though the isolation does not prove that *C. tarsalis* could carry enough virus through an entire winter to infect birds and mammals in the spring, it furnishes the best supportive evidence for this theory that has yet been obtained. The ratio of positive pools to total pools tested of hibernating mosquitoes is not incompatible with the results of summer collecting and testing.

Congregation of *C. tarsalis* in certain mines is noteworthy, and relatively large numbers of mosquitoes found in these mines suggests that temperature and relative humidity therein are tolerable for mosquito hibernation. Although mines are artificial hibernating sites, and it is obvious that the majority of individuals of this widespread species must hibernate in locations other than mines, conditions found in these mines may furnish clues as to types of natural mosquito hibernation sites.

Summary. A total of 1361 hibernating female *C. tarsalis* mosquitoes, comprising 31 pools, was collected in mines in Colorado foothills from Dec. 15, 1953, to Feb. 29, 1954. One of these pools, collected Dec. 30, 1953, contained WEE virus upon inoculation into freshly hatched chicks. The finding of such large numbers of hibernating *C. tarsalis* and the recovery of WEE virus from them, may contribute to the knowledge of the overwintering mechanism of this virus.

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Recent Isolations of Encephalomyocarditis Virus. (22194)

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Strains of encephalomyocarditis (EMC) virus have been isolated on several occasions. The earliest of these isolations was made by Jungeblut during poliomyelitis investigations, first from cotton rats in 1940 (Columbia-SK strain)(1) and from hamsters in 1943 (MM strain)(2), but these were initially regarded as strains of poliovirus. A virus producing encephalomyocarditis in animals, and thus named encephalomyocarditis (EMC) virus, was isolated in 1945 by Helwig and Schmidt from a chimpanzee dying of myocarditis at Dania, Fla.(3,4). This virus was later found to be immunologically identical to Columbia-SK and MM viruses(5). In 1948 Mengo encephalomyelitis virus was isolated at Entebbe, Africa, from a rhesus monkey(6) and this virus was similar or related to the EMC virus. Since then similar types of virus have been isolated in Africa from man, mosquitoes, and monkeys(7). The present report describes 3 more isolations of viruses belonging to the EMC group.

On June 12, 1952, specimens of lung, liver, spleen, kidney and stomach contents from a mandrill baboon which had died of unknown cause at the Anthropoid Ape Research Foundation Farm in Dania, Fla., were received at the Communicable Disease Center from the Miami Regional Laboratory of the Florida State Board of Health for study. The baboon had died 24 hours after the first visible signs

of illness. All that was noted by the veterinarian in charge during this 24-hour period was that the baboon could not stand and refused food. The animal had been at the Foundation farm in Florida for 3 or 4 years.

Methods. The liver and spleen were pooled as were kidney and lungs. Ten per cent suspensions of these pools and the stomach contents were prepared and, after addition of suitable amounts of penicillin and streptomycin, each of the 3 suspensions was inoculated intracerebrally into groups of 5 mice. All inoculated mice died between the 4th and 14th days. Cultures of the brains revealed bacterial contamination. These brains were inoculated into a second group of mice which died on 3rd to 6th day. No evidence of bacterial contamination was observed when brains of these mice were cultured on blood agar and in thioglycollate broth. A suspension of 2 of these 2nd mouse passage brains had a mouse intracerebral LD_{50} titer of $10^{6.3}$. The titer of 3rd and 4th mouse passage material was $10^{6.3}$ to $10^{6.8}$.

Results. Neutralization tests were carried out by incubating mixtures of 10-fold dilutions of the agent and undiluted antiserums to various viruses at 4°C overnight and then inoculating each of the mixtures intracerebrally into 5 mice. Neutralization was not considered positive unless the LD_{50} endpoint of test serum was 1.5 logs lower than normal serum control. The agent was not neutralized by antisera to eastern or western equine encephalomyelitis or St. Louis encephalitis viruses, but was neutralized by EMC antiserum. Antiserums to EMC virus and the new agent were prepared by a single subcutaneous inoculation of guinea pigs with live material. Reciprocal neutralization tests were made with these serums. Both EMC virus and the new agent were neutralized to essentially the same degree by heterologous as well as homologous antiserum.

A second isolation of EMC virus was made from a monkey brain submitted by the Florida State Health Department on Feb. 9, 1953. The virus was also isolated from brain of a mouse which had been inoculated at the Florida State Health Department Laboratory with brain material from this same monkey. This monkey had been housed at the same farm in Dania, Florida, where the baboon mentioned earlier had been kept. The monkey species and its clinical history were not provided with the specimen and could not be obtained later. A suspension of the brain of this monkey, upon inoculation into mice, yielded a virus which behaved essentially like that isolated earlier from the baboon.

EMC virus was isolated again from a squirrel brain submitted by the Florida State Health Department Laboratory on Feb. 11, 1954. This squirrel, which originated in Palm Beach County, Fla., had appeared sick and bit a child who handled it. Therefore, it was captured to have it examined for evidence of rabies infection. As far as we are aware, this inadvertent finding represents the first isolation of EMC virus from a wild animal indigenous to North America, although antibodies to the EMC group of viruses has been previously demonstrated in wild rats in the southern United States by Warren and coworkers(8).

The second mouse passage material of the baboon strain was titrated in 3-week-old mice by the subcutaneous, intramuscular, intraperitoneal, intranasal, and intracerebral routes. An inoculum of 0.03 ml brain suspension material was used. The agent titered about 10-fold higher by the intracerebral than by the intramuscular, subcutaneous, and intraperitoneal routes, and about 1000-fold higher than by the intranasal route. The incubation periods or symptoms did not vary significantly by the various routes. Mice receiving the lower dilutions died in 3 to 5 days after inoculation, while death from the more dilute suspensions could be delayed up to the 10th day. Those mice receiving the low dilutions of brain suspension became weak, with ruffled hair coats, and they would be found dead the day after these symptoms appeared. Occasionally convulsions would be seen. Mice receiving higher dilutions would first be seen walking with an unsteady gait. This would be followed by a complete paralysis of the hind legs. These symptoms would continue for one to 4 days and then be followed by prostration

and death.

Cotton rats inoculated intranasally or subcutaneously with a 10^{-3} dilution of virus became weak and died within 48 to 72 hours after inoculation, while guinea pigs displayed a febrile response only and survived subcutaneous inoculation.

The lesions in inoculated mice consisted of glial nodules scattered through the corpus callosum, internal capsule, thalamus and midbrain. There was almost complete destruction of the Purkinje cells of the cerebellum. The cerebral cortex was essentially free of lesions. The lumbar portions of the spinal cord showed an occasional necrotic neuron in the ventral gray horn. Demyelinization was present in certain fiber tracts and nerve trunks. The heart showed interstitial round cell infiltration and mild fiber fragmentation in the auricular myocardium. The spleen, lungs, liver, gut, and kidneys were free of demonstrable lesions.

Summary. During the past 3 years encephalomyocarditis virus was isolated twice from captive primates imported to Florida and once from a wild squirrel originating in Florida.

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Effect of Temperature on Activity of Guinea Pig Complement. (22195)

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When human complement (hu C′) and sensitized sheep erythrocytes (EA) interact, both extent of lysis and shape of the lytic curves obtained are markedly dependent on temperature(1). These findings have recently been explained(2) on the basis of proportionality between temperature and rates of formation and decay of an intermediate complex $EAhuC'_A$.

Since the great majority of both experimental and routine laboratories have used guinea pig (g.p.) rather than hu C′ in their investigations, and since the choice of 37°C as reaction temperature appeared to be quite arbitrary, the effect of temperature on lysis by g.p.C′ was investigated. These studies were greatly facilitated by data from several laboratories(3,4) on EAg.p.C′1,4,2, the complex between EA and g.p.C′1, C′4 and C′2.

Methods. Sheep blood in 500 ml lots was preserved with the addition of 2000000 units of penicillin "G" and 25 ml of 0.15 *M* Trisodium ethylenediamine tetraacetic acid (Na_3E.D.T.A.)(4). Veronal buffer(5) containing 5 x 10^{-4} *M* Mg^{++} and 1.5 x 10^{-4} *M* Ca^{++} was used for all dilutions and washings. Pooled guinea pig sera, stored in small lots at –25°C, were the source of g.p.C′. Kinetic analyses were carried out according to the technic of Mayer and Levine(6). Bath temperatures were held constant to ± 0.1°C. All solutions were equilibrated at the temperature of the experiment before mixing.

Results. Two flasks, each containing 10 ml of EA, 10 ml of buffer and 5 ml of g.p.C′/170 were incubated at 37°C, and 27°C respectively. After sampling was completed, 2 more flasks were run at 37°C and 32°C. The reaction curves are plotted in Fig. 1. The extent of reaction at 37°C was considerably less than at either 27°C or 32°C.

The next experiment was designed to study