workers(8). Another possibility to be considered is a change in the relative rates of synthesis and hydrolysis of ACh produced by a lowered body temperature. Further studies are necessary to decide whether these or other alterations are responsible for the ACh increase.

Summary. Exposure to −12°C for 2 hours significantly increased both the free and total acetylcholine concentration of rat brain.

The statistical help given by Dr. William F. Taylor, Department of Biometrics, is acknowledged.

8. Elliott, K. A. C., Swank, R. L., and Henderson, N., *Am. J. Physiol.*, 1950, v162, 469.

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Isolation from Human Sera in Egypt of a Virus Apparently Identical to West Nile Virus.* (18884)

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This report describes the isolation from human sera collected in Egypt, of 3 strains of a virus characterized by the capacity to produce encephalomyelitis in monkeys, mice and hamsters, and immunologically related to West Nile virus. The circumstances under which these virus strains were isolated were as follows. In the course of an "antibody survey," carried out in July 1950, one of us (J.R.P.), collected serum from 251 Egyptians living within a Rural Health District known as Markaz Callub, established by the Ministry of Public Health with the cooperation of the Rockefeller Foundation, and located some 30 km. north of Cairo.‡ Most of the Egyptians were children and most of them were attending a Dispensary. Apparently at least 3 of the children happened to be in stages of viremia at the time they were bled, for it was from their sera that the strains were isolated.

All 3 strains were subsequently found to be antigenically related to each other and to the West Nile virus. The latter virus was isolated at the Yellow Fever Research Institute in Entebbe from a human serum obtained in 1937 in Uganda, about 2000 miles south of Cairo(1), but it has not been recovered in nature since then. However, serological evidence obtained by Smithburn(2) pointed to widespread human infection with this virus in Central Africa, with 1 to 46% of the various population groups studied there yielding positive tests for neutralizing antibodies to West Nile virus. In like manner evidence has been obtained in the present study to show that antibodies to the newly isolated Egyptian virus are present in a very large percentage of

* Laboratory work carried out in New Haven under the auspices of the Commission on Virus and Rickettsial Diseases, Armed Forces Epidemiological Board, Office of the Surgeon General, U. S. Army, Washington, D. C.

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We are also indebted to Dr. W. A. McIntosh and Dr. J. M. Weir of the International Health Division of the Rockefeller Foundation at Cairo, for indicating sites for the collection of clinical material and data; and for providing professional and technical help, and other facilities.

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

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

normal individuals living in the vicinity of Cairo.

Isolation of virus. Each of the 251 Egyptian sera referred to above were tested for the presence of poliomyelitis antibodies by the addition of Lansing poliomyelitis virus and subsequent inoculation of the serum-virus mixture into the brain of adult Swiss mice. During the course of 3 of these 251 tests, (on sera Nos. 19, 21 and 101), the inoculated mice (8 used for each test) developed signs of encephalitis rather than the myelitis typical of the Lansing virus. From these mice 3 strains of a virus were recovered which did not prove to be Lansing virus. Re-examination of each of the 3 sera alone yielded viruses capable of producing encephalomyelitis in mice. In one instance the serum had an intracerebral titer of $10^{-2.5}$ in mice.

The virus in one serum was found to grow directly in the chick embryo, and a line of the virus has been maintained in embryonated eggs without its ever having been in mice. Infection in the egg may be recognized by the death of the embryo and by passage of embryo suspensions to mice in which the typical encephalitic disease is produced.

The agent readily passed bacterial filters (Seitz EK, Corning UF glass fritted).

Prior to and at the time these strains were isolated, there was no West Nile virus in our laboratory nor have we ever had a strain of this virus in our laboratory before.

Host range of Egyptian virus. Species susceptible to infection by the Egyptian virus include the mouse, hamster, monkey, chimpanzee, and chick embryo. Disease has not been produced in cotton rats, guinea pigs, and rabbits; however these latter species produce antibodies in high titer following a single intracerebral inoculation of virus. Rabbits also failed to respond to the application of virus to the scarified cornea.

Adult mice are more susceptible to the virus following brain inoculation, the titer of infected mouse brain by the intracerebral route being $10^{-7.5}$ and by the intraperitoneal route, about 10^{-3} and irregular. On the other hand newborn mice are equally susceptible to both intracerebral and intraperitoneal routes of inoculation, the titer by either route usually being $10^{-7.5}$. The titer in chick embryos is over 10^{-4} by either dropping the virus on the chorio-allantoic membrane or inoculating it into the yolk sac.

Rhesus monkeys are very susceptible to intracerebral (*i.c.*) inoculation of the virus: cynomolgus monkeys perhaps less so. The disease in monkeys (resulting from *i.c.* inoculation) is characterized by viremia, fever, ataxia, and prostration. Some monkeys may be left with a severe flaccid paralysis of the extremities resembling poliomyelitis, others show marked improvement with no signs of the disease after a period of one or 2 months. Histologically the intracerebrally inoculated rhesus monkeys have an extensive encephalomyelitis involving the cerebellum as well as other areas of the brain and cord. Following intraperitoneal and intramuscular inoculation, the picture is different, some monkeys exhibit a temporary viremia without fever about 3 days after injection, while none show signs of encephalomyelitis, nor has it been evident histologically in sacrificed monkeys.

Chimpanzees develop a silent infection without fever, following intracutaneous inoculation of virus (Fig. 1). Following a period of viremia which lasts for 3 days, neutralizing antibodies begin to appear, and are present in significant titer by the 6th day reaching their maximum by the 2nd to 3rd week. Complement fixing antibodies make their appearance a few days after neutralizing antibodies are detectable. Virus does not appear in the throat or feces during the 2 week period following its inoculation. The response of these animals to feeding of virus is unlike that following ingestion of poliomyelitis and Coxsackie (C) viruses by these animals. Again Egyptian virus cannot be detected in the throat or stools, and antibodies fail to appear.

Immunological reactions. The 3 strains of the Egypt virus are related to each other by cross neutralization and cross complement fixation tests. The virus is neutralized by and fixes complement in the presence of West Nile immune serum. It is neutralized in an irregular fashion and to a lesser degree by certain Japanese B and St. Louis immune sera, in similar fashion to West Nile virus

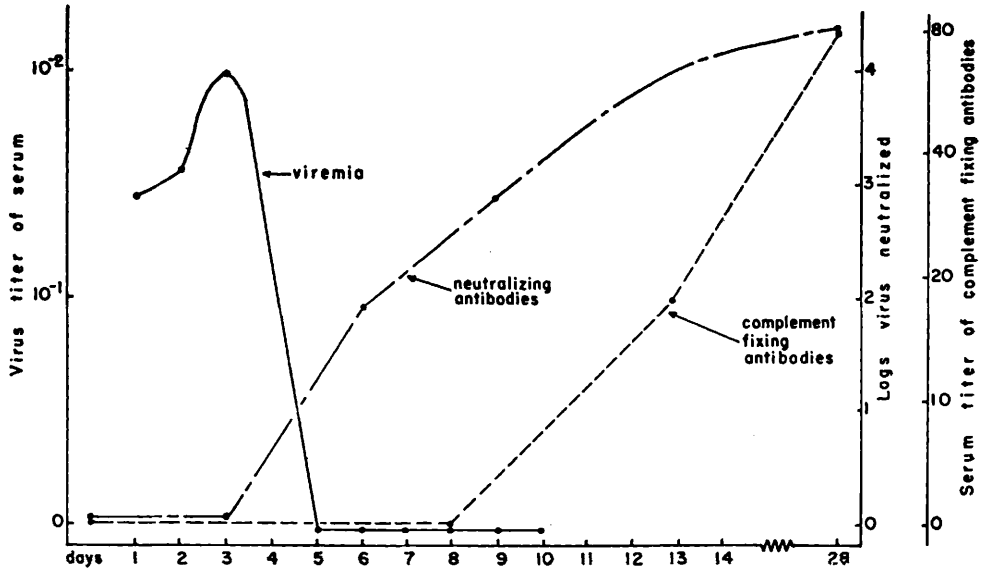


FIG. 1. Infection of 4 chimpanzees with the Egyptian virus. Each point represents the average of 4 determinations, each made on a different chimpanzee.

(3-5), while some Japanese B and St. Louis sera fail to neutralize the virus. Neutralization tests with the Egyptian virus may be carried out in adult mice (intracerebral route) or in newborn mice (intraperitoneal route). In many tests no difference has been observed in the sensitivity of the test carried out in either animal; however as with West Nile virus(6), in some tests the neutralization index of a serum has been 1 to 1.5 logs greater when infant mice were used.

The virus is not neutralized by sera containing antibodies to the following viruses:§

Dengue
Eastern equine encephalomyelitis (EEE)
Western equine encephalomyelitis (WEE)
Louping ill
Russian spring-summer encephalitis
Venezuelan encephalitis
Rift valley fever
Lymphocytic choriomeningitis (LCM)
Encephalomyocarditis (EMC, Mengo, Col.-SK, MM)
Vaccinia
Semliki
Bunyamwera
Uganda S
Zika
Ntaya
Bwamba
Mengo (EMC)
Coxsackie, Type Conn.—5
" " Texas—1
" " Ohio—1
Poliomyelitis, Type Lansing
" " Brunhilde
Mouse encephalomyelitis, Theiler's FA
" " " GD7

In addition the virus was not neutralized by 4 pools of human gamma globulin prepared in the U. S. in 1945, 1949, and 1951.

Sera obtained from monkeys and chimpanzees following infection with the Egyptian virus have been found to react with West Nile virus in both neutralization and complement fixation tests.‖ In addition certain Egypt virus immune sera have been found by Dr. I. Ruchman to neutralize Japanese B encephalitis, epidemic Keratoconjunctivitis, and St. Louis encephalitis viruses, but these crossings have been less pronounced than the reaction with homologous virus. In the complement fixation test, the

3. Smithburn, K. C., *J. Immunol.*, 1942, v44, 25.
4. Casals, J., *J. Exp. Med.*, 1944, v79, 341.
5. Lennette, E. H., and Koprowski, H., *J. Immunol.*, 1946, v52, 235.
6. Lennette, E. H., and Koprowski, H., *J. Immunol.*, 1944, v49, 375.

§ We are particularly indebted to Dr. Joel Warren of the Army Medical Center, to Dr. Kenneth C. Smithburn of the Rockefeller Foundation, to Dr. Albert B. Sabin of the Children's Hospital Research Foundation, Cincinnati, Ohio for some of the sera used in these tests.

TABLE I. Antigenic Relationship of Egyptian Strains by Complement Fixation.

Antigen	Dilution of serum*									
	Mouse. Egypt-19	Monkey 5166. Egypt-19	Monkey 5160. Egypt-101	Monkey 5433. West Nile	Mouse. St. Louis	Mouse. Jap B.	Monkey. LCM	Guinea pig. EEE	Monkey. Dengue	Monkey. Normal
EV-19	64	128	256	32	4	16	0	0	0	0
EV-21	64	256	512	256	32	16	0	0	0	0
EV-101	>64	256	512	256	<8		0		0	0
West Nile	64	128	512	256	32	32	0	0	0	0
St. Louis	16		4	0	256	8	0	0	0	0
Jap B.	32		16	0	32	64	0	0	0	0
L.C.M.	0		0	0	0	0	64	0	0	0
EEE	0		0	0	0	0	0	>256	0	0
WEE	0		0	0	0	0	0	0	0	0
Normal	0		0	0	0	0	0	0	0	0

* These tests were carried out by a modified Kolmer technic as used in this laboratory (7). Species is listed in which serum was prepared for indicated virus. Many of the antigens used and some of the sera were kindly furnished by Dr. Herald R. Cox, Lederle Laboratories, Pearl River, N. Y.

TABLE II. Clinical Record of 3 Egyptian Children with Viremia.

No.	Initials	Sex	Age in years	Diagnosis
19	S.A.F.	M	1	Impetigo
21	N.A.I.	F	10	? Malaria
101	M.A.	M	2	? Bronchitis

Egyptian strains cross react strongly with the West Nile virus and to a lesser degree with Japanese B and St. Louis encephalitis viruses. They fail to show any cross reactions with the viruses of western and eastern equine encephalomyelitis, lymphocytic choriomeningitis, and dengue (Table I).

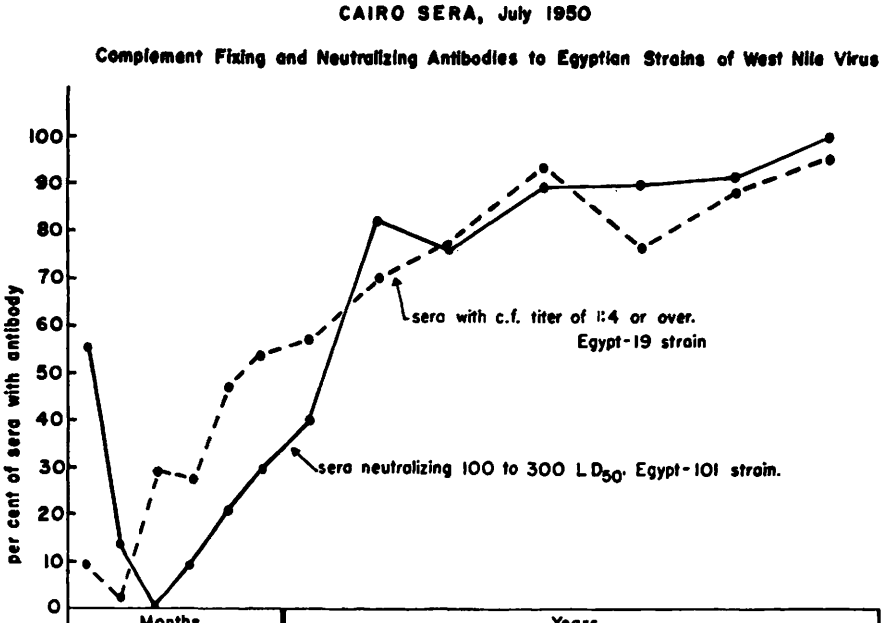
Type of "Patient". The great majority of children from whom these sera were collected were thought to be normal at the time, although some had minor ailments, such as skin diseases, mild respiratory infection, or diarrhea. The Dispensary diagnoses in the 3 children with viremia are listed in Table II. There was no evidence that any of these 3 children was severely ill at the time blood was taken, but from the Dispensary diagnoses, it is likely that fever may have been present in N.A.I. (No. 21).

Local serological evidence of infection. Following the isolation of this virus from 3 of the 251 sera collected in Egypt, a search was made for neutralizing and complement fixing antibodies to the virus in the other local sera. Neutralization tests were carried out on 228 sera using undiluted serum and 2 to 2.5 logs of the No. 101 strain of the Egyptian virus, and at least two-thirds of the inoculated mice had to survive for a serum to be called positive. The complement fixation tests were carried out on 215 sera, using the No. 19 strain as antigen and reading the end point at 50% hemolysis. A positive serum was one in which at least 3 fifty per cent units of complement were fixed when the serum had been diluted 1:4. As shown in Fig. 2, both neutralizing and complement fixing (*c.f.*) antibodies were found in this human population to a marked degree. Both curves are similar except for the infants under 6 months who were found to contain neutralizing but not *c.f.* antibody. It appears that infection with this agent was widespread in this population in 1950. Maternal antibodies disappeared by the age of

‖ We wish to acknowledge the generous cooperation in this work of Drs. Kenneth Smithburn, New York, Joel Warren, Washington, and Isaac Ruchman, Cincinnati, who have also found that our Egyptian immune sera neutralize the strain of West Nile virus used in their respective laboratories. Using the complement fixation test, Dr. J. A. Kerr, New York, also found that the sera of 2 monkeys convalescent from Egypt virus infection reacted with the West Nile virus used in his laboratory.

7. Kraft, L. M., and Melnick, J. L., *J. Exp. Med.*, 1950, v92, 483.

** These tests were largely carried out by one of us (N.G.).



		Months						Years						
Age		0 to 5	6 to 11	12 to 17	18 to 23	24 to 29	30 to 35	3	4	5 to 7	8 to 10	11 to 20	21 to 40	over 40
No. tested	c.f.	10	16	14	11	13	15	16	16	22	16	29	27	10
	neut.	13	16	16	13	15	17	18	17	21	17	30	24	11

FIG. 2. Distribution of neutralizing and complement fixing antibodies in the normal population in the vicinity of Cairo at the time the virus-containing sera were obtained.

6 months, and then the children began to build up their own antibodies quite rapidly so that at the age of 4, 70 to 80% of the group had both neutralizing and *c.f.* antibodies. Both antibodies were maintained in the population through the age of 40 and above.

The occurrence of a viremia both in man and in experimental hosts and the failure to detect virus in the throat or stools of infected primates lead us to suspect that a biting insect acts as a vector in the transmission of the infectious agent. Obviously further work on sera collected in subsequent years from this area will be required to settle the many questions which have been raised by this study.

Summary. 1. Three strains of a filterable virus closely related to West Nile have been isolated from the blood of children living on the outskirts of Cairo, Egypt. 2. The 3 strains of virus appear to be immunologically identical. 3. In addition to a strong relationship and apparent identity with West Nile virus, the Egyptian virus crosses, although to a lesser degree, with both Japanese B and St. Louis encephalitis viruses. This was found by both neutralization and complement fixation tests. 4. The virus produces a marked encephalomyelitis in monkeys inoculated intracerebrally. Lesions are found throughout the spinal cord and brain including the cerebellum. 5. Chimpanzees develop a silent infection following intracutaneous infection of the virus. This is characterized by a viremia lasting 3 days followed by the appearance of both neutralizing and complement fixing antibodies. 6. The Egyptian virus was not neutralized by human gamma globulin collected in the United States, nor by 24 different hyperimmune sera, each containing antibodies to an antigenically distinct virus type. 7. Infection with this virus has been widespread in the local Egyptian population in 1950, with more than 70% of the inhabitants aged 4 years and over having both neutralizing and complement fixing antibodies.

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