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A novel biocompatibility test for disperse materials

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Abstract

Introduction. Development of novel biocompatibility tests represents an urgent problem. Use of chicken embryos minimizes the effect of exogenous factors on the experimental course (as the chicken embryo develops in medium it is almost completely isolated from external effects), enables the observation of physiological and pathological processes in the dynamics and assessment of the response of the body response to various materials in many cell populations.

Objective. We aimed to show the possibility of using chicken embryo as a test system for evaluation of the biological effects of powdered materials.

Materials and methods. In this study, we applied developing chicken embryos produced by incubation of Highline white eggs. Test and control groups (200 embryos in total) were used. Powdered materials were introduced into the embryo yolk sac in the form of suspension in biocompatible dextran (rheopolyglucine).The material was sterilized for 60 min at 120 °C. Two disperse materials, activated charcoal and asbestos powders, were selected to assess the capabilities of the method. Morphological (review, selective histochemical, and electron microscopy) examination methods were applied in testing, which produced the following results.

Results. Model efficacy was confirmed by testing certain substances, such as activated charcoal and asbestos. Faster growth and accelerated development of chicken embryos, the absence of tissue pathological reactions, was indicative of the biocompatibility of activated charcoal. Poor biocompatibility of asbestos was concluded from its multiple teratogenic effects detected for the first time for this material.

Conclusions. The paper contains motivation and experimental data regarding the usability of chicken embryos in integrated testing of disperse material biocompatibility.

Key words

chicken embryos,activated charcoal, asbestos

INTRODUCTION

Development of novel, simple and reliable material biocompatibility tests still remains an urgent problem. In particular, the development of tests conforming to the following criteria is expedient:

- the tests should allow the detection of the biological effects of substances and materials with various dispersity, including those of nano-size range;
- the tests should allow assessment of the body (living tissue) responses to materials in many cell populations, including their interactions during body development;
- the tests should not require considerable expenses;
- the tests should be reproducible in standard laboratory conditions.

For that purpose, Laboratory of Endoecology and Anthropogenic Induced Pathology of the Institute of Sorption and Problems of Endoecology of the National Academy of Sciences of the Ukraine applied a 'method for assessing biological effects of substances and materials' [1]. Chicken embryos in their dynamics were applied.

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Usually, chicken embryos serve medical science in practice as virus culturing media. Thus, it is known as a method of using chicken embryos for culturing human and animal viruses for laboratory in the diagnostics of viral infections, investigation of pathogenesis and immune system state in the above diseases, and production of diagnostic and vaccine preparations [2]. According to this method, a hole is made in embryonated 5-day chicken egg, wherein the tested material, i.e. viruses, is introduced. After incubation for a few days, the embryo coats and body are extracted, and the situation is evaluated for signs of virus reproduction in the living embryo tissues. This model is intended for testing a separate group of purely microbial agents (viruses) only, although, it has certain limitations, as many viruses do not propagate in avian embryos.

The second variant involving chicken embryos as test systems is related with the use of their chorio-allantoic membrane to assess penetrability of various substances [3]. The authors of this method suggest making holes for observation in an egg with a 4-day embryo. An irritant, i.e. a cotton thread or a silicone tube, is introduced through a hole into the chorio-allantoic membrane. After that, the embryo is incubated for 8 days, chorio-allantoic membrane is extracted, and cellular response to materials applied is assessed by visual examination or using either histological or electron microscopy studies. According to the authors of [3], responses to irritations are similar to those in mammals and

vary depending on material types, and spatial structure of the tested materials exerts a significant impact on the manifested signs of response to the chorio-allantoic membrane irritants applied. Although agreeing with our colleagues, we should, nevertheless, state the limited amount of material which may be introduced into the chorio-allantoic membrane and limited number of varieties of embryo cell populations applied in complex studies, in view of the tiny size and cellular composition of the chorio-allantoic membrane.

OBJECTIVE

In our research we aimed to show the possibility of using chicken embryo as a test system for evaluation of the biological effects of powdered materials.

MATERIALS AND METHOD

In this study, we applied developing chicken embryos to assess the biocompatibility of powdered materials as the most complicated objects for biocompatibility testing. Here, in contrast to previously applied methods, we assessed biocompatibility by the aggregate of morphological characteristics at all stages of embryo development.

Powdered materials were introduced into the embryo yolk sac. Tested substances/materials administration term (third day of incubation) was selected due to the formation of yolk sac vessels by the said time [4]. The yolk sac is a provisory organ performing the function of yolk digestion and absorption, and simultaneously serving as the first hemopoietic and respiratory organ at the first fetus development stages, as yolk sac blood vessels approximate the subshell tunic. The embryo absorbs nutrients through the yolk sac; thus, there are reasons to introduce the materials being investigated for their effects on a living organism into it. This approach simultaneously prevents lesion of the embryo body itself, and assures penetration of the tested object into it, in view of the above structures' topography.

Two disperse materials, activated charcoal and asbestos powders, were selected to assess the capabilities of the method.

Powdered and granulated charcoals has long been applied in medical practice as an element of pathogenetic and sanogenetic therapy. Nevertheless, we considered it expedient to collect additional information on the biocompatibility of a growing body (developing chicken embryo).

Negative effects of the second powdered material (asbestos) on living organisms are well known; however, the available information included pneumoconioses (chronic pulmonary diseases induced by prolonged inhalation of harmful substances with specific fibrous tissue development) and mesotheliomas (serous epithelium tumors). We found no information pertaining to other pathogenic defects of asbestos, its tissue affinity and biocompatibility.

Chicken embryos produced by incubation of Highline white eggs in a domestic household incubator served as the study material. Test and control groups (200 embryos in total) were used in the experiments.

Activated charcoal of ZL-302 grade with density of 0.42 g/cm^3 and pore diameter of 30–70 μm was administered. Ash content in this charcoal is less than 4 %. The material was sterilized for 60 min at 120 °C.

Asbestos (serpentine, chemical formula $[Mg_6Si_4O_{10}(OH)_8]$) had a particle size of less than 0.25 mm, and was sterilized in similar conditions.

Both materials were injected into eggs in the form of suspension in biocompatible dextran (rheopolyglucine). Morphological (review, selective histochemical, and electron microscopy) examination methods were applied in the testing, which produced the following results.

RESULTS AND DISCUSSION

It was found that activated charcoal administration promoteed faster growth of chicken embryos and their accelerated development, especially at late incubation stages. Most likely, this is related with the localization of harmful recrements formed during embryo vital activity on this sorbent. Activated charcoal had no significant effect on yolk sac choroid formation – normal and pathologically deformed vasculature parameters were comparable to those of control specimens.

Subsequent to the complex study of the experimental material, we have documented the effect of activated charcoal particles penetration through cellular membranes, their migration and deposition in the body. As these phenomena (according to results of morphological methods applied) were not accompanied by pathological reactions; in our opinion, the biocompatibility of powdered charcoal material may be concluded (Tab. 1, Fig. 1).

Table 1. Results of activated charcoal and asbestos powders biocompatibility studies using chicken embryos

Manifested parameters	Tested substances	
	Activated charcoal	Asbestos
Chicken embryo growth	Increase	Inhibition
Chicken embryo development	Acceleration	Deceleration, death
Yolk sac choroid	Has no significant effect	Has pronounced pathological effect
Ability for migration in embryonal tissues	Found	
Ability for causing pathological changes of embryonal tissues	Not detected	Detected (multiple lesions)
Conclusion	Biocompatible	Has poor biocompatibility

During the second stage of the experiments, we detected a negative effect, including teratogenic action (accompanied by the development of malformations), of asbestos on chicken embryos. Besides, their death rate during incubation was five times as high as that of the control specimens. Moreover, the inhibitory effect of asbestos on fetal growth and its pronounced pathological effect on yolk membrane vessels has been documented. These signs, as well as the presence of multiple lesions in tissues of asbestos group embryos, enable concluding pthe oor biocompatibility of this material (Tab. 1).

CONCLUSIONS

1. Chicken embryo studies represent convenient and effective means for the integrated testing of the biocompatibility of powdered materials, which allow the prevention of

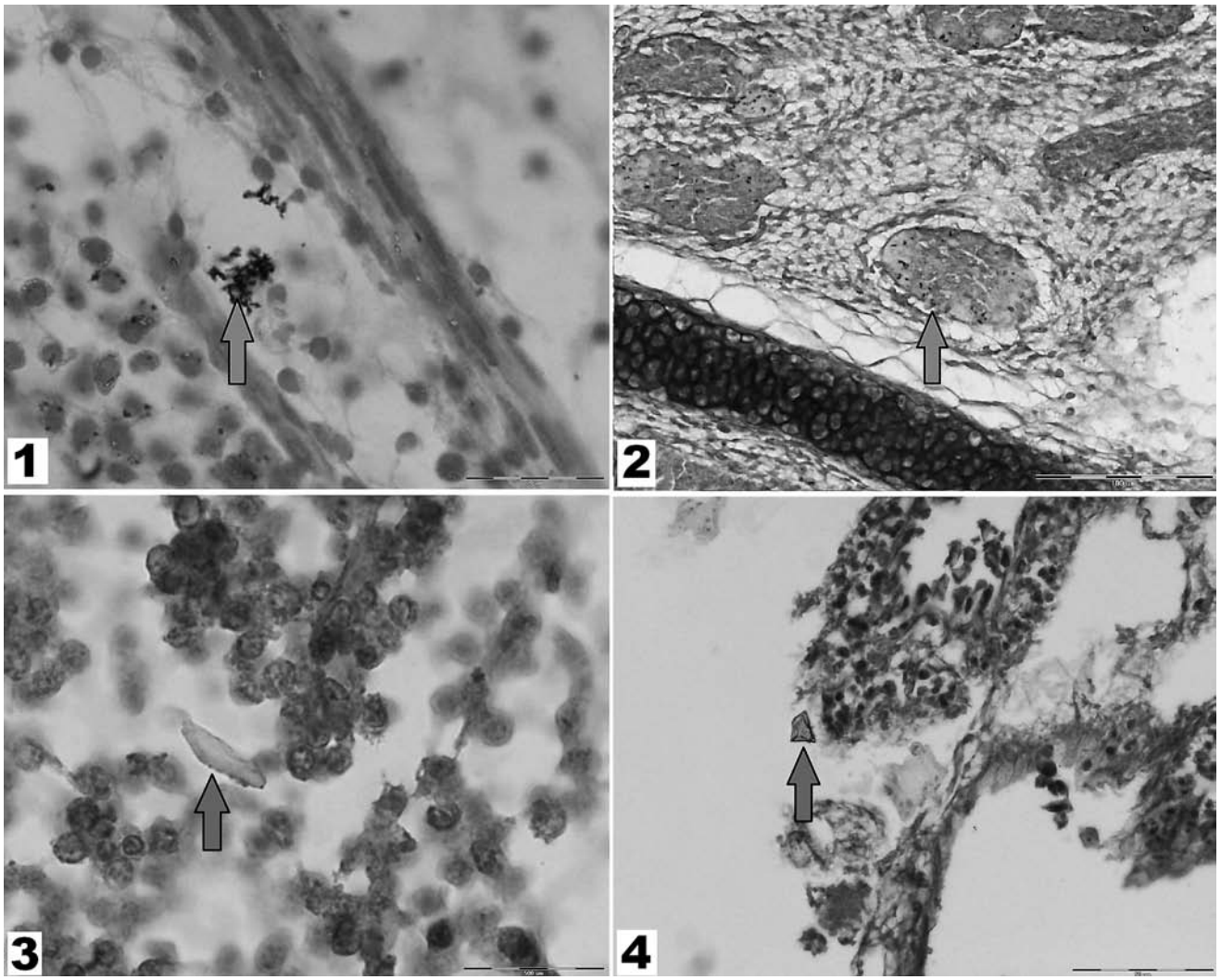

Figure 1. Test application results.
Activated charcoal particle depositions (shown by arrows) in 17-day chicken embryo tissues are not associated with any lesions or inflammatory response, which is indicative of inertness of the administered substance. 1 – Congo red staining, ×1000; 2 – Hematoxylin and eosin staining, ×200.
Asbestos crystal depositions (shown by arrows) in damaged (necrobiotically altered) 10-day chicken embryo tissues without pronounced inflammatory response. Hematoxylin and eosin staining: 3 – ×1000; 4 – ×400

any extraneous effects, thus assuring the integrity of experiments.
2. In spite of the ability of activated charcoal to penetrate through cellular membranes and migrate in the body, the absence of tissue pathological reactions is indicative of its biocompatibility.
3. The faster growth and accelerated development of chicken embryos (especially at late incubation stages) detected after activated charcoal injection into yolk sacs allows assuming the localization of harmful embryo recrements on this sorbent.
4. Poor biocompatibility of asbestos was concluded from its multiple teratogenic effects detected for the first time for this material.
5. The aggregate of accumulated data enables identification of the developed biocompatibility assessment method as an integrated test.

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