8. Comar, C. L., Davis, G. K., and Taylor, R. F., *Arch. Biochem.*, 1946, v9, 149.

9. Comar, C. L., and Davis, G. K., *Arch. Biochem.*, 1947, v12, 257.

10. Comar, C. L., *Nucleonics*, 1948, v3, No. 4, 30.

11. Ulrich, F., and Copp, D. H., *U.S.A.E.C. Document*, 1950, UCRL-839.

12. Monroe, R. A., Patrick, H., Comar, C. L., and Goff, O. E., *Poultry Sci.*, 1952, v31, 79.

13. Barbee, K. W., and Johnson, B. C., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 720.

14. Chow, B. F., Rosenblum, C., Silber, R. H., Woodbury, D. T., Yamamoto, R., and Lang, C. A., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 393.

15. Hansard, S. L., Comar, C. L., and Plumlee, M. P., *Nucleonics*, 1951, v9, No. 1, 13, and No. 2, 38.

16. Woodruff, H. B., and Foster, J. C., *J. Biol. Chem.*, 1950, v183, 569.

17. Fantes, K. H., Page, J. E., Parker, L. F. J., and Smith, E. Lester, *Proc. Royal Soc., B.*, 1950, v136, 592.

18. McCance, R. A., and Widdowson, E. A., *Ann. Rev. Biochem.*, 1944, v13, 315.

19. Smith, S. E., Becker, D. E., Loosli, J. K., and Beeson, K. C., *J. Animal Sci.*, 1950, v9, 221.

20. Gall, L. S., Smith, S. E., Becker, D. E., Stark, C. N., and Loosli, J. K., *Science*, 1949, v109, 468.

21. Tosic, J., and Mitchell, R. L., *Nature*, 1948, v162, 502.

22. Hale, W. H., Pope, A. L., Phillips, P. H., and Bohstedt, G., *J. Animal Sci.*, 1950, v9, 484.

23. Smith, S. E., Koch, B. A., and Turk, K. L., *J. Nutri.*, 1951, v44, 455.

24. Becker, D. E., and Smith, S. E., *J. Nutri.*, 1951, v43, 87.

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Viability of the Lansing Strain of Poliomyelitis Virus in the Bat (*Eptesicus fuscus*). (19587)

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The present study was undertaken in order to determine whether the bat (*Eptesicus fuscus*) might be a reservoir for poliomyelitis virus. Since under normal conditions the most probable method of exposure for the bat would be by the oral or intranasal routes, these 2 methods were used in the following experiment. As a control on the susceptibility of the bats, they were also inoculated by the intracerebral route.

Materials and methods. The Lansing strain of poliomyelitis virus was used. The strain was originally obtained by this laboratory in the form of mouse-brain suspension of the 200th mouse passage from Dr. David Bodian of the Poliomyelitis Research Center, Johns Hopkins University, Baltimore, Md. The virus had been passed 5 additional times in mice and once in cotton rats at the initiation of the present study. A 10% virus-bearing cotton-rat brain suspension was used as the inoculum for all bats. According to the Reed-Muench method of calculation(1) this material titrated $10^{-4.5}$ in cotton rats by intracerebral inoculation. The bats used in the present experiment were of the species *Eptesicus fuscus* and were in hibernation when they were obtained. They were kept kept in a heated room at the laboratory for 10 days before inoculation. During this period, all weak or inactive bats were discarded. The bats were divided into 3 groups of 28 bats each, and each group was exposed to the virus by the oral, intranasal, or intracerebral route. Bats of the intranasal and intracerebral groups were lightly anesthetized with ether before administration of the virus. The inoculum for each group was as follows: intracerebral, 0.03 ml per bat; intranasal, 0.02 ml per bat; and oral, 0.1 ml per bat. Following exposure to the virus, the bats of different groups were kept in separate cages in an isolation room with a lower temperature. Most of the bats went into hibernation and the remainder were very lethargic. On the 5th day post inoculation, 6 bats from each group were

sacrificed and the following pools of tissues and fecal material made from bats of the same group: brains and spinal cords, hearts, lungs and tracheas, spleens, livers, kidneys and bladders, and intestines and feces. Aseptic technics were maintained and different instruments were used in removing each type of tissue. These 21 pools were stored in a —40° C deep freeze cabinet. On the 12th, 20th, and 30th days after inoculation the same procedure was repeated using 5 bats from each group. Similar pools of tissues were made from one bat of the oral group which died 18 days after inoculation. The bats which remained after the 30-day period were discarded. The pools of tissue were removed from the deep freeze, thawed, ground with alundum, and diluted in as small an amount of physiological saline as possible. The suspensions were centrifuged at 1500 r.p.m. for 2 minutes. The sediment was discarded and the supernatant was treated with penicillin and streptomycin in order to eliminate any possible bacterial contamination.

The eastern cotton rat (*Sigmodon hispidus hispidus*) rather than the mouse was chosen as the test animal because of its higher susceptibility to Lansing strain poliomyelitis. Four hundred fifty-five cotton rats were divided into 91 groups of 5 rats each. Each of the 5 rats from one group were inoculated intracerebrally with 0.06 ml of one of the suspensions described above. These rats were observed twice daily for 18 days. Rats dying within the first 48 hours were discarded. In cases where 3 or more rats died within this period, more rats were injected so that a minimum of 3 rats survived after 48 hours. The brains of rats evidencing symptoms of central nervous system (CNS) involvement or dying after the first 48 hours were removed aseptically. Histopathological examinations were made on sections of each of these rat brains by Dr. Theodore Winship and Dr. David Bell of Garfield Memorial Hospital, Washington, D.C. One-half of each brain was stored in 50% glycerine until the end of the 18-day observation period. They were then ground separately with alundum and diluted to 20% suspensions with physiological saline. Each of 5 rats was injected intracerebrally with 0.06 ml of one of these suspensions. Those rats which died within the first 48 hours were discarded, and the remainder were held for an 18-day observation period.

Results. None of the bats inoculated showed any evidence of CNS involvement following inoculation. The one bat from the oral group which died on the 18th day after exposure was found dead in the morning and no symptoms had been noted the previous day.

Table I shows that 9 rats became sick or died following injection with the bat-brain suspensions. The 3 columns under the heading "Inoculum" indicate the bat material from which the inoculum was prepared. Of the 9 rats succumbing, 4 were injected with suspensions of feces and intestines, 3 with suspensions of brains and cords, and only one each with a suspension of hearts and of kidneys and bladders. A considerable number of rats inoculated with the pools of kidneys and bladders died within the first 24 hours. This high mortality rate might have been due to a toxic factor in the suspensions.

Histopathological examinations of the rat brain sections showed no specific changes that might be due to infectious agents. The predominant feature in all cases was capillary dilatation and engorgement with blood. No perivascular cuffing or degenerative changes in the neuronal or glial elements were seen.

Attempts to pass the lethal agent were unsuccessful. Rats of the 2nd passage remained normal during the 18-day observation period.

Discussion. The fact that the bats were in hibernation during the period following exposure to the virus made it rather difficult to determine symptoms of illness. However, no symptoms of CNS involvement such as paralysis or tremors were noted.

In the 4 cases where the rats succumbed following inoculation with suspensions of bat feces and intestines, the inoculum was still contaminated with bacteria in spite of the treatment with penicillin and streptomycin. It is therefore possible that the symptoms and deaths were due to a bacterial infection. However, although symptoms of CNS involvement were present in the rats succumbing on the 11th and 12th days after inoculation, no evidence of a bacterial encephalitis or menin-

TABLE I. Rats Dying or Showing CNS Involvement Following Injection with Bat-Brain Suspension. One rat in each experiment.

Bat group	Inoculum: Days after inj. tissues were harvested	Inoculum: Pool	Incubation, days	Symptoms or death
Oral	5	Feces and intestines	11	Paralysis hind legs
	12	" " "	12	Slight tremors; partial paralysis
	12	Hearts	6	Paralysis
	30	Brains and cords	8	Dead*
	30	Kidneys and bladders	4	Paralysis hind legs
Intranasal	12	Feces and intestines	6	Dead*
	30	Brains and cords	7	" *
Intracerebral	5	" " "	8	" *
	12	Feces and intestines	6	" *

* These rats were dead when examined in the morning although they had shown no symptoms the previous afternoon.

gitis were noted on histopathological examinations nor could the agent be passed serially to more rats. Furthermore, the pathological findings in all 4 cases were almost identical with those in the other 5 rats which succumbed following injection with bacteria-free material.

Summary. Different groups of bats (*Eptesicus fuscus*) were exposed to the Lansing strain of poliomyelitis virus by the oral, intranasal, and intracerebral routes. Attempts were made to isolate the virus in cotton rats from suspensions of various tissues and feces of bats sacrificed on the 5th, 12th, 20th, and 30th days after exposure. Nine of the rats died or were paralyzed on the first passage. Four of these rats had been injected with bat feces and intestines, 3 with bat brains and cords, one with bat hearts, and one with bat kidneys and bladders. However, histopathological examinations showed no typical lesions of poliomyelitis, and attempts to produce symptoms in rats of the 2nd passage were unsuccessful.

1. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, v31, 493.

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Effects of Isonicotinic Acid Derivatives on Tubercle Bacilli.* (19588)

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The mechanism underlying the action of synthetic chemicals and antibiotics on bacteria remains a debated question. The possibility that the tuberculocidal or tuberculostatic effect of some chemicals and antibiotics may be due to their action on the respiratory enzymes of tubercle bacilli has long been under investigation in this laboratory.

The effect of isonicotinic acid hydrazide and 1-isonicotinyl-2-isopropylhydrazine on oxygen consumption, succinic dehydrogenase and catalase activity of virulent, streptomycin-sensitive and streptomycin-resistant strains of human type tubercle bacilli, as well as on the attenuated, streptomycin-sensitive, bovine type tubercle bacilli BCG, was studied.

Material and methods. Cultures of tubercle bacilli were grown on Sautons synthetic medium for 7 to 19 days, during which time they produced a heavy wrinkled film on the surface of the medium while the underlying culture medium remained clear. The bacilli

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