appears and growth occurs to an extent greater than that attributable only to the L-dihydroorotic acid present.

The data of Fig. 1 form the basis for possible microbiological methods of determining uracil, orotic acid, and dihydroorotic acid in a mixture of the three compounds. The uracil content would be given by the difference between the results obtained by *L. bifidus* assay and that obtained by *L. bulgaricus* 09 assay. The orotic acid content would be given by the difference between the result obtained by *L. brevis* assay and the value previously found for uracil. The dihydroorotic acid content would be given by the difference between the result obtained by *L. bifidus* and that obtained by *L. brevis*. Obviously other interfering compounds such as, for example, cytosine, thymine, and ureidosuccinic acid must be absent.

Summary. L-Dihydroorotic acid is utilized by a number of "pyrimidineless" lactobacilli to promote growth. D-Dihydroorotic acid in comparable amount is not utilized and under suitable conditions is an antimetabolite of L-dihydroorotic acid. Methods are outlined by which the microbiological determination of uracil, orotic acid or dihydroorotic acid in a mixture of the three is possible by differential assay.

1. Wright, L. D., Huff, J. W., Skeggs, H. R., Valentik, K. A., and Bosshardt, D. K., *J. Am. Chem. Soc.*, 1950, v72, 2312.

2. Wright, L. D., Valentik, K. A., Spicer, D. S., Huff, J. W., and Skeggs, H. R., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 293.

3. Wright, L. D., Miller, C. S., Skeggs, H. R., Huff, J. W., Weed, L. L., and Wilson, D. W., *J. Am. Chem. Soc.*, 1951, v73, 1898.

4. Bachstez, M., and Cavallini, G., *Ber.*, 1933, v66, 681.

5. Spicer, D. S., Liebert, K. V., Wright, L. D., and Huff, J. W., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 587.

6. Lieberman, I., and Kornberg, A., *Fed. Proc.*, 1953 ,v12, 239.

7. ———, Material presented in a paper and the subsequent discussion before 37th Ann. Meet. of Fed. Am. Soc. for Exp. Biol., Chicago, Ill., April 6-10, 1953.

8. Miller, C. S., Gordon, J. T., and Engelhardt, E. L., *J. Am. Chem. Soc.*, 1953, v75, 6086.

9. Wright, L. D., and Miller, C. S., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 131.

Received November 20, 1953. P.S.E.B.M., 1953, v84.

Isolation of West Nile Virus from Hooded Crow and Rock Pigeon in the Nile Delta.* (20764)

TELFORD H. WORK,† HERBERT S. HURLBUT,‡ AND R. M. TAYLOR.§
(Introduced by J. Casals.)
From the U. S. Naval Medical Research Unit No. 3, Cairo, Egypt.

West Nile virus has repeatedly been isolated from the blood of febrile children(1-4) and from *Culex* mosquitoes(5) collected in an endemic area in the Nile Delta of Egypt. Because of the high incidence of immunity found in the human population during these studies, investigation was extended to the com-

* The study was conducted under the auspices of the Ministry of Public Health of the Egyptian Government, the U. S. Naval Medical Research Unit No. 3, and the Division of Medicine and Public Health of The Rockefeller Foundation.

The opinions or assertions contained herein are the private ones of the authors, and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

† Staff member of the Division of Medicine and Public Health, The Rockefeller Foundation, New York, assigned to the Department of Virology, U. S. Naval Medical Research Unit No. 3, Cairo, Egypt.

‡ CDR MSC USN, Department of Entomology.

§ Consultant to the Division of Medicine and Public Health, The Rockefeller Foundation, New York, assigned to the Department of Virology, U. S. Naval Medical Research Unit No. 3, Cairo, Egypt.

mon indigenous birds of the area in an attempt to find potential warm-blooded virus circulators which might be serving as a virus source for widespread infection of mosquitoes during the peak transmission season. Serological survey of certain avian species indicated a high incidence of immunity to West Nile virus in domestic rock pigeons, *Columba livia,* and in wild hooded crows, *Corvus corone sardonius.* Experimental infection by the feeding of infected *Culex* mosquitoes on crows showed that this species circulated West Nile virus in high titer for at least 5 days(6).

Considerable attention was directed to examination of these birds as collected in their natural habitat beginning early in June 1953. This report deals with the isolation of West Nile virus from two pigeons and a crow.

Materials and methods. Blood for examination was routinely drawn by cardiac puncture into a sterile 2cc or 10cc syringe. Captured birds were bled by needle puncture through intact skin. In specimens obtained by shotgun collection, the chest was incised with a scalpel, exposing the base of the heart. Blood was drawn directly through a 21-gauge needle inserted into the cardiac chambers or great vessels. Blood samples were emptied into sterile 13 x 100 mm or 12 x 75 mm test tubes closed with sterile rubber stoppers. These tubes were placed on ice in a wide-mouth thermos for transport to the laboratory, where they arrived within two to three hours after collection. In the laboratory the clot was broken up and the specimen centrifuged at 1500 rpm for 10 minutes. The serum was drawn off by sterile pipette, cultured on blood agar, and deposited in a numbered screw-top glass vial for reference storage at −20°C. A portion of each serum was inoculated, .02 ml intracerebrally and .03 ml subcutaneously, into each of 7 two- or three-day-old white Swiss mice. The few specimens contaminated by bacteria were filtered through Seitz apparatus and reinoculated. The mice were observed daily for signs of sickness, paralysis, or death. When abnormal signs appeared, the mouse was sacrificed. The brain harvest was triturated into a 10% suspension in 10% inactivated normal rabbit serum. This material was passed as above into a group of infant mice, and .03 ml intracerebrally into a group of 6 three-week-old mice. When any strain of virus produced paralysis and death consistently in the entire group in four to seven days, the brain harvest was titrated in a neutralization test with West Nile-immune and nonimmune serum. Observation of characteristic paralysis and death in three-week-old mice and neutralization of more than 100 LD_{50} of the virus strain by West Nile-immune serum were considered adequate evidence that the virus was West Nile.

Results. On July 5, 1953, one of us (HSH), on a visit to Chenuda Farm 15 kilometers east of Kafr el Sheikh in the north central area of the Nile Delta, had his attention called by the farm owner to a sick pigeon, *Columba livia.* It was a young bird, fully feathered and estimated to be 3 to 4 weeks old. Although of flying age it was too weak even to walk. No paralysis or tremor was noted at the time. The bird (AN-1230) was received alive in our laboratory on July 6. It was exsanguinated by cardiac puncture, and 10% suspensions of brain and spleen in 10% normal rabbit serum were prepared. These suspensions and the serum were each inoculated into a group of infant mice. The mice showed characteristic paralysis on the fifth postinoculation day and all were dead on the sixth. A 10% suspension of brain harvest was passed into infant and 3-week-old mice; paralysis and death, characteristic of West Nile virus infection, occurred on the fourth and fifth days. A 10% suspension of brain harvest material from these mice was titrated against nonimmune and West Nile-immune baboon sera which showed a 2.89 log difference. It was therefore concluded that this strain was a West Nile virus.

Beginning early in June 1953, periodic collections of 6 to 15 specimens of the hooded crow, *Corvus corone sardonius,* were made at 5- to 14-day intervals in the environs of Sindbis village about 30 kilometers north of Cairo. A specimen (AN-1246) of serum obtained on July 14, 1953, and prepared by the methods described previously, was inoculated into a group of 7 infant mice. One mouse died on the first day; the remaining 6 died on the sixth postinoculation day. A Seitz-filtered 10% suspension of two brains harvested was

passed intracerebrally into 3-day-old mice. These were paralyzed and died on the sixth and seventh postinoculation days. Brain harvest material from these mice, passed intracerebrally into 7 two-day-old and 6 three-week-old mice, produced paralysis and death on the fourth and fifth postinoculation days in all animals. Brain harvest material was titrated against nonimmune and West Nile-immune sera of the same baboon and 3.5 logs of difference resulted. It was concluded that this strain also was a West Nile virus.

In following up the isolation of West Nile virus from the sick pigeon (AN-1230), a return was made to Chenuda Farm on August 6, 1953, when 33 apparently healthy pigeons were captured there and 2 cc of blood was collected by cardiac puncture before the birds were released. Serum from all these specimens was inoculated into one-day-old mice for attempted virus isolation according to the procedure described. On the seventh postinoculation day one mouse was found with characteristic paralysis. The animal was sacrificed and a 10% brain suspension was passed into a group of 2-day-old mice. All became paralyzed and died on the third and fourth postinoculation days. Brain harvest material from these mice was passed in a 10% suspension into 3-day-old and 3-week-old mice. All developed paralysis and died by the fifth postinoculation day.

Brain harvest material was titrated against nonimmune and West Nile-immune sera of the same baboon and a difference of 2.4 logs specific neutralization was calculated. This was taken as adequate evidence that the strain isolated was an additional West Nile virus.

The failure of 6 of the 7 mice initially inoculated with the pigeon serum to show paralysis or death may have been due to low titer of virus in the serum. It should also be noted that this same serum (AN-1324) neutralized 100 LD_{50} of West Nile virus in a routine neutralization test using 33rd passage strain of West Nile virus originally isolated by Smithburn *et al.* in Uganda(7).

Discussion. Eastern equine encephalitis virus has repeatedly been isolated in North America from naturally infected avian hosts; *i.e.,* from the brains of ring-necked pheasants (8-11) and domestic pigeons(12) and the blood of a purple grackle(13). Western equine encephalitis virus has been recovered from the brain and spleen of a prairie chicken (14) and the blood of a nestling magpie and nestling redwing blackbirds(15).

Studies of western equine and St. Louis viruses in regard to isolation from wild-caught mosquitoes, precipitin testing of mosquito stomach contents, neutralizing antibody surveys of wild and domestic avian species, and experimental infection of some species have rather firmly established the potential role of certain avian hosts in the epidemiology of these encephalitides(16-19).

The immunological relationship of West Nile to Japanese B and St. Louis encephalitis viruses has repeatedly been shown(20-23). The incidence of positive neutralization of 100 LD_{50} of West Nile virus by the sera of pigeons and crows, as well as of human residents, in areas where the virus was recovered from bird blood is 50% or more. It is of some significance therefore that West Nile virus has been isolated from birds in nature in an endemic area of West Nile virus infection in the Nile Delta of Egypt.

The isolations of these strains from birds in July and early August coincided with the period during which most West Nile virus isolations were made from *Culex* mosquitoes in 1952 and 1953. Over 400 acres contiguous with the location of the pigeon loft were planted in well-watered rice, which provided breeding ponds for large numbers of *Culex* mosquitoes.

The pigeons from which the isolations were made were a feral type deliberately attracted to large cone-shaped lofts in order to produce guano for use as fertilizer on the farm. The smaller sleek form of the feral type distinguishes it from the domestic permanent resident pigeons which are raised for food by the fellahin.

The Chenuda farm owner stated that epidemics of illness such as were manifested by the sick pigeon had occurred in previous years. He observed that during these epidemics the apparently healthy birds deserted the loft to move to a new and distant locality. Several months passed before another feral flock took

up residence in the deserted loft. This presents an interesting possible mechanism for dissemination of the virus by local pigeon movements.

A cursory survey of nesting hooded crows in the Sindbis area indicates that this is the third commonest breeding species and may contribute as many as 75 nonimmune juveniles per square kilometer each year.

Finding circulating West Nile virus in birds commonly resident in endemic areas of human infection in Egypt adds further support to the concept that avian species play an important if not essential role in the transmission of certain of the arthropod-borne virus encephalitides.

Summary. 1. Strains of West Nile virus were isolated from the blood of two pigeons, *Columba livia,* and one hooded crow, *Corvus corone sardonius,* in an endemic area of the Nile Delta of Egypt in July and August 1953. The virus was also obtained from the brain and spleen of one of the pigeons. 2. The relation of these isolations to the environment is briefly discussed.

Appreciation is expressed to Harry R. Dressler HMC USN. to Del Thrasher HMC USN, and to Rostom Bablanian for technical assistance in the laboratory and in the field.

1. Melnick, J. L., Paul, J. R., Riordan, J. T., Barnett, V. H., Goldblum, N., and Zabin, E., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 661.

2. Taylor, R. M., *Lebanese Med. J.,* 1953, May, 134.

3. ———, *Proc. Vth Internat. Cong. Trop. Med. and Malaria,* 1953, in press.

4. Smithburn, K. C., Taylor, R. M., Rizk, F., and Kader, A., *Am. J. Trop. Med. and Hyg.,* in press.

5. Taylor, R. M., and Hurlbut, H. S., *J. Roy. Egypt. Med. Assn.,* 1953, v36, 199.

6. Work, T. H., Hurlbut, H. S., and Taylor, R. M, to be published.

7. Smithburn, K. C., Hughes, T. P., Burke, A. W., and Paul, J. H., *Am. J. Trop. Med.,* 1940, v20, 471.

8. Tyzzer, E. E., Sellards, A. W., and Bennett, B. L., *Science,* 1938, v88, 505.

9. Van Rockel, H., and Clarke, M. K., *J. Am. Vet. Med. Assn.,* 1939, v94, 466.

10. Beaudette, F. R., and Hudson, C. B., *ibid.,* 1945, v107, 384.

11. Beaudette, F. R., and Black, J. J., *ibid.,* 1948, v112, 140.

12. Fothergill, L. D., and Dingle, J. H., *Science,* 1938, v88, 551.

13. Kissling, R. E., Rubin, H., Chamberlain, R. W., and Eidson, M. E., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 398.

14. Cox, H. R., Jellison, W. L., and Hughes, L. H., *Pub. Health Rep.,* 1941, v56, 1905.

15. Sooter, C. A., Howitt, B. F., Gorrie, R., and Cockburn, T. A., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 393.

16. Hammon, W. M., Reeves, W. C., *et. al.,* Collected Papers of the George Williams Hooper Foundation, 1946-1950. Vol. 22. Passim.

17. Hammon, W. M., Reeves, W. C., and Sather, G. E., *J. Immunol.,* 1951, v67, 357.

18. Sooter, C. A., Howitt, B. F., and Gorrie, R., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 507.

19. Thompson, G. A., Howitt, B. F., Gorrie, R., and Cockburn, T. A., *ibid.,* 1951, v78, 289.

20. Smithburn, K. C., and Jacobs, H. R., *J. Immunol.,* 1942, v44, 9.

21. Smithburn, K. C., *ibid.,* 1942, v44, 25.

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

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

Received December 2, 1953. P.S.E.B.M., 1953, v84.