tion than at 24 hours. In animals exposed to 550, 750, 1200 or 2500 r the percentage of liver glycogen was not greatly different at 48 hours and 24 hours after the time of irradiation. In no case did the liver glycogen levels in the irradiated rats equal or exceed that found in normal, fed rats. 3. Whole-body x-irradiation increased the blood sugar levels in fasting rats. Larger increases were noted at 48 hours after the time of irradiation than at 24 hours. At 48 hours after the time of irradiation the blood sugar level was, in every case, greater than that of fed, normal rats.

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A Winter Isolation of Western Equine Encephalitis Virus from Hibernating *Culex tarsalis* Coquillett. (22193)

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The means of survival of Western equine encephalomyelitis virus (WEE) during the winter months has been a perplexing question since the disease was first recognized. Several explanations have been proposed for reintroduction or reappearance of the virus every year in certain endemic areas. The 3 most common theories are: 1. Migrating birds bring the virus northward each spring. 2. Arthropods, other than mosquitoes, harbor the virus during winter and infect susceptible birds during the most active stages of the arthropod life cycle. 3. Infected mosquitoes hibernate over the winter and infect susceptible birds and mammals during the following spring. It is the purpose here to present our findings and discuss the last possibility. As pointed out by Eklund(1,2) the conditions under which a mosquito-vertebrate cycle fails to account for maintenance of virus must be better understood before alternative mechanisms can be explored intelligently.

During the summer months WEE virus has been recovered from *Culex tarsalis* Coquillett frequently and from other mosquitoes occasionally in endemic areas(3-8). Hibernating *C. tarsalis* have not previously been reported in such numbers, and few of these have been tested for the presence of WEE virus. Although winter isolations of WEE virus from this species have been made in California during November and January(9) it is felt that these mosquitoes did not represent true hibernators. We consider the following factors to be indicative of true hibernation: the presence of fat bodies, lack of feeding activity of mosquitoes, absence of male mosquitoes, quiescence of female mosquitoes, and temperatures prohibitive for mosquito survival outside of the hibernating sites.

Methods. Mosquito hibernation sites. C. tarsalis mosquitoes were found hibernating in abandoned mines during winter months. These mines were in the foothills in Boulder and Larimer Counties, Colo., between elevations of 5000 and 8000 feet above sea level. The temperature from Dec. 22 through Feb. 20, was between 12.2°-13.3°C in a mine near

TABLE I. Hibernating *Culex tarsalis* Mosquitoes Collected in Colorado during the Winter of 1953-1954.

Date collected	Date tested	No. mosquitoes	No. pools	From	Location
12/15/53	1/19/54	38	1	6 mines	7 miles W of Masonville
30	"	587	12	3 "	3 " E " Jamestown*
"	"	1	1	1 mine	7 " W " Boulder
1/ 7/54	"	612	12	1 "	3 " E " Jamestown
13	2/16	1	1	1 "	1 " NW " Sunshine Canyon
14	22	63	1	Mines in vicinity of Jamestown	
21	16	3	1	" " "	" Central City
22	22	41	1	5 coal mines	12 miles NE of Fort Collins
2/ 9	"	15	1	1 mine	2 miles E of Masonville
Total		1361	31		

* Isolation made from one pool of 50 mosquitoes.

Masonville, and relative humidity in this mine fluctuated from 30 to 80%.* However, during the latter part of February, after a long dry period, the relative humidity dropped to 15%. No mosquitoes were found in this mine after this sharp drop. The mine shaft entrances varied from 4 to 6 feet in diameter; the shafts proper extended from 120 feet straight back from the entrances to side shafts which extended into unexplored depths. *Collection of mosquitoes.* From Dec. 15, 1953 to Feb. 9, 1954, mosquitoes (*C. tarsalis*) were collected at random from the abandoned mines. The mosquitoes were resting approximately 10 to 50 feet from mine entrances. In no cases where mine shaft was absolutely dark during daylight hours were any mosquitoes found. A few mosquitoes were found in mines after the date in spring when presence of active adult *C. tarsalis* in the open indicated that they were leaving hibernation sites. Collections were made by locating mosquitoes on rock surfaces with a flashlight and aspirating them with a hand aspirator. The mosquitoes were taken to the laboratory in small cages, identified, then stored at –20°C in 10 x 75 mm tubes until tested for virus. *Processing of mosquitoes.* The mosquitoes were ground by chilled mortar and pestle in inactivated 20% normal horse serum (pH 7.4). A volume of 2 ml of diluent was used for pools of 25 to 75 mosquitoes, and 1 ml for those pools of less than 25 mosquitoes. The suspensions were centrifuged 10 min. at 1000 x G at room temperature. Three-tenths ml of buffered saline (pH 7.4) containing 2 mg of streptomycin sulfate and 1000 units of penicillin G sodium were added to 1 ml of supernatant materials and the mixtures incubated overnight at 4°C. Each antibiotic-treated supernate was inoculated subcutaneously in 0.03 ml amounts into 4 freshly hatched chicks, which were then observed for a 72-hour period(10). The remainder of supernatant material was ampuled, then frozen in alcohol and dry ice and stored at –70°C for reference.

Results. A total of 1361 female *C. tarsalis* mosquitoes, as shown in Table I, was collected and made into 31 pools. One pool of 50 mosquitoes collected on Dec. 30, 1953, killed 4 out of 4 chicks within 24 hours. None of the other pools killed chicks. The brains of the 4 chicks showed no evidence of bacterial contamination when cultured in thioglycollate medium. Upon passage of the 20% brain suspension, 3 of 4 chicks died in 24 hours with no evidence of bacterial contamination.

The brains of these chicks, as well as the original infected mosquito suspension, were sent in a frozen state to the Communicable Disease Center, Virus and Rickettsia Section, Montgomery, Ala., where the agent was confirmed as WEE virus by complement-fixation tests and serum neutralization tests in mice.

Discussion. The isolation of WEE virus from hibernating *C. tarsalis* mosquitoes collected in December shows this species to be capable of harboring the virus through a period of adverse weather conditions. Al-

* As measured by a recording hygrothermograph manufactured by Instruments Corp. of Baltimore, Md.

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