FUNGICIDAL PROPERTIES OF THE ALBUMEN MEMBRANE OF THE HEN'S EGG IN VARIOUS STAGES OF FETAL GROWTH

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The significance of the albumen membrane (egg-white) of the hen's egg for the defense of the embryo against various infections cannot be doubted. In connection with the tackling of the problem of the immunity of the embryos, the necessity has arisen of studying the changes which occur in the antibiotic properties of the "white" in the growth process of the chicken embryo.

A number of authors have studied the change in the bactericidal and protozoal properties of the natural "egg-white." One can conclude from the work of Kh. Girfanova [1] and L. Priezzheva [2] that upon use of the various bacteria, one observes essentially different curves of activity of the "egg-white" in various stages of fetal development. The same results were obtained by us in work with protozoas [4]. In relation to the large number of bacteria and protozoas, one did not succeed in observing a fall or increase of the antibiotic properties of the "egg-white" during the first six days of growth. The weakening of the activity of the "egg-white" towards the sixth day of growth was still observed in connection with two organisms; Bac. prodigiosium and Spirostomum ambiguum.

In seeking to decide the question as to whether the fungicidal and fungistatic properties of the albumen membrane change in the process of growth, we investigated the influence of the "egg white" on mold fungi (Aspergillus niger and Penicillium glaucum), air-borne yeasts (Torula utilis) and actinomycetes (Actinomyces albas and Actinomyces griseus), organisms of varying resistance to the effect of the natural "egg white".

The least resistance to the "egg white" effect was shown by the actinomycetes, since the spores of the Actinomyces albas immediately perished upon contact with the "egg white" [5]. The cells of the Torula utilis were resistant to the effect of the "egg white" [5]. In connection with Aspergillus niger and Penicillium glaucum, we found a distinct fungistatic effect [3].

EXPERIMENTAL METHODS

In the present work, we used eggs in the first six days of growth (in later stages of growth, the "egg white" is very thick, and becomes of little use for experiments). The surface of chicken eggs (white leghorns), was treated with alcohol and roasted in a jet flame; through the aperture in the shell, the "egg white" was extracted. For the experiments, 2 ml each of "egg white" from nonincubated eggs and eggs at different periods of incubation were collected in test tubes. As control medium, a meat-peptone bouillion (for the actinomycetes), must (for the mold fungi and yeasts), and also physiological solution were utilized. To all the test tubes, 0.2 ml of physiological solution, containing the spores of the fungi (the number of spores varying in the various experiments) was added. At specific intervals, seeding was carried out on Petri dishes (meat-peptone - agar or must-agar). The Petri dishes and the test tubes were kept at a temperature of 25° C. For inoculation, we used the spores of a three day culture of the fungi. All in all, three series each of tests were carried out with Torula utilis, Aspergillus niger and Penicillium glaucum, and five series each of tests with actinomycetes. The results of the experiments proved to be analogous.

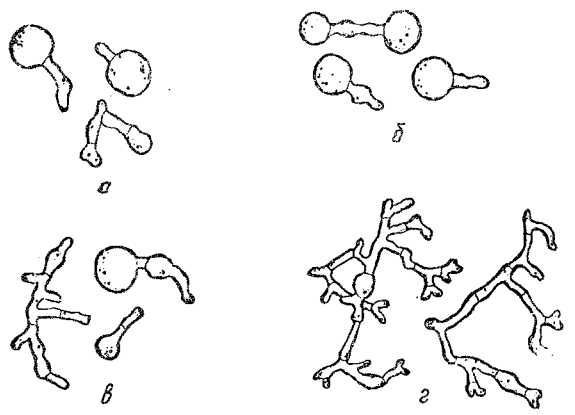

Fig. 1. Aspergillus niger on seventh day after introduction of spores on "egg white";
a) nonincubated, b) second day of incubation, c) fourth day of incubation, d) sixth day of incubation.

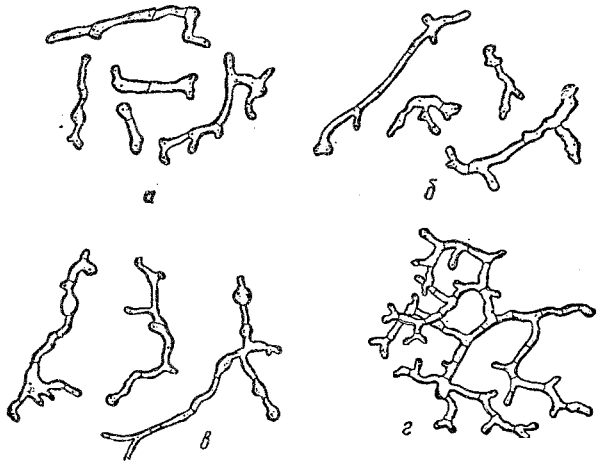

Fig. 2. Pencillium glaucum on seventh day after introduction of spores on "egg white":
a) nonincubated, b) second day of incubation, c) fourth day of incubation, d) sixth day of incubation.

EXPERIMENTAL RESULTS

Table 1 shows the results of the experiment with fungus (Actinomyces griseus). To 2 ml of "egg white" and culture medium was added 0.2 ml physiological solution containing 5000 spores Actinomyces griseus.

As seen from Table 1, the fungicidal properties of the "egg white" in relation to Actinomyces griseus, do not change in the course of the first six days of growth.

TABLE 1

Effect of the "Egg White" of Hen's Eggs at Different Stages of Incubation on the Actinomyces griseus Spores *

No.	Medium	Time of seeding: Immediately	Within: 2 hrs.	4 hrs.	8 hrs.	12 hrs.	18 hrs.	48 hrs.
1	Albumen of nonincubated eggs	++	++	++	++	+	0	0
2	"White" of egg incubated for 1 day	++	++	++	++	+	0	0
3	"White" of egg incubated for 2 days	++	++	++	++	+	0	0
4	"White" of egg incubated for 3 days	++	++	++	++	+	0	0
5	"White" of egg incubated for 4 days	++	++	++	++	+	0	0
6	"White" of egg incubated for 5 days	++	++	++	++	+	0	0
7	"White" of egg incubated for 6 days	++	++	++	++	+	0	0
8	Physiological soln.	++	++	++	++	++	++	+
9	Meat-peptone bouillon	++	++	++	+++	+++	+++	++++

* 1) Results of the experiments to demonstrate the influence of the "egg white", removed from the eggs in various stages of incubation, on Actinomyces (Table 1) and on mold fungi (Table 2) are presented not in numbers but in conventional signs. The reason is that on the growth of fungi intertwining mycelia are formed, which cannot be separated from each other in seedings on the Petri dish. Consequently, the number of colonies recorded does not correspond to the number of spores.

TABLE 2

Effect of the "Egg White" of Hen's Eggs at Different Stages of Incubation on the Aspergillus niger Spores *

No.	Medium	Time of seeding: Immediately	Within: 1 day	3 days	5 days	7 days
1	Albumen of nonincubated eggs	++	++	++	++	++
2	White of egg incubated for 1 day	++	++	++	++	++
3	White of egg incubated for 2 days	++	++	++	++	++
4	White of egg incubated for 3 days	++	++	++	++	++
5	White of egg incubated for 4 days	++	++	++	++	++
6	White of egg incubated for 5 days	++	++	++	++	++
7	White of egg incubated for 6 days	++	++	++	++	++
8	Physiological soln.	++	++	++	++	+
9	Must	++	++	Spore-bearing		

Conventional signs — see Table 1.

* See footnote to Table 1.

Similar results were obtained in connection with Actinomyces albas, the spores of which immediately perished on contact with the "egg white" taken from the eggs at various stages of incubation.

Table 2, presents the results of the test with Aspergillus niger.

In this case, to 2 ml "egg white" and culture medium was added 0.2 ml of physiological solution containing 2500 spores of Aspergillus niger.

The fungistatic properties in relation to Aspergillus niger proved to be equally inherent to the "egg white" in different periods of growth of the hen's egg. An identical result was obtained with Penicillium glaucum.

The results of the experiments with Torula utilis testify to the maintenance by the "egg white", taken from the eggs at different periods of incubation, of properties promoting the development of cells of this species of air-borne yeast.

The material of the experiments bears out the fact that the fungicidal and fungistatic properties of the "egg white" of the hen egg, in relation to the studied organisms, do not change in the course of development of the chicken fetus. However, when the growth of the spores of Aspergillus niger and Penicillium glaucum in the "egg white" from eggs at different periods of incubation was observed, it was found that the "egg white" on the 5th and 6th days of incubation became a more favorable medium for the development of the spores. On the 7th day after the commencement of the experiment, the mycelia of aspergilla and penicillin were more highly developed in the "egg white" than in the eggs on the 5th and 6th day of incubation (Fig. 1 and 2). However, reproduction of the fungi, even in these "egg whites", did not occur, i. e., the fungicidal properties were retained.

The material we and other authors have obtained on the stability of the antibiotic properties of the albumen membrane of chickeneggs during their development is of interest in connection with the subsequent fate of the albumen membrane. It is well known that on the 14th day of incubation the "egg white" falls into the amnion sac, and then is actively utilized by the embryo [6]. If the albumen membrane was to gradually lose in the course of growth the capacity to kill microorganisms, it could easily become a focus of infection, and with the subsequent fall into the amnion sac, could produce infection of the embryo. Thus the biological purpose of the phenomenon of stability of the antibiotic properties of the albumen membrane is clear. The chick will eat the sterile food "egg white", which at the same time may serve both as nutritional material and as a factor in passive immunization akin to lysozyme contained in mammalian milk in the period of rearing of the young.

Further study of the properties of the albumen membrane in the process of development of the egg, and also study of the properties of the amniotic and other fluids in developing chick embryos, will enable us to get a clearer idea of the complicated system of the defense mechanism in a developing organism, and also to establish the subsequent development and change in these mechanisms.

LITERATURE CITED

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