

Rickettsiostasis in Fertile Eggs from Use of Antibiotic Residues in Poultry Feeds.* (19183)

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During the past 8 months we have obtained irregular and usually very scanty growth of murine typhus rickettsiae in the yolk sacs of fertile eggs, in striking contrast to the uniformly heavy infection previously observed over a period of 7 years. This definitely altered susceptibility coincided with the adoption of new types of feeds by the flock owners from whom we have obtained our eggs. The feeds used in recent months are supplemented by fermented mash left over from the manufacture of antibiotics and vit. B₁₂.

The experiments to be reported here were planned to test the possibility that our recent failure to cultivate rickettsiae satisfactorily in eggs might be the result of the factors newly introduced into the diet of laying hens. Another objective was to determine the effect of these new factors on the multiplication of Influenza A virus.

Materials and methods. To carry out this investigation it was necessary to obtain fertile eggs from a flock that had never been fed the new type of ration. After visiting many small farms in the vicinity of our laboratory, a flock (Flock A) meeting these requirements was located. The owner of this flock had supplemented his ground corn only with meat meal, fish meal, dried milk and minerals, following the procedures used in past years in the preparation of poultry feeds. It was possible to trace this flock back to the hatchery, where records showed that Flock A was only a small portion of the original hatch, the remainder (Flock B) having been sold to a nearby poultry man. Flock B had received, since hatching, a diet identical with that given to Flock A, but supplemented with 1 pound of antibiotic residue per 100 lb of final ration. This antibiotic residue was guaranteed

by the processor to contain, by assay, not less than 1 g of antibiotics (aureomycin and terramycin) per lb, as well as an unspecified amount of vit B₁₂. Aureomycin and terramycin are known to be powerful rickettsiostatic agents. Eggs from Flock A were gathered at 12 hr intervals and stored at approximately 15°C, at which temperature they remain viable for 4 to 6 weeks. After gathering eggs for several days, (Group 1) the diet of the hens of Flock A was changed to the new type described above, which Flock B had received since hatching. Six days later, eggs were again gathered twice daily for 2 days (Group 2). Flock A was continued on the new diet and eggs were gathered and stored at the end of the second week (Group 3) and at the end of the third week (Group 4). Eggs from Flock B were collected and stored during the same periods (Groups 1, 2, 3, and 4). After the last eggs had been gathered, all, with the exception of those used for influenza studies, were incubated for 5 days at 37.5°C and then injected with rickettsiae. The methods used for preparing the inoculum, infecting fertile eggs, making and staining yolk sac smears, and determining the degree of infection were those described in previous papers(1,2). Five eggs from Group 1, Flock A, and 5 from Group 1, Flock B, were incubated for 11 days at 37.5°C and then tested for ability to support the growth of the PR8 strain of influenza virus. The technique used for inoculation and measuring growth was that recently published by Blumenthal *et al.*(3).

Results. The effect of changing the diet of Flock A hens from the original one, presumably free from antibiotics, to the new one containing aureomycin and terramycin, is shown in Table I. The eggs in Group 1, fed the original diet, showed the characteristic pattern of heavy infection, with embryonic death on or before the 7th day after injection. The eggs collected after feeding the new diet

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into the diet of a flock of laying hens resulted in progressively increasing resistance of the eggs to rickettsial growth, the resistance becoming almost complete at the end of 3 weeks. Similar dietary factors had no appreciable effect on the growth of Influenza A virus in eggs. These results, with other data submitted, indicate that fertile eggs obtained from commercial hatcheries may, under present conditions, be unsatisfactory for controlled microbiological studies.

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Counteraction of Cortisone Inhibition of Body, Hair and Thymus Growth by Vitamin B₁₂ and Aureomycin.* (19184)

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Large doses of cortisone have been shown to inhibit both body(1,2) and hair growth (3,4) in rats. These effects can be at least partially attributed to the reduction in availability of protein to the organism(5,6). Large doses of cortisone have also been reported to reduce normal food intake in rats(7), which may further contribute to reduced body and hair growth. Vit. B₁₂ partially or completely counteracted growth-inhibition in rats induced by administering large doses of thyroid-active substances(8-10), diethylstilbestrol (11), or 0.1% thiouracil(12). Several antibiotics have also been shown to be effective in prolonging survival time(13) and overcoming growth-inhibition(14) in hyperthyroid rats. In general, these studies are believed to indicate that marked changes in estrogen or thyroid levels within the body can increase the need for vit. B₁₂ and perhaps other factors by the organism.

It was the purpose of the present study to determine whether vit. B₁₂ and aureomycin

could prevent large doses of cortisone from depressing body and hair growth in rats fed a ration deficient in vit. B₁₂. If large amounts of cortisone can aggravate an existent dietary deficiency, and thus account to some extent for inhibition of body and hair growth, then supplementing the diet with the missing nutrients should help to prevent these manifestations.

Methods. A total of 130 immature male albino rats of the Carworth strain were used in 3 separate experimental trials. All rats were divided into uniform groups by weight of 10 each, and were placed for 30 days on the following vit. B₁₂-deficient ration: yellow corn meal, 35%; ground wheat, 25%; linseed oil meal, 10%; soybean oil meal, 20%;‡ alfalfa leaf meal, 6%; brewers' yeast, 3%; and table salt, 1%. *Cortisone acetate* (*Cortone*)§ was injected subcutaneously, daily, in a 0.1 ml volume of saline containing 0.5 mg of the hormone. Crystalline vit. B₁₂§ was

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‡ Low fiber, solvent extracted, containing 50% protein. Manufactured by Archer-Daniels-Midland Co., Minneapolis, Minn.

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