TRANSLOCATION OF ANTIBIOTICS IN DEVELOPING AVIAN EMBRYOS

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SUMMARY

Kanamycin, penicillin, and streptomycin were injected individually into air cells of newly set chicken and turkey hatching eggs, and assay was made throughout the incubation period to determine the location, potency, and duration of efficacy. By 24 hours, each antibiotic had diffused throughout the albumen. By six days, penicillin residues could not be detected, whereas kanamycin or streptomycin was found within certain areas of the developing embryo and in newly hatched chicks or poults. The migration patterns for kanamycin and streptomycin were markedly similar, and for turkeys the following results apply: antibiotic residues were detected in albumen (incubation days 1–22); yolk (day 13 to 6th day after hatching); amniotic fluid (days 16–23); and cloacal or cecal content (day 24 to 6th day after hatching). The findings indicate that the antibiotics did not diffuse into the yolk or amniotic fluid from the albumen but were translocated to those sites during normal embryonic development, and subsequent movement of drugs was not via the blood, since none was found in whole blood or serum (tested day 13 to 11th day after hatching). Antibiotic residues were not detected in allantoic fluid (tested days 6–24), and this could be a site of persisting pathogens in treated eggs. Evidence of drug levels in postembryonic yolk sac contents and intestines suggests that treatment of hatching eggs may offer some protection in newly hatched birds.

INTRODUCTION

Although it has been suggested that the introduction of antibiotics or chemotherapeutic agents into hatching eggs controls some egg-borne diseases (9,11,15,16), such treatments have not
always proven entirely effective in eradicating arizonae, mycoplasmas, and salmonellae from within the egg (2,4,7,8,12,13,16,17). Carlson and Snoeyenbos (3) tested seven antibacterials and concluded that none destroyed *Salmonella typhimurium* within eggs when practical, nontoxic doses were administered. Elliott and Romoser (5) mixed antibiotics of the tetracycline group with albumen and noted that 30–40% of the functional drug activity became masked. They observed that treated albumen held at 49 C developed an unexplained peak of activity at five days, after which the potency gradually declined. Carlson and Snoeyenbos (3) studied the persistence of antibacterials in the albumen of chicken eggs and concluded that kanamycin was detectable until the 16th day of incubation, which is approximately that time that the albumen is completely absorbed. Lee et al. (10) placed streptomycin on the inner surface of the chorioallantoic membrane, and 12 hours later detected it in circulating blood. Allen et al. (1) injected 1 mg of chlortetracycline into the allantoic cavity of nine-day-old chick embryos and subsequently detected the drug in allantoic fluid and membrane, blood, and liver, but not in the brain or heart.

In an attempt to explain the survival of bacteria in treated eggs, selected antibiotics were introduced individually into the air cells of newly set hatching eggs, and assay was made regularly throughout the incubation period to detect drug activity within separate areas of the developing embryo.

**MATERIALS AND METHODS**

The antibodies tested were kanamycin sulfate (Kan.), penicillin-G potassium (Pen.), and streptomycin sulfate (Strep.). Each antibiotic (0.3 ml) was injected in the air cell of separate labeled groups of eggs in the manner described by Greenfield and Bigland (7). The test cultures used were *Arizona* 7:1,7,8 and *Pasteurella multocida*.

**Drug assay.** Diagnostic Sensitivity Test Agar Base (DST, Oxoid) was freshly prepared in 50-ml volumes and allowed to cool to 48 C in a water bath. To each flask was added 1 ml of either an arizona or pasteurella suspension, and the mixture was swirled and poured into a 25 × 150-mm petri dish (Integrid Sterile Disposable Petri Dish, BioQuest). The arizona suspension was a $10^{-4}$ dilution in 1% tryptone–0.85% saline of a stock broth culture maintained in the maximum stationary phase of growth (7). The pasteurella suspension was a $10^{-3}$ dilution in Eugon broth (BioQuest) of a 24-hr culture grown in the same medium.
Antibiotics were acquired with defined potencies (Kan., Bristol Laboratories, Syracuse, New York; Pen. and Strep., Ayerst Laboratories, Montreal), and dilutions were made to obtain solutions containing 1000, 500, 250, 100, 50, 25, 10, and 1 μg/ml (Kan. and Strep.) or I.U./ml (Pen.). From each of 10 separately prepared series of solutions two volumes of 0.1 ml from each dilution were inoculated into individual sterile porcelain assay cylinders (Penicylinders, 8 × 10 mm, Fisher Scientific Co.) which had been placed on the dry surface of freshly seeded DST plates. Plates seeded with arizona were used to detect Kan. and Strep., and plates with pasteurella to detect penicillin. After incubation at 35 C for 24 hr, the diameter of the clear area around each cylinder was measured, and the zone of bacterial inhibition was determined by subtracting the outer diameter of the cylinder. Standard curves were drawn relating antibiotic potency to zone of inhibition.

**Test systems.** The investigation consisted of two separate studies. Study A assessed the toxicity and duration of efficacy of antibiotics introduced into chicken eggs and served as a pilot project for study B, which examined in detail the translocation of bacteria-inhibiting levels of the antibiotics within developing turkey eggs.

![Graph](image)

**Fig. 1.** Standard curves for inhibition of *Arizona 7:1,7,8* by kanamycin and streptomycin. Numbers in parentheses indicate potencies in μg/ml.
Study A. Antibiotic toxicity. Chicken hatching eggs were divided into 13 groups of 20 eggs. One group was not treated. The air cells of the remaining groups were injected with either Kan. or Strep. (25, 20, 15, and 10 mg), or Pen. (200,000, 150,000, 100,000, and 50 I.U.) contained in a 0.3-ml volume. The eggs were incubated to term, and percent hatchability was determined.

Duration of drug efficacy. Chicken hatching eggs were divided into 4 groups of 30 eggs. One group was not treated, two groups were injected via the air cell with 20 mg Kan. or Strep., and the remaining group injected with 100,000 I.U. Pen. On alternate days throughout the incubation period, a single egg was removed from each group, two 0.1-ml samples were taken from albumen, yolk, amniotic fluid, allantoic fluid, whole blood, serum, cloacal fluid, and cecal content during the period that each was available, and the whole egg was blended and sampled. Specimen materials were also taken from newly hatched chicks.

Each sample (0.2–2 ml) was collected in a suitable disposable syringe through a needle of convenient size (14–27-gauge). During the first 10 days of incubation the yolk membrane is fragile and albumen and yolk samples were obtained through a 2-cm hole made in the shell at the small end of the egg, then the egg contents were poured carefully into a petri dish, and other materials were taken. After day 10, the chicken yolk sac does not rupture so readily, so, to facilitate sampling, the whole embryo completely invested in the inner shell membrane was deposited in a petri dish. This was achieved by cracking the egg around the equator and very slowly and carefully teasing apart the two shell hemispheres. Blood samples were taken from vessels beneath the membrane under the air cell, after the shell and outer shell membrane had been removed from the area, but after day 17 it was easier to obtain a suitable volume from the vitelline vein close to the yolk stalk. After the 19th day of incubation a portion of shell was removed, the head and neck were exposed, the embryo was decapitated, and blood was collected in a small tube. Samples were tested to detect residual antibacterial levels of each drug, using DST plates seeded with the appropriate organism in the manner described previously. Materials from untreated control eggs were tested, using individual plates seeded with arizona and pasteurella.

Study B. Six hundred turkey hatching eggs were obtained, and, immediately prior to incubation, 250 were injected with 15 mg Kan., 125 with 15 mg Strep., and 50 with 100,000 I.U. Pen.
Controls were 175 untreated eggs. Throughout the incubation period, four Kan.-treated, four Pen.-treated, four untreated, and two Strep.-treated eggs were removed daily, and tests were made for antibacterial drug residues in the specimen materials listed in Study A. As soon as embryo development was discernible, only viable eggs were sampled. All eggs were candled on the eighth day of incubation and periodically thereafter, and all infertile or early-embryo-death specimens were discarded. Tests for Pen. residues were discontinued after the tenth day of incubation, whereas the study was extended to examine continuing Kan. and Strep. residues in hatched poults. Two poults from each of the three remaining groups were sacrificed daily, and tests were made on whole blood, serum, yolk sac content, cecal content, and whole blended poult. Since the latter three materials became progressively consolidated, each was diluted with an equal volume of physiological saline prior to testing.

RESULTS AND DISCUSSION

Drug assay. Fig. 1 shows standard curves which relate inhibition of arizona growth to potency of Kan. and Strep. The cultures chosen to detect antibiotic residues (Ar. 7:1,7,8 for Kan. and Strep., and P. multocida for Pen.) were selected after lengthy comparative sensitivity trials. These species were clearly sensitive to low concentrations of the antibiotics in tests by the in vitro
Table 1. Comparative toxicity of antibiotics injected into the air cell of chicken eggs immediately prior to incubation.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Drug inoculum</th>
<th>% mortality</th>
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<tbody>
<tr>
<td>Kanamycin sulfate</td>
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<td>79</td>
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<td></td>
<td>20</td>
<td>42</td>
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<td>15</td>
<td>28</td>
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<td>10</td>
<td>17</td>
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<tr>
<td>Penicillin-G potassium</td>
<td>200,000 I.U.</td>
<td>87</td>
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<tr>
<td></td>
<td>150,000</td>
<td>79</td>
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<tr>
<td></td>
<td>100,000</td>
<td>39</td>
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<tr>
<td></td>
<td>50,000</td>
<td>22</td>
</tr>
<tr>
<td>Streptomycin sulfate</td>
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<td>22</td>
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<tr>
<td></td>
<td>10</td>
<td>21</td>
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<tr>
<td>Untreated</td>
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disc method (Sensi-discs, BioQuest), and growth was not inhibited by purified lysozyme (Lysozyme Grade 1, Sigma Chemical Co., St. Louis) in high concentrations (1–10 mg/ml). Lysozyme occurs naturally in egg white and is capable of inhibiting the growth of certain bacteria (6). However, growth of P. multocida in DST was slightly inhibited (zone of approximately 0.2 cm) by 0.1 ml of albumen from untreated eggs. The existence of this undocumented lytic substance in albumen possibly explains why fowl cholera (pasteurellosis) is not an egg-borne disease.

Study A. Knowledge was required of injectable antibiotic doses which were not excessively toxic, and Table 1 indicates the mortality produced by injecting various doses of Kan., Pen., and Strep. into the air cells of chicken eggs immediately prior to incubation. Doses considered suitable were 20 mg Kan. or Strep., and 100,000 I.U. Pen. The investigations on duration of drug efficacy showed that the three antibiotics diffused through the albumen within 24 hr postinoculation, but by the fifth day Pen. residues could no longer be detected. The patterns of antibiotic persistence for Kan. and Strep. were markedly similar and are reported together. Both drugs could be detected in albumen until it had been completely absorbed, on the 17th day. Antibiotics were found in yolk after the ninth day and persisted in the yolk sac contents of newly hatched chicks. On the 11th day the amniotic fluid commenced to show high drug levels, which were maintained until the fluid was totally reabsorbed (or swallowed) by the embryo on the 17th day, and thereafter it was detectible in cloacal fluid and cecal content. Allantoic fluid was collected from the 9th to 17th days, and whole blood and serum were tested from day 15 until several days after hatching, but no antibacterial levels of antibiotics were
detected in any of these fluids by the method used. It is possible that more sensitive methods would have detected antibiotic residues, but we used a procedure to detect conditions which were bacteriostatic or bactericidal, and thus considered protective. No inhibition of arizona was produced by any fluid from untreated eggs, and growth of pasteurella was inhibited only by albumen.

**Study B.** This study was designed to examine with greater accuracy the translocation of antibiotics within the eggs. Turkey eggs were selected because their incubation period (28 days) is longer than that of chicken eggs (21 days), and because we are particularly interested in estimating the usefulness of introducing antibacterials into turkey eggs to control arizona disease (7). Figs. 2 and 3 show the translocation of Kan. and Strep. These graphs are markedly similar in form, a fact which might have been even more obvious had it been possible to test more eggs each day. If Figs. 2 and 3 are condensed to represent a 21-day period, the results are similar to those obtained for chicken eggs (Study A). Figs. 2 and 3 show that antibiotic residues were detected in albumen (days 1–22), yolk (day 13 to 6th day after hatching), amniotic

![Diagram](image_url)

**Fig. 3.** Streptomycin activity in regions of the developing turkey embryo and newly hatched poult after introduction into the air cell of the egg.
Translocating antibiotics in embryos (days 16–23), and cloacal or cecal content (day 24 to 6th day after hatching). Penicillin residues could not be detected in treated eggs after the sixth day of incubation, and Kan. and Strep. at arizonacidal levels were not found in allantoic fluid (tested days 7–24) or in whole blood or serum (tested day 13 to 11th day after hatching). No inhibition of bacterial growth was produced by fluids from untreated eggs except for the effect of albumen on pasteurella reported above. These results imply that the antibiotics did not diffuse from the albumen through the bounding membranes of the allantois, amnion, and yolk. The movement of drugs into the amniotic fluid after the 12th day could be attributed to normal embryonic development. At this time an influx of albumen occurs through the ruptured sero-amniotic connection (14). Prior to the 13th day, antibiotics might be excluded from the yolk in the turkey while a vestige of the vitelline membrane or the presence of chorionic tissue remains intact as a partition between albumen and yolk (14). Antibiotics did not appear to be transferred between embryonic tissues by the blood, although it is possible that transport was made at levels undetectible by the method employed and concentrations developed in new sites. The allantoic fluid remained permanently devoid of arizonacidal levels of antibiotics, and the existence of such an environment could explain the occurrence of pathogens in antibiotic-treated eggs. However, evidence of persisting drug levels in postembryonic yolk sac contents and intestines suggests that treatment of eggs may give a measure of protection to newly hatched chicks and poults.

REFERENCES