

laboratory and industrial chemistry of organic fluorine compounds⁸ may, in the light of the results reported here, encourage further work in the rather neglected field of the water-immiscible volatile antiseptics.

⁸ Symposium on Organo-fluorine Chemistry, 1947, *Ind. Eng. Chem.* (many authors), **39**, 235.

Summary. A mixture by volume of 1 part *o*-fluorotoluene, 2 parts *n*-butyl chloride, and 1 part 1,2-dichlorethane, is recommended as a chemically nearly inert but nevertheless effective preservative for biological solutions. This preservative, used at approximately the 1% level, is completely removed under the usual conditions of steam sterilization.

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Isolation of an Agent in Chicken Embryo Causing Infectious Sinusitis of Turkeys.

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The term infectious sinusitis of turkeys was suggested by Dickenson and Hinshaw¹ to differentiate it from sinusitis which may occur in connection with reduced vitamin A intake² or follow inoculation with *Hemophilus gallinarum*.³ Infectious sinusitis occurs in all sections of the country and although mortality is low, morbidity varies from 10% to 90% of a flock.^{1,4} Thus, losses to the grower resulting from inferior quality of the birds are often quite heavy. According to the literature Delaplane⁵ has reported a *Pasteurella*-like organism to be associated with an infectious type of sinusitis. In transmission experiments Hart⁶ and Hinshaw⁷ were unable to filter the causal agent through collodion membranes, Chamberland or sintered glass filters.

Serial transmission of the disease in this

laboratory from a case of sinusitis submitted to the laboratory for examination was carried out in the usual manner.⁷ Sinus exudate was aspirated from infected turkeys and 0.5 ml amounts were inoculated into the right infra-orbital sinus of normal turkeys. The inoculated sinus became swollen 5 to 10 days after inoculation and contained a viscous exudate which resembled egg albumen and was bacteriologically sterile. Later the sinuses became greatly distended and often contained caseated material from which a variety of bacteria were isolated. On the assumption that these organisms represented secondary bacterial invaders an attempt was made to isolate the causal agent early in the course of the disease from the third serial turkey passage. Accordingly, sinus exudate was aspirated on the second day after the appearance of clinical symptoms and was inoculated into embryonating eggs by the following routes: onto the chorioallantois, into the allantoic cavity and into the yolk sac. No embryo deaths or significant macroscopic lesions were observed in any of the eggs inoculated onto the chorioallantois or into the allantoic cavity. However, all of the 10 embryos inoculated into the yolk sac died 6 to 12 days after inoculation. Yolk sacs from these embryos were bacteriologically sterile and were subsequently passed in

¹ Dickenson, E. M., and Hinshaw, W. R., *J. Am. Vet. Med. Assn.*, 1938, **93**, 151.

² Hinshaw, W. R., and Lloyd, W. E., *Hilgardia*, 1934, **8**, 281.

³ Beach, J. R., and Schalm, O. W., *Poult. Sc.*, 1936, **15**, 466.

⁴ Lee, C. D., *No. Am. Vet.*, 1942, **23**, 715.

⁵ Delaplane, J. P., *Poult. Sc.*, 1944, **23**, 247.

⁶ Hart, L., *Austral. Vet. J.*, 1940, **16**, 163.

⁷ Hinshaw, W. R., from *Diseases of Poultry*, edited by H. E. Biester and L. Devries, The Iowa State College Press, 1945.

chicken embryos by yolk sac inoculation for a total of 28 consecutive passages.

After the second yolk sac passage 0.5 ml of a 10% yolk sac suspension was inoculated into the right sinus of each of 2 normal turkeys. The sinuses became swollen 5 and 8 days after inoculation respectively. Sinus exudate aspirated on the first day after the appearance of clinical symptoms was bacteriologically sterile and killed all of 10 chicken embryos 6 to 12 days after inoculation into the yolk sac. Clinical sinusitis was produced in a total of 10 additional turkeys with yolk sac material from the 3rd, 4th, and 16th egg passages respectively.

Filtration studies on the egg-adapted strain of the agent of turkey sinusitis were carried out using a 1/100 dilution of infected yolk sacs. At least 50.0 ml amounts of the suspension were filtered through Berkefeld V, Berkefeld N, and Seitz E K filters respectively. The filtrates obtained, together with the unfiltered suspension, were tested for infectivity by inoculation into groups of 15 embryonating eggs. All embryos inoculated with the unfiltered suspension and the Berkefeld V filtrate died 4 to 8 days after inoculation. However, all embryos inoculated with Berkefeld N and Seitz E K filtrates respectively were still living 14 days after inoculation. It is evident that the causal agent passed through a Berkefeld V filter but did not pass readily Berkefeld N or Seitz E K filters.

During the first 10 egg passages micro-

scopic examination of impression smears of infected yolk sacs stained by Machiavello's technic revealed only an occasional red staining coccoid body. However, beginning with the 14th egg passage when the infective titer of yolk sacs reached 10^{-8} or more small, coccoid bodies were regularly observed both intra- and extracellularly. These bodies were found to vary in size from approximately 0.5μ to 1.0μ or more in diameter and were frequently imbedded in a matrix. The bodies described above closely resembled morphologically the bodies found in similar impression smears of yolk sacs infected with the etiological agents of the lymphogranuloma-psittacosis group (*Chlamydozoaceae*).

Further studies on the characteristics of the agent of turkey sinusitis and its possible etiological relationship to an agent isolated from air sac infection in turkeys by Minard and Jungherr⁸ are in progress.

Summary. 1. An agent capable of producing clinical sinusitis in turkeys was isolated and propagated in the yolk sac of the developing chicken embryo. 2. Impression smears of infected yolk sacs stained by Machiavello's technic revealed morphologic forms closely resembling those found in the lymphogranuloma-psittacosis group of agents (*Chlamydozoaceae*).

⁸ Minard, E. L., and Jungherr, E., 1944, Proc. 16th An. Conf. Lab. Workers in Pullorum Dis. Control, Coll. of Agr., Univ. of Conn., mimeog. report.

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Observations on the Effect of 4-Amino-Pteroylglutamic Acid on Mice.

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The production of a syndrome in mice by feeding a crude "antagonist" of pteroylglutamic acid (PGA) and its prevention by raising the dietary level of PGA was described.¹ The syndrome was marked by slow growth followed by a loss in weight, low hemoglobin

and low white blood cell count. Recently, another substance, "4-amino" pteroylglutamic acid ("4-amino-PGA"), which is strongly

¹ Franklin, A. L., Stokstad, E. L. R., and Jukes, T. H., Proc. Soc. Exp. Biol. and Med., 1947, **65**, 368.