the writer when the mouth of the pipette is small. It also seems to be a contributory factor in the causation of colds, possibly due to the effort and the contamination of the old saliva. Pressure and suction can be applied and regulated by the device shown in Fig. 2. A well of mercury (A) is attached to the pipette by the T-tube B mounted in a block of wood. The tube holding the spring clamp D leads to the pipette; the tube holding clamp E to the air. When pressure is desired, the bulb C is raised by pressing a foot treadle and the clamp D is opened. When suction is desired, the spring clamp E is opened and the bulb C is raised until the well A is nearly full of mercury. Then the clamp E is closed, the bulb C is dropped and suction is applied to the pipette by opening the clamp D.

CARL C. LINDEGREN

INSTITUTE OF PATHOLOGY
DR. R. R. MELLON, DIRECTOR
WESTERN PENNSYLVANIA HOSPITAL
PITTSBURGH, PA.

SPECIAL ARTICLES

INSECT TRANSMISSION EXPERIMENTS WITH HERPESENCEPHALITIS VIRUS[1]

THE recent demonstration by Kelser of the fact that the virus of equine-encephalomyelitis can be transmitted by *Aedes aegypti* suggested the possibility that some of the other neurotropic filterable viruses might likewise be transmissible either through this mosquito or through other species of insects. Also, in spite of the present uncertainty as to the etiological relationship between such viruses and epidemic human encephalitis, it was believed that insect transmission experiments might add to our limited information concerning the methods by which this disease is spread.

With this possibility in mind preliminary experiments were begun in April, 1933, using a laboratory-bred strain of *Aedes aegypti* and several neurotropic viruses including (a) the well-known E1–1–Perdrau strain of herpes-encephalitis virus isolated in 1925 from a human case of encephalitis, (b) a virus designated as "W," more recently isolated from an acute fatal human case of ascending paralysis, and (c) the Le Fevre strain of herpes virus originally isolated from a case of herpes genitalis. For the first two viruses we are indebted to Dr. F. P. Gay and Dr. M. Holden, of Columbia University, and for the other to Dr. E. B. McKinley, of George Washington University.

The various mosquito-transmission experiments have conformed to the following general plan: (1) An infective dose of the tissue containing the virus was inoculated into one or more normal animals. (2) On each succeeding day of the test these animals were immobilized and placed in a sterilized feeding cage containing 50 to 100 normal female *Aedes aegypti*. At the end of the feeding period all mosquitoes which failed to ingest blood were caught and destroyed; those remaining in the cage were counted, given a lot number and reserved for the transmission tests. (3) After arbitrarily selected intervals, ranging from 5 to 55 days, the potentially infected mosquitoes of each lot were tested by allowing them to feed on normal animals. These feeding tests were carried out in clean rooms never previously used for virus experiments; after being bitten, each test animal was placed in a sterilized cage and isolated throughout the entire period of its observation. (4) When an animal died the brain and in some instances the spinal cord was removed as soon as possible. Half of the brain was fixed in Zenker's solution or in formalin and prepared for histological study; a portion of the remaining half was used for transfer to other animals by subdural or intracutaneous inoculation; and the rest of the brain was preserved in 50 per cent. glycerin at 5° C.

At the present stage of the investigation we are unable to draw definite conclusions as to the transmissibility of these three viruses by *Aedes aegypti*. However, some of the results strongly suggest that this may have occurred; and it is to certain of these observations that we wish to call attention in this brief progress note.

The results obtained in one experiment with the E1–1–Perdrau virus are indicated diagrammatically in Chart I. On April 6 infective amounts of the virus were inoculated subdurally and intracutaneously into two normal rabbits (R–1 and R–2); both of which died 4 days later. Forty-eight and 72 hours after inoculation, normal *A. aegypti* were fed on both animals and these mosquitoes were designated as Lots 1B and 2B, respectively. The *A. aegypti* of Lot 1B, which had ingested blood of the inoculated rabbits at the end of 48 hours, were first tested on April 14, when five mosquitoes bit a normal rabbit (R–384), and again on April 21, when six fed on another (R–396). Both animals died; the former after 50 days and the latter after 16 days. Histological sections of the brain from one animal (R–384) were negative, while those from the other (R–396) contained lesions characteristic of encephalitis. Transfer of a suspension of the fresh brain of R–384, by intracutaneous inoculation into a normal rabbit (R–495) was followed by death 10 days later; and transfer of brain from R–396 to four normal rabbits (R–416,

[1] A preliminary note.

R–417, R–456 and R–457) was followed by the death of two of these animals after 17 and 32 days, respectively. The histological reports on the three animals which succumbed were negative.

The Lot 2B mosquitoes which had ingested blood from the inoculated rabbits at the end of 72 hours were tested on April 15, when seven bit a normal rabbit (R–390); on April 17, when four bit another (R–386); and again on April 24, when eight fed on a third normal rabbit (R–406). These test rabbits died after intervals of 12, 39 and 31 days, respectively; and one of them (R–390) became paralyzed in the hind legs six days before death. Histological examination of the brain of one of them (R–406) was negative. Suspensions of brain from each of these three animals were inoculated into normal rabbits. In a group of three animals which received the brain of R–390, one is alive, while two died after 36 and 65 days, respectively, and the histological examinations indicate that one of these had encephalitis. Of the three rabbits inoculated with the brain of R–386, two died after intervals of 76 and 17 days, and one of these was paralyzed nine days before death. The histological examination of the paralyzed animal has not been completed, but the brain of the other showed lesions of encephalitis. In the third group of rabbits, which was inoculated with brain from R–406, two lived and one died after an interval of 14 days; but no pathological lesions were found in the brain.

In addition to the feeding experiments outlined above, the *A. aegypti* of Lots 1B and 2B were tested for infectivity on April 26, by macerating in saline two mosquitoes from each lot, and injecting this suspension intracutaneously into a normal rabbit (R–408). This animal became paralyzed after 33 days and died on the 35th day. Histological examination of the brain was negative, but transfer of a saline suspension of the fresh brain by intracutaneous inoculation into another rabbit (R–491) was likewise followed by paralysis after 12 days and by death on the 14th day. The histological report on this animal was negative.

Thus, of the six rabbits used in testing the mosquitoes of Lots 1B and 2B, all died after periods of 12 to 50 days; and two were paralyzed before death. The brains of four animals have been examined histologically, and one of these showed the lesions of encephalitis. Of the fifteen animals inoculated with suspensions of brain tissue obtained from the mosquito-test rabbits, nine died after 10 to 76 days, two were paralyzed before death, and of the eight which have been examined histologically two showed lesions of encephalitis. It is of interest to note here that of

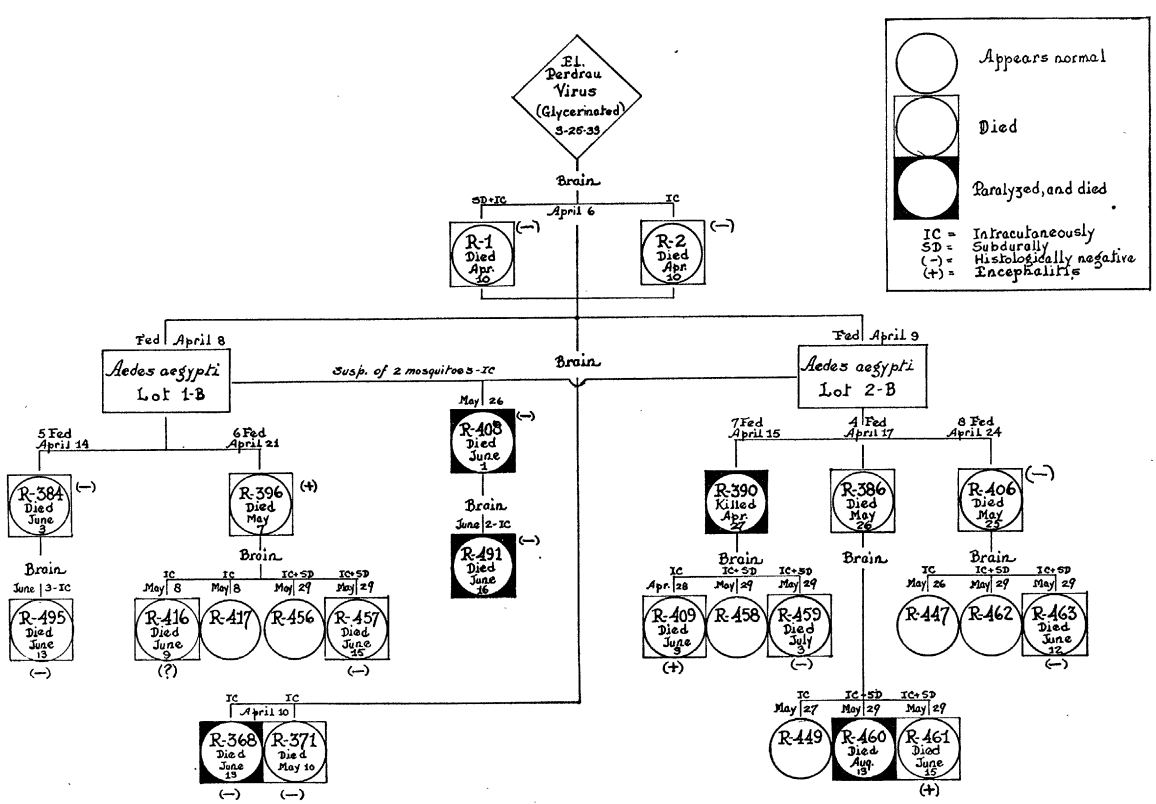

CHART I

seven rabbits inoculated either subdurally or intracutaneously with material known to contain original virus, all died after periods of 3 to 64 days; only one was paralyzed, and lesions of encephalitis were found in brain sections in only one, although all have been examined histologically.

In a second transmission experiment, begun on June 5, the Perdrau virus was inoculated subdurally and intracutaneously into three rabbits, which died after 3, 4 and 6 days, respectively. Large groups of normal *A. aegypti* fed on one or more of these rabbits 1, 2, 3, 4 and 5 days after inoculation, respectively, and mosquitoes of each of these lots were tested on normal rabbits after intervals of 8, 16 and 24 days. A total of 33 rabbits were bitten, of which 19 died after four to fifty-five days. None of these showed signs of paralysis. Brain sections have been examined from 16 of these animals and in two of these the diagnosis was encephalitis. Brain suspensions from the 19 mosquito-test animals were inoculated into 19 normal rabbits, of which 10 died after intervals of 2 to 11 days; while the remaining 9 are still being observed. Brain sections from four of these animals were negative in three instances but showed lesions of encephalitis in one. The mosquitoes used in this experiment (lots 1F to 5F inclusive) were again tested on August 22, when 470 of them fed on a normal monkey (*Cebus capucinus* No. 2). This animal showed no elevation of temperature and no paralysis, but died six days later on August 28. Brain sections of this monkey show lesions of encephalitis.

The Le Fevre virus has been used in two experiments. In one begun on April 1 the virus was inoculated subdurally and intracutaneously into three guinea pigs, two of which died 25 and 29 days later. At intervals of 2, 3, 4, 5, 6, 7, 8, 9 and 10 days following the inoculations, these three animals were exposed to groups of normal *A. aegypti*. The nine lots of mosquitoes were tested after intervals of 7 to 55 days by allowing them to feed on normal rabbits. Of the 15 animals bitten 14 died after periods of 11 to 50 days; and five were paralyzed before death. Brain sections from four of these rabbits have been examined, and in two there was evidence of encephalitis. Suspensions of brain tissue from the mosquito-test animals were transferred to 37 rabbits, of which 28 died after intervals of 6 to 71 days, and 6 of these were paralyzed before death. Five of six brains examined were negative and one was positive for encephalitis.

The second experiment with Le Fevre virus was begun on May 29, when the original virus was inoculated subdurally and intracutaneously into 3 normal rabbits, one of which died 13 days later, and brain sections show lesions of encephalitis. It appears that at least one of the survivors (R–477) was also infected, since a series of animals inoculated with 0.5 cc amounts of its blood drawn on successive days indicated that virus was present on the 1st, 2nd, 3rd, 7th and 9th days. The other two inoculated rabbits (R–471 and R–483) were exposed to large groups of mosquitoes at the end of 2, 3 and 4 days; and these 4 mosquito lots were each tested by feeding on 2 normal rabbits after intervals of 8, 16 and 24 days. Of the 27 animals bitten in these tests, 24 died after periods of from 5 to 64 days; and of these none was paralyzed. Sections of brain were examined from 23 of these mosquito-test rabbits, of which 20 were negative and 3 showed lesions of encephalitis. Suspensions of brain from the mosquito-test animals were inoculated into 24 normal rabbits, of which 19 have died within 2 to 24 days, and 2 of these were paralyzed before death. Brain sections from 10 of these were negative and one showed lesions of encephalitis.

The four lots of *A. aegypti* were again tested on August 23, when 377 mosquitoes bit a normal monkey (*Cebus capucinus* No. 3). At the time of this report the monkey is moribund and will be killed for further study.

The "W" virus, which has been used in only one transmission experiment on May 4, is unique in that following intracutaneous injection into rabbits it usually causes a paralysis of the extremities, followed by death on or about the eighth day. However, on histological examination of brain sections from 4 so infected, three have shown no lesions and the other contained lesions which were considered as suspicious but not diagnostic. Two normal rabbits were inoculated intracutaneously with this virus, and were later bitten by groups of normal *A. aegypti* after intervals of 1, 2, 3, 4, 5, 6, 7 and 8 days, respectively. The mosquitoes of each lot were tested by feeding on normal rabbits at the end of 7 days and again after 12 to 14 days. Of the 16 rabbits bitten, 11 died within 5 to 71 days, and none was paralyzed. Brain sections from only four rabbits have been examined, three being negative and one positive for encephalitis. Suspensions of brain have been transferred intracutaneously to 9 normal rabbits, of which 3 died within 3 to 33 days, and one was paralyzed before death. The brain sections from two rabbits have been examined histologically; one was negative and the other positive for encephalitis. The eight lots of mosquitoes were again tested on June 1, when a total of 90 bit a Cebus monkey (*Cebus capucinus* No. 1). This animal's temperature rose to 104 or more on the 11th and 21st days after the test, but otherwise it appeared normal until August 4, when it suddenly became weak, refused food and drink, was unable to raise its right arm above its head, and soon appeared to be moribund. X-ray examination failed to show any evidence of fracture or dislocation. Histologi-

TABLE 1

RESULTS OF TRANSMISSION EXPERIMENTS WITH NEUROTROPIC VIRUSES
Preliminary Summary

Viruses			El–1–Perdrau	Le Fevre	"W"	Total
I. Rabbits inoculated with original virus			7	9	5	21
Fatalities	Number of deaths		7	6	5	18
	Period of survival (days)		3–64	9–54	8–9	
	Paralysis		1	1	5	7
	Histological examination of brain	Not completed	0	0	1	1
		Negative	6	1	3	10
		Encephalitis	1	5	1(?)	7

II. Test rabbits bitten by potentially infected mosquitoes			Exp. 1	Exp. 2	Exp. 1	Exp. 2	16	97
			6	33	15	27		
Fatalities	Number of deaths		6	19	14	24	11	74
	Period of survival (days)		15–50	4–55	11–50	5–64	5–71	
	Paralysis		2	0	5	0	0	7
	Histological examination of brain	Not completed	2	3	10	1	7	23
		Negative	3	14	2	20	3	42
		Encephalitis	1	2	2	3	1	9

III. Rabbits inoculated with brain material from the test rabbits (II)			15	19	37	24	9	104
Fatalities	Number of deaths		9	10	28	19	3	69
	Period of survival (days)		10–76	2–11	6–71	2–24	3–33	
	Paralysis		2	0	6	2	1	11
	Histological examination of brain	Not completed	1	6	22	8	1	38
		Negative	6	3	5	10	1	25
		Encephalitis	2	1	1	1	1	6

cally the sections of brain and spinal cord were negative. Specimens of blood collected before death and suspensions of brain have been inoculated into normal rabbits, some of which have died and are now being studied.

In conclusion we wish to reemphasize the fact that the studies outlined in this report are not yet complete; and that additional work will be necessary to determine whether or not the tissues of those animals which died after being bitten by the test mosquitoes contain the specific viruses with which the respective experiments were initiated. It is also considered possible that, as in all extensive experiments with rabbits, some of the animals may have died of unrecognized extraneous causes. However, a large number of untreated control rabbits were observed during this period and only a few of these died with diarrhea, none of them showing paralysis. *Encephalitozoon cuniculi* was not found in the brain sections of any of the experimental animals.

As indicated in Table 1, irregular results were obtained with the rabbits of Group II which were bitten by the test mosquitoes, and this was also true of the animals in Group III which had received brain inoculations from the Group II rabbits. However, taken as a whole, they do not differ greatly from the results obtained in the rabbits of Group I, which were inoculated directly with the original viruses.

In view of the incidence of death and paralysis among the experimental animals, and particularly because of the nine rabbits and one monkey which showed the histological lesions of encephalitis after being bitten by the mosquitoes, we feel that the work already done strongly indicates that the viruses used have been transmitted by *A. aegypti*, but that further investigation is required to furnish absolute proof.

JAMES S. SIMMONS
RAYMOND A. KELSER
VIRGIL H. CORNELL[2]

ARMY MEDICAL SCHOOL
WASHINGTON, D. C.

BOOKS RECEIVED

CHAMBERLIN, ROLLIN T. and PAUL MACCLINTOCK. *College Text-Book of Geology:* Part I: *Geologic Processes and Their Results.* Second edition, revised. Pp. xi + 445. Holt. $3.00.

CHANT, C. A. and E. F. BURTON. *Text-Book of College Physics.* Pp. xiv + 541. 574 figures. Holt. $3.25.

HATFIELD, H. STAFFORD. *The Inventor and His World.* Pp. v + 269. Dutton. $2.40.

[2] With the technical assistance of Staff Sergeants George F. Luippold and Jesse F. Rhodes, Medical Department, U. S. Army.