actively fibrinolytic but lysed clots containing prolysins in longer times than the prolysin-free control (lysis time: 15 min.) so that its action is presumably due solely to a proteolytic enzyme from the bacteria themselves. All other materials gave negative results in concentrations of 0.06-1.0%. There were insufficient materials for testing at higher concentrations.

Discussion. Omitted from these studies were a number of bacterial types, some of which were known to have been tested by earlier workers with the older "fibrinolytic" technics. Tillett(10) reviews a number of these and particularly states that the pneumococcus gives negative results. Our data clearly show that fibrinolytic activity is encountered in cultures of a wide variety of bacteria, but only in the case of certain hemolytic streptococci and staphylococci can a definite lysokinase be demonstrated. The results with *Clostridium botulinum* show the need for the prolysin-free control test. Fibrinolytic activity appears to be unrelated to the gelatine liquefaction test.

Summary. In a survey test of 77 satisfactory cultures of 40 bacterial types, fibrinolytic phenomena were encountered in 19 types (33 cultures) of bacteria tested. Only certain hemolytic streptococci and staphylococci gave positive results with a specific test for *lysokinase,* however, and these organisms were not directly fibrinolytic nor able to activate bovine profibrinolysin. *Direct* fibrinolytic activity may occur in cultures when (except in the case of *Cl. botulinum*) it is not found in the alcoholic precipitate. The failure to obtain a lysokinase in at least 6 types of bacterial cultures which gave good results in the survey test is disappointing, as is the failure to find a kinase for bovine profibrinolysin.

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A Substance in Egg Yolk Which Inhibits Deterioration of Aureomycin Activity.* (17550)

C. RAY WOMACK, EDWARD H. KASS,† E. BUIST WELLS AND MAXWELL FINLAND

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services, (Harvard), Boston City Hospital and the Department of Medicine, Harvard Medical, School, Boston, Mass.

In the treatment of chick embryos infected with psittacosis virus(1) the single dose of aureomycin required to protect 50% of infected embryos for 7-9 days was found to be one-third to one-fourth as great as the dose of chloromycetin required to produce the same effect. This finding was unexpected in view of the rapidity with which aureomycin deteriorates *in vitro* at 37°C under neutral or slightly alkaline conditions(2-6) as well as the relative stability of chloramphenicol under similar circumstances.(7-9) It appeared possible, therefore, that some factor, present in the embryonated egg, protected aureomycin from deterioration. In preliminary experiments, de-

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† Senior Fellow in Virus Diseases, National Research Council.

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TABLE I.
Effect of Fertile Eggs Upon Deterioration of Aureomycin at 37°C.

Hr of incubation	Medium		
	Infusion broth	Intact egg	Homogenized egg
0	50.*	50	50
24	<12.5	25–50	25–50
48	2.0	32	16
72	—	16	8
96	2.0	—	6
144	1.0	12	4
168	0.5	12	3

* Aureomycin concentrations (μg/ml).

signed to test such a possibility, it was found that when 7-day old embryonated eggs were inoculated by the yolk sac route with aureomycin most of the aureomycin activity could still be demonstrated in the egg fluids as long as 7 days after inoculation. The presence of actively multiplying psittacine virus did not affect the persistence of the aureomycin activity. The following experiments were, therefore, performed to determine whether a substance exists in eggs which permits aureomycin to retain its potency despite prolonged incubation under conditions which usually destroy the activity of the antibiotic agent.

Methods. Aureomycin hydrochloride‡ was made up to the desired concentration in brain heart infusion broth (Difco), pH 7.2. Aureomycin levels were determined by a serial 2-fold dilution method similar to that used for penicillin(10) using separate pipettes for each dilution. Bacillus No. 5§ was the standard test organism. All test fluids were incubated at 37°C.

Experimental. Tests in Embryonated Eggs. In the first experiments it was sought to determine whether the protective factor was found only in relation to intact chick embryos. Seven-day old embryonated eggs, with an average weight of 61.0 g and an assumed average volume of 40.0 ml of fluid were used. A dose of 2.0 mg of aureomycin in 0.25 ml of broth was injected into the allantoic sac of each embryo. Control eggs received the same amount of broth containing no drug. Three uninoculated embryonated eggs were each homogenized aseptically in a Waring blendor; 2.0 mg of aureomycin in broth were added to 2 of them and a comparable volume of broth alone was added to the third. The homogenized eggs and the intact fertile eggs were then incubated. At intervals thereafter fertile eggs were homogenized and their contents of aureomycin assayed and compared with those in the eggs originally homogenized. A control tube containing 2.0 mg of aureomycin in 40 ml of broth was also included. The results appear in Table I. It is seen that both in the intact and in the homogenized embryos the rate of deterioration of aureomycin at 37° was much slower than in the broth. The protective effect was probably unrelated to differences in hydrogen ion concentration:—the pH of recently homogenized eggs, as determined with a Beckman potentiometer, was found to vary between 7.1 and 7.3; that of homogenized eggs after prolonged incubation, was 7.1-7.2, and that of the control broth was 7.2. In no case did eggs without aureomycin exert any significant effect on the growth of the test organism.

Tests with Nonfertile Eggs. Nonfertile eggs were examined for their capacity to delay the deterioration of aureomycin, and the relative potencies of yolk and albumen, in this regard, were also determined. An egg showing no evidence of fertility was homogenized in the blendor and 1.0 mg of aureomycin added to 20 ml of the emulsion. The yolk and albumen of another nonfertile egg were separated aseptically and aureomycin added to each in the same concentration. Broth and a 25% solution of human serum albumin, each containing the same concentration of aureomycin,

‡ Supplied in crystalline form in sterile vials by Lederle Laboratories.

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§ Obtained from Dr. B. M. Duggar.

TABLE II.
Effect of Nonfertile Eggs, Egg Yolk and Egg Albumen on Deterioration of Aureomycin at 37°C.

Hr of incubation	Medium				
	Infusion broth	Homogenized nonfertile egg	Egg yolk	Egg albumen	25% human serum albumin
0	64.0*	64	64	64	64.0
24	8.0	32	64	8	16.0
72	2.0	16	32	4	0.5
144	1.0	4	16	4	<0.1

* Aureomycin concentrations (μg/ml).

served as controls. Aureomycin levels were determined immediately and at intervals after incubation. The results, which appear in Table II, show that the nonfertile egg also has the capacity to protect aureomycin from deterioration. Furthermore, the protective substance was found in the yolk. Thus, whereas the broth controls and the tubes containing egg and serum albumins retained but one-eighth of the original activity after 24 hours of incubation, there was no significant loss in the presence of whole egg or egg yolk alone. Even after 72 hours of incubation, when a 32-fold loss had occurred in the broth controls and a 16-fold loss in the presence of egg albumen, there was but a 4-fold deterioration in the presence of whole egg and a 2-fold deterioration in the presence of egg yolk. A 2- to 4-fold variation in activity has generally been considered to be within the limits of error of the method. The effect of dilution of egg yolk on its capacity to protect aureomycin from loss of potency is demonstrated in Table III. Egg yolks from nonfertile eggs were mixed with broth in varying proportions and sufficient aureomycin added to achieve a standard concentration of 50 μg per ml. It is seen that when egg yolk has been diluted beyond 1:4 its capacity to protect aureomycin from deterioration diminishes.

Subsequent experiments have shown that the protective substance in yolk resists heating at 60°C for 80 minutes. Studies still in progress indicate that this protective substance remains active after heating for one hour at 100°C and, under certain conditions, even after autoclaving at 120° for 15 minutes.

Discussion. The rapid rate of deterioration of aureomycin activity upon standing has been a constant source of concern to those working with this antibiotic. Stringent precautions are necessary to avoid loss of aureomycin activity in body fluids withdrawn for assay, and even then aureomycin deteriorates during the period of incubation required for assay.(3) Whether similar deterioration occurs within the body is not clear; however, significantly large amounts of injected aureomycin remain unaccounted for after assay of body fluids. The mechanism of the destruction of aureomycin is quite unknown,(6) but it is manifestly of value to seek a substance which will inhibit this destruction. It has been shown in the present studies that such a substance exists in egg yolk. Further studies on the isolation, characterization and properties of this substance are now in progress.

TABLE III.
Inhibition of Deterioration of Aureomycin at 37°C by Varying Dilutions of Egg Yolk in Broth.

Final dilution of egg yolk	Hr of incubation		
	0	24	72
1:2	32*	32	8
1:4	32	32	4
1:10	32	16	2
1:20	32	16	2
1:100	32	8	1
Broth alone	**32**	**8**	**1**

* Aureomycin concentrations (μg/ml).

Summary. A substance (or substances) found in egg yolk, inhibits the deterioration of aureomycin activity which ordinarily occurs upon incubation at 37°C. The protective agent is heat stable and of sufficient concentration to permit dilution of egg yolk to 1:4 without significant diminution of aureomycin-protective action. Egg albumen and human serum albumin are not protective in the concentrations used.

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