A commercial yeast extract sold under the name of Vegex was found to be ineffective when 1 gm. daily was fed, but was prophylactic when 2 gm. daily were used. This same extract was employed by Strauss and Castle[3] as a source of the dietary anti-anemia factor. Desiccated hog stomach given in 2 gm. amounts daily as Ventriculin was found to be ineffective, as would be expected if it contained only the gastric without the dietary anti-anemia factor. If 1 gm. of Ventriculin plus 1 gm. of Vegex were administered, however, complete prophylaxis occurred, although neither substance was effective alone in the dosage given. The results suggest that the deficiency which is causative of canine black tongue is closely allied to the deficiency which is etiologic in pernicious anemia. Moreover, the death or survival of the guinea pig fed the diet producing black tongue may serve as a useful test for evaluating the potency of various substances used in the treatment of pernicious anemia in the human being.

Conclusion. Guinea pigs fed a diet causative of canine black tongue lose weight rapidly and die within a short period. This effect may be prevented by substances which are capable of causing remissions in pernicious anemia.

7707 C

Multiplication of Equine Encephalomyelitis Virus in Mosquitoes.

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While Davis, Frobisher and Lloyd[1] failed to find evidence of multiplication of yellow fever virus in infected *Aëdes aegypti,* the experiments of St. John, Simmons and Reynolds[2] and of Holt and Kintner,[3] though limited in number, suggest that the dengue fever virus does multiply in its transmitting insect host. We have reported[4] that *Aëdes sollicitans* fed on animals infected with the

[1] Davis, N. C., Frobisher, M., Jr., and Lloyd, W., *J. Exp. Med.*, 1933, **58**, 211.

[2] St. John, J. H., Simmons, J. S., and Reynolds, F. H. K., *Am. J. Trop. Med.*, 1930, **10**, 23.

[3] Holt, R. L., and Kintner, J. H., *Phil. J. Sci.*, 1931, **46**, 593.

[4] Merrill, M. H., Lacaillade, C. W., Jr., and Ten Broeck, C., *Science*, 1934, **80**, 251.

eastern strain of equine encephalomyelitis virus show, by titration of the suspension of a given number of insects, a greater concentration of virus at 5 days than immediately after feeding. Titration experiments are not absolutely convincing and we therefore adopted a modification of the method used by St. John, Simmons and Reynolds in order to determine whether the equine encephalomyelitis virus could be carried by serial passage through mosquitoes. If it can be carried from mosquito to mosquito directly the proof of the multiplication of the virus is convincing.

Thirty female *Aëdes aegypti* infected 5 days previously by feeding on brain virus of the western strain of equine encephalomyelitis were suspended in 4 cc. salt solution plus 1 cc. normal horse serum. An equal amount of defibrinated horse blood was added and a pledget of cotton in a Petri dish was moistened with the mixture. A small amount of sugar was sprinkled over the surface of the cotton and the Petri dish was placed in a cage containing female *Aëdes aegypti* that had had no sugar solution for 4 days and no water for one day. Since the virus deteriorates rapidly when in contact with the air at room temperature, the Petri dish was replaced in an hour's time by one containing the mixture that had been kept in the refrigerator. After another hour this was removed, so that the mosquitoes that fed took up active virus. Those that did not feed were eliminated by withholding water for 24 hours and sugar solution for 48 hours from the entire lot. The infected mosquitoes were kept in cages at a room temperature of from 24-28°C.

At 6 to 7 day intervals from 25 to 30 mosquitoes from the last feeding have been suspended and fed to starved females as outlined above. At each transfer virus has been demonstrated in the suspension of crushed mosquitoes by guinea pig inoculation and in many instances dilutions as high as 10^{-5} have proven infectious. Control inoculations of 3 kinds into guinea pigs have all been negative: a suspension of mosquitoes from our healthy stock; the horse serum and saline used; and a boiled suspension of infected mosquitoes. Since the virus has now been passed in series through 10 lots of mosquitoes and since the dilution at each transfer is at least 1:100 we must conclude that multiplication has taken place.

No difference has been demonstrated between the mosquito passage virus and the original strain. Its serological characters are unchanged, the virulence has been modified little if at all, and it passes Berkefeld "N" filters readily. Mosquitoes infected with the passage strain readily infect guinea pigs by biting.

The virus seems to be generally distributed in the bodies of the

mosquitoes, for it has been demonstrated by guinea pig inoculation in suspensions of legs removed from uncrushed insects, as well as in suspensions of the body fluid, heads, thoraces, and abdomens. Since this virus kills horses and other mammals so readily, we might expect that a general invasion of the mosquito would likewise be fatal. This is, however, not the case, for the mortality in the cages containing infected mosquitoes is no higher than in those containing normal ones.

7708 C

Injections of Combined Paratyphoid Colon Bacillus Filtrate and Poliomyelitis Virus by Way of Gastrointestinal Tract.*

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Poliomyelitis was produced in monkeys by injecting saline suspensions of virus into the intestine between clamps or subserosally.[1] What effect would the addition of paratyphoid colon bacillus culture filtrate have on weak suspensions of virus if introduced in the same fashion?

Monkey I was injected directly into the intestines between intestinal clamps with 40 cc. of a 2% suspension of poliomyelitis virus and 40 cc. of P. C. B.† filtrate. The first day after injection, the animal had furring and weakness of the right foot; it was very sick and obviously paretic on the 3rd day. By the 5th day, both feet were weak, the right leg showed paresis, and the extensors of the right hand were weak. It recovered and on the 17th day was active again, although it still had some paresis with beginning atrophy of the muscle groups described.

Monkey II, control for monkey I, was injected directly into the intestines between intestinal clamps with 40 cc. of a 2% suspension

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[1] Toomey, John A., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1015.

† A number of paratyphoid colon organisms listed in a previous communication[2] were planted in glucose broth, grown for 10 days and the material passed through an N and W filter. For convenience, I will term the paratyphoid colon bacillus culture filtrate, the P. C. B. filtrate.

[2] Toomey, John A., *J. Infect. Dis.*, 1934, **54**, 74.