breathed ambient air, but a greater fall in pressure after pulmonary hypertension had been produced by hypoxia. The fall in pressure was associated with either a constant or an increased cardiac output. The evidence suggests that acetylcholine causes vasodilatation in the lungs and that this action is largely dependent on the pre-existing tone of the pulmonary vessels.

1. Motley, H. L., Cournand, A., Werkö, L., Himmelstein, A., and Dresdale, D., *Am. J. Physiol.*, 1947, v105, 315.

2. Westcott, R. N., Fowler, N. O., Scott, R. C., Hauenstein, V. D., and McGuire, J., *J. Clin. Invest.*, 1951, v30, 957.

3. Doyle, J. T., Wilson, J. S., and Warren, J. V., *Circulation*, 1952, v5, 263.

4. Fritts, H. W., Jr., Braunwald, E., Fishman, A. P., and Cournand, A., *Fed. Proc.*, 1956, v15, 68.

5. Harris, P., Thesis, Univ. of London, 1955.

6. Cournand, A., Fritts, H. W., Jr., Harris, P., and Himmelstein, A., *Tr. A. Am. Physicians*, in press.

7. Harris, P., *Brit. Heart J.*, 1955, v17, 85.

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Relationship of Bat Salivary Gland Virus to St. Louis Encephalitis Group of Viruses.* (22669)

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The existence of rabies in bats in the United States was first suspected in 1951 when a woman in Big Spring, Texas died of rabies 3 weeks after being bitten by a bat. This experience was reported(1) after the first demonstration of rabies in bats in the United States when the virus was demonstrated in a Florida yellow bat (*Dasypterus floridanus*) which had attacked a child(2). Three months after this incident another case was reported in Pennsylvania where, without provocation, a woman was bitten on the arm by a bat (probably *Lasciurus cinereus*) from which the rabies virus was isolated(3,4). These and other experiences prompted several groups to initiate studies to assess the importance of these animals as reservoirs and carriers of the infection(5-10). The rabies virus has now been recovered from bats in widely scattered areas in the United States (Florida (2), Pennsylvania(3), Texas(6,7), California (10), and Montana(11).)

During the course of their studies on rabies in nonsanguivorous bats in Texas, Burns and Farinacci(8,12,13) recovered a number of viral agents in addition to the rabies virus from the salivary glands of encephalitic bats. Following preliminary tests(13) which suggested that these agents were antigenically related to the St. Louis encephalitis virus, one strain was referred to us for determination of its antigenic relationship to the Japanese (JE)-West Nile (WN)-St. Louis (SLE)-Murray Valley (MVE) encephalitis group(14-19). This report is concerned with the relationship of the bat salivary gland (SG) virus to the JE-WN-SLE-MVE group as established by cross complement-fixation tests using hyperimmune guinea pig sera.

Viruses. The bat salivary gland virus (SG) designated #1410-19 and in its 5th mouse brain passage was sent to us by Lt. Col. Kenneth F. Burns. The Nakayama strain of JE virus in the 41st mouse brain passage, a strain of MVE in the 10th passage, and the WN virus (J7259) in the 25th passage were obtained from the Viral and Rickettsial Disease Registry, American Type Culture Collection, Washington, D.C. The Hubbard strain of SLE was obtained from Dr. Isaac Rushman.

Antigens and antisera. The antigens and the antisera for the complement fixation tests and the grid type test using these materials are essentially the same as described by Pond and his associates(19) and by Hammon and Espana(20). Antisera against the JE, WN, SLE and MVE viruses were obtained from

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guinea pigs inoculated intracerebrally with suspensions of infected hamster brain. In each instance the virus contained in infected mouse brain tissue in 10% normal rabbit serum was passed intracerebrally through 3 serial passages in weaned hamsters. The guinea pigs were given 2 injections, 10 days apart, each consisting of 0.15 ml of a 10% suspension of 3rd passage infected hamster brain tissue. The sera obtained 2 weeks after the last injection were stored at —40°C. Each serum was inactivated at 56°C for 30 minutes before it was used in the complement fixation test. Since the SG virus is not infective for young hamsters, antiserum against this agent was prepared as described above, except 6th mouse brain passage material in 10% normal guinea pig serum in saline was used for immunizing the guinea pigs. A control group of guinea pigs each received two injections of 10% normal mouse brain in 10% guinea pig serum in saline.

In the initial tests it was found that all of the viruses except SG yielded effective complement-fixing antigens prepared by the technic of Havens *et al.*(21) or Casals *et al.*(22). Since highly effective antigens with the SG virus were obtained by the benzene extraction technic of DeBoer and Cox(23), all complement fixation tests were carried out with antigens prepared by this method.

Complement fixation tests. The complement fixation tests used in these studies are essentially the same as those described by Pond *et al.*(19). Serial 2-fold dilutions of each antigen were tested against serial 2-fold dilutions of each of the guinea pig antisera. In addition to the usual serum and antigen anti-complementary controls, normal guinea pig serum in serial dilution was tested against each dilution of the various antigens. In the test 2 exact units of complement were used. Following overnight incubation at 4°C the indicator system consisting of 3 units of hemolysin and 3% washed sheep cells was added. The tests were read after an additional incubation for 30 minutes at 37°C. Tubes with 50% or more fixation were considered positive.

Results. The results of a grid-type complement fixation test are presented graphically in Fig. 1 using the method previously described by Pond *et al.*(19). The shaded areas indicate mixtures in which 50% or more fixation of complement occurred. The results with the JE, MVE, WN and SLE antigens are in some respects the same as described by Pond *et al.*(19), although the higher titered SLE antigen used in these tests demonstrated serological relationships with other members of the family which were not evident in their study. For example, it can be seen that the JE antiserum reacted with its homologous antigen and to some extent with the SLE antigen, but failed to react with the MVE antigen. Pond *et al.*(19), however, found that JE antiserum reacted with the MVE antigen but not with the SLE antigen. In the results described in Fig. 1 it can be seen that the SLE antigen reacted to some extent with each of the antisera tested.

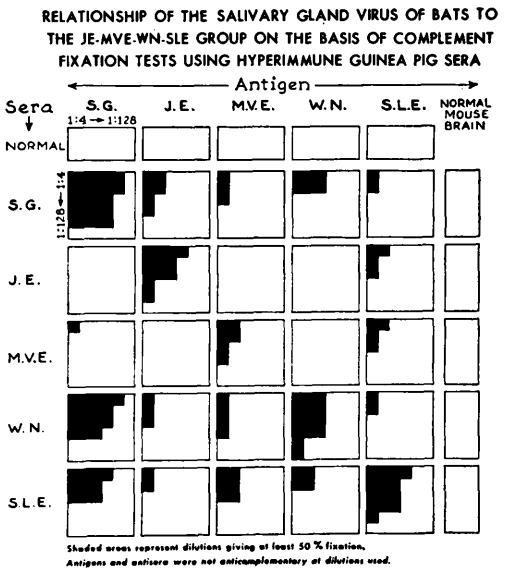

FIG. 1.

The results indicate that the SG agent appears to be a new member of the JE-WN-SLE-MVE group of viruses and that it is more closely related antigenically to WN than to other members of the group. These data, together with the fact that the SG antigen failed to fix complement in the presence of antisera prepared against a variety of other neurotropic viruses, confirm the preliminary

observations of Burns and Farinacci(13). Additional studies are in progress to establish whether the serological relationships observed in the complement fixation test can be more effectively demonstrated by other immunologic technics.

Summary. A virus recently isolated from the salivary glands of Mexican free-tailed bats (*Tadarida mexicana*) appears to be a new member of the JE-WN-SLE-MVE group of viruses. On the basis of the results of complement fixation tests it appears that the salivary gland virus of bats is more closely related to the West Nile virus than to other members of the group.

1. Sulkin, S. E., and Greve, M. J., *Texas State J. Med.,* 1954, v50, 620.

2. Venters, H. D., Hoffert, W. R., Scatterday, J. E., and Hardy, A. V., *Am. J. Pub. Health,* 1954, v44, 182.

3. Witte, E. J., *ibid.,* 1954, v44, 186.

4. Kough, R. H., *J. Am. Med. Assn.,* 1954, v155, 441.

5. Malaga-Alba, A., *Texas Health Bull.,* 1953, v6, 4.

6. Sullivan, T. D., Owens, J. E., Eads, R. B., Menzies, G. C., and Irons, J. V., *Pub. Health Rep.,* 1954, v69, 766.

7. Burns, K. F., and Farinacci, C. J., *Science,* 1954, v120, 548.

8. ———, *J. Inf. Dis.,* 1955, v97, 211.

9. Sulkin, S. E., Krutzsch, P. H., Wallis, C., and Allen, R., to be published.

10. Enright, J. B., Sadler, W. W., Moulton, J. E., and Constantine, D., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 94.

11. Bell, J. F., Hadlow, W. J., and Jellison, W. L., *Pub. Health Rep.,* 1955, v70, 991.

12. Burns, K. F., Farinacci, C. J., and Murnane, T. G., *J. Am. Vet. Med. Assn.,* 1956, v128, 27.

13. Burns, K. F., and Farinacci, C. J., *Science,* 1956, v123, 227.

14. Kudo, M., Uraguchi, K., Matusda, S., and Hoshimoto, H., *J. Immunol.,* 1937, v32, 129.

15. Smith, M. G., and Reames, H. R., PROC. SOC. EXP. BIOL. AND MED., 1940, v45, 838.

16. Smithburn, K. C., *J. Immunol.,* 1942, v44, 25.

17. Casals, J., *J. Exp. Med.,* 1944, v79, 341.

18. Lennette, E. H., and Koprowski, H., *J. Immunol.,* 1946, v52, 235.

19. Pond, E. L., Russ, S. B., Rogers, N. G., and Smadel, J. E., *ibid.,* 1955, v75, 78.

20. Hammon, W. McD., and Espana, C., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 113.

21. Havens, W. P., Watson, D. W., Green, R. H., Lavin, G. I., and Smadel, J. F., *J. Exp. Med.,* 1943, v77, 139.

22. Casals, J., Olitsky, P. K., and Onslow, R. O., *ibid.,* 1951, v94, 123.

23. DeBoer, C. J., and Cox, H. R., *J. Immunol.,* 1947, v55, 193.

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Choline Antimetabolites. Studies with Intestinal Muscle. (22670)

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The report of Keston and Wortis(1) that frog muscle contracts in response to choline and that this contraction is inhibited by triethylcholine was of interest to us in our studies of compounds structurally similar to choline. Triethylcholine is only a very weak inhibitor of choline oxidation by choline dehydrogenase(2) and has usually been found to have choline-like activity (*Neurospora crassa* growth(3); lipotropic(4); antihemorrhagic (5)). In view of this anomalous behavior of triethylcholine with muscle we have extended our studies to include the effects of choline and related compounds on muscle contraction.

In this study segments of rabbit ileum were used instead of frog muscle. This acetylcholine sensitive tissue contracts in response to choline. We found that the response to choline was inhibited competitively by triethylcholine, β-methyltriethylcholine and α-methyl-α-hydroxymethylcholine. Triethylcholine was not, however, an inhibitor of acetylcholine. Methyldiethylcholine, triethylhomocholine, and α-methyl-α-hydroxymethyltrie-