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# Use of Bacteriostatic Agents in Preparation of Seed Cultures of Psittacosis Virus.*

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Previous experiments[1] have indicated that psittacosis virus (strain 6 BC) is resistant to the action of streptomycin when grown in roller tube tissue cultures of minced chick embryo tissue. Experiments[1] also indicated that this virus would survive in as much as 50 mg % sodium sulfadiazine in the buffered salt solution-serum ultrafiltrate fluid[2] containing a small amount of dead chick embryo tissue when kept at room temperature for 5 days although it was inhibited by sulfadiazine when actively growing in tissue cultures and eggs.[1] The resistance of the virus to these 2 agents, suggested their use in the control of contamination in seed cultures where the virus is not actively multiplying.

*Preparation of Seed Cultures.* Eight-day-old embryonated eggs were injected by the yolk sac route with 0.25 ml of a $10^{-6}$ dilution in nutrient broth of yolk sacs infected with psittacosis virus (strain 6 BC). These embryos died on the fourth day, at which time yolk sacs were harvested and made into a 20% suspension in broth by shaking with glass beads for ½ hour at 0°C. This suspension was stored overnight at 0°C.

The 20% suspension of infected yolk sacs was divided into 2 parts. Lot I was diluted to 10% by the addition of an equal volume of nutrient broth. An equal amount of nutrient broth containing 50 mg % sodium sulfadiazine and 250 u/ml of streptomycin was added to Lot II to give a final concentration of 25 mg % sodium sulfadiazine and 125 u/ml streptomycin. These seed cultures were sealed in glass vials, shell-frozen, and stored in the $CO_2$ ice box.

Repeated simultaneous comparative titrations were made on Lots I and II by injecting serial dilutions of the virus suspensions in nutrient broth as follows: 0.03 ml intracerebrally and 0.25 ml intravenously in 18-20 g mice and 0.25 ml into the yolk sacs of 6-day-old embryonated eggs. There were no significant differences in the titers of the virus obtained on Lots I and II by any of the 3 methods used. Titers were calculated as $LD_{50}$ by the method of Reed and Muench.[3]

These results indicated that 25 mg % sodium sulfadiazine and 125 u/ml of streptomycin can be added to 10% yolk sac seed cultures of psittacosis virus (strain 6 BC) as a safeguard against possible contamination without any deleterious effect on the titer of the virus when sealed in glass vials and stored in a $CO_2$ ice box.

Preliminary experiments also indicated that the use of these bacteriostatic agents in diluting fluids (50 mg % sodium sulfadiazine and 250 u/ml of streptomycin) is of considerable value in the isolation of psittacosis virus by intracerebral injection in mice from such contaminated sources as urine, feces and soil.

*Summary.* The addition of 25 mg % sodium sulfadiazine and 125 u/ml of streptomycin to yolk sac seed cultures of psittacosis virus stored with carbon dioxide ice had no effect on the titer of the virus.

* Studies conducted at Camp Detrick, Md., from March to May, 1945.

[1] Early, R. L., and Morgan, H. R., in press.

[2] Simms, H. S., and Sanders, M., *Arch. Path.*, 1942, **33**, 619.

[3] Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.