines may be useful for MBP quality control.

## **SUMMARY**

1. MBP used for prophylaxis and treatment in pediatrics, although approved for usage by current control methods, including animal testing, indeed may be toxic in cell cultures.
2. By means of passaged cell cultures, it is possible to accurately determine the non-toxic MBP concentration by the absence of cell damage. Toxicity of specific and nonspecific MBP ingredients may also be determined.
3. Cultures of continuous cell lines may effectively be used for MBP quality control.
4. Merthiolate usage for MBP preservation should be discontinued due to its high toxicity.