FUNGICIDAL PROPERTIES OF THE ALBUMEN MEMBRANE OF CHICK'S EGG

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The albumen membrane of chick egg is a powerful defense barrier protecting the developing embryo from a variety of infections.

It kills or inhibits the development of microorganisms in the environment of the chick egg [1, 2, 3, 7, 8, 10].

The question as to whether the albumen membrane possesses antibiotic properties in relation to lower fungi and actinomycetes has been little studied. Kossowicz (1912, 1916) showed the presence of fungicidal properties in the albumen of non-incubated chick egg in relation to Aspergillus niger, Penicillium glaucum, Cladosporum herbarum and vine yeasts. The effect of the albumen membrane on Aspergillus and Penicillium spores and mycelia [5] has been studied in greater detail.

In the present work the results are given of experiments on the influence of the albumen membrane of chick egg on the cells of Torula utilis and the spores of Actinomyces albas and Actinomyces griseus. In connection with the problem of the immunity of the fetus, studied in our laboratory, we were interested in the properties of the albumen membrane with which microorganisms may come into contact in natural conditions. For the purpose of comparison, experiments were also conducted with the influence of the albumen denatured by high temperature and the yolk of a non-incubated chick egg on yeasts and actinomycetes.

Since we earlier succeeded in establishing that the outer, middle and inner layers of the albumen membrane possess different bactericidal and protozoacidal properties [4, 5] we conducted differentiating investigations of the fungicidal properties of the different layers.

We used in the experiments white leghorns' eggs not later than 10 days after hatching. The contents of the egg was decanted aseptically into a Petri dish. In the test tube 2 ml of albumen was accumulated to which was added 0.2 ml suspension of the spores of Actinomyces or the yeast cells in physiological solution (number of spores in the various experiments varied). The control media were a meat-peptone bouillon (for the actinomycetes) or must (for the yeasts) and also physiological solution. At definite time intervals, we made seedings on a Petri dish with a solid nutritive medium. The Petri dish and test tubes were kept in a thermostat at 25°C. For the seedings we used three day old cultures of yeasts and actinomycetes. Altogether 5 experiments with actinomycetes and 4 experiments with yeasts were conducted. The results of the experiments proved to be analogous.

Torula utilis was uniformly resistant to the effect of the albumen from various layers of the membrane of the non-incubated chick egg.

Below are given the results of an experiment in which to 2 ml of chick egg albumen and of the control medium was added 0.2 ml physiological solution containing 1600 cells of Torula utilis (Table 1).

Unlike the yeasts the actinomycetes rapidly perished on contact with the chick egg albumen. The representatives of two species of Actinomyces albas and Actinomyces griseus which we used in the experiments proved

to be sensitive in differing degrees to the action of this albumen. In Table 2 are set out the results of the experiment in which the effect of various layers of the albumen membrane on Actinomyces griseus was studied. In this experiment to 2 ml of chick egg albumen and the control medium was added 0.2 ml physiological solution containing 18,000 spores of Actinomyces griseus.

TABLE 1

Effect of Different Layers of Albumen Membrane of Non-incubated Chick Egg on Cells of Torula utilis.

Medium	Seedings				
	immediate	after 1 day	after 3 days	after 5 days	after 7 days
Layer of albumen membrane: outer	+	++	+++	+++	++++
Layer of albumen membrane: middle	+	++	+++	+++	++++
Layer of albumen membrane: inner	+	++	+++	+++	++++
Physiological solution	+	++	++	++	++
Must	+	+++	++++	++++	++++

Significance: ++++ continuous growth; +++ weakly marked growth; ++ over 100 colonies; + single colonies.

TABLE 2

Effect of Various Layers of the Albumen Membrane of a Non-incubated Egg on Spores of Actinomyces griseus.

Medium	Seedings						
	immediate	after 2 hours	after 4 hours	after 8 hours	after 24 hours	after 2 days	after 3 days
Layer of albumen membrane: outer	++	++	++	++	+	0	0
Layer of albumen membrane: middle	++	++	++	++	+	0	0
Layer of albumen membrane: inner	++	++	++	++	+	0	0
Physiological solution	++	++	++	++	++	++	+
Meat-peptone bouillon	++	++	++	++	+++	++++	++++

Conventional signs: O-sterile, absence of growth; other signs as in Table 1.

All three layers of the albumen membrane showed themselves to be uniformly active in relation to Actinomyces griseus. Upon insertion into the albumen of the chick egg of a smaller number of Actinomycetes spores, absence of growth was observed in the sub-cultures after 18-24 hours in this medium.

Table 3 gives the results of one of the experiments with Actinomyces albas in which to 2 ml of chick egg albumen and control medium was added 0.2 ml physiological solution containing 50,000 Actinomycetes spores.

All three layers of the albumen membrane possessed exceptional anti-microbial properties in relation to Actinomyces albas; the spores of this species of actinomycetes die immediately on contact with the layers.

TABLE 3

Effect of Various Layers of Albumen Membrane of Non-incubated Chick Egg on Spores of Actinomyces Albas.

Medium	Seedings			
		after		
	Immediate	1 hour	24 hours	48 hours
Layer of albumen membrane: outer	0	0	0	0
Layer of albumen membrane: middle	0	0	0	0
Layer of albumen membrane: inner	0	0	0	0
Physiological solution	++	++	++	+
Meat-peptone bouillon	++	++	+++	++++

Conventional signs as in Tables 1 and 2.

Experiments with liquid chick egg albumen from the inner and outer layers diluted with physiological solution were also carried out. We established the maximum dilution at which the fungicidal effect is observed. It was shown that with the albumen effect on Actinomyces albas diluted with physiological solution (1:10,000) growth was not observed in the sub-culture after 24-28 hours with this influence. Upon greater dilution the effect was the same as that of the pure physiological solution.

The material we obtained on the fungicidal properties of the albumen membrane is of interest in connection with the problem of embryonic immunity.

Particularly noteworthy is the fact discovered by us of the exceptional toxicity of chick egg albumen in relation to Actinomyces albas. This agent, as in the case of Micrococus lysodecticus, may serve as an indicator of the antibiotic activity of the albumen membrane.

It is interesting that the chick egg albumen denatured by high temperature loses the capacity to kill actinomycetes and when the spores are planted on it, on the third day intensive development of them and fertilization takes place.

The yoke of fresh non-incubated chick egg is an excellent nutritive medium for the development of the investigated actinomycetes and air yeasts. Thus in the system of the chick egg only the albumen membrane insures the function of a defense barrier. Upon penetration by the bacterial cells and the spores of the fungi through the shell and sub-shell membranes, the former either perish or do not develop and remain, as it were, preserved in the albumen membrane.

Further investigations of the antibiotic properties of chick egg albumen and the changes in these properties in the course of development of the chick embryo are necessary.

LITERATURE CITED

[1] Girfanova, Kh. N. Doklady Akad. Nauk SSSR 68, 5, 941-944 (1949).

[2] Girfanova, Kh. N. Doklady Akad. Nauk SSSR 68, 6, 1105-1108 (1949).

[3] Korotkova, G. P. and Priezzheva, L. S. Vestnik Leningrad Univ. 7, pp. 3-22 (1952).

[4] Korotkova, G. P. Doklady Akad. Nauk SSSR 92, No. 1, pp. 197-200 (1953).

[5] Korotkova, G. P. Byull. Eksptl. Biol. i Med. 40, No. 10, pp. 60-63 (1955).

[6] Kossowicz, Mh. Landwirtsch I. (1912).

[7] Kossowicz, Neue tierartztl. Mschr. 3, 1916; 1, 1917. Cited by J. Grossfeld, Handbuch der Eirkunde, Berlin, (1938).

[8] Lomakin, I. R. Izvest. Imper. Tomsk. Univ. 40, pp. 1-21 (1910).

[9] Rettger, L. F. and Sperry, J. A.,U. Mech. Research 26 (1912).

[10] Hadley, P. B. and Caldwell, D. W. Bull. Agr. Exp. Station of the Rhode Island State College No. 164 (1916).