aware of the androgenic potentialities of the mouse ovary(4,5).

Deanesly(6) and subsequent workers have extended Hill's original observations, finding an androgenic activity in the rat ovary. However, in no case has the rat exhibited the same quality of androgenic activity in its ovaries as that found in the mouse. Likewise the androgenic response in the mouse ovary is much more closely correlated with temperature changes than is the rat ovary. Too often it is assumed that, because of gross similarity, and sometimes a physiologic similarity, that those reactions occurring in one species will occur in the other species.

Summary. Two separate investigators, working in different laboratories at different times and with unrelated rat colonies have correlative data which shows, within the limits of the experiments, that spleen grafted ovaries will not noticeably extend the post adrenalectomy life of the host animal. The difference between the positive response in mice and the negative response in rats is noted. Histology of the grafted tissue was not correlated with the physiological response obtained.

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Interval Between Inoculations as a Factor in Interference between Neurotropic Viruses. (20623)

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In recent studies(1,2) reported from this laboratory it was found that 3- to 4-week-old rats, which are ordinarily highly susceptible to Western equine encephalomyelitis (WEE) virus, were much less susceptible to the virus, following nasal instillation, if St. Louis encephalitis (SLE) virus had been inoculated by the same route 72 hours previously. The purpose of the studies reported here was to determine the interval that must elapse between the intranasal inoculation of SLE virus and the subsequent inoculation of WEE virus for interference to occur and also to determine how long the interfering effect persisted.

Materials and methods. The 2 viruses used have been described previously(1). Two different methods were employed in preparing the different virus suspensions used. They were prepared from virus-infected brains of 2- to 4-week-old Swiss mice by either grinding the brains in a mortar with alundum and sufficient cold, inactivated, undiluted rabbit serum to make a 10% suspension of infected brains or by homogenizing the infected brains in a Waring blendor together with sufficient of the above diluent to make a 10% suspension. The suspensions were centrifuged at about 2000 RPM for 5 minutes to sediment gross particles. The supernatant fluid was removed, distributed in 2 ml amounts in screw-capped vials and "quick-frozen" by rolling the vials in a mixture of dry-ice and alcohol. The vials were then stored in a CO_2 cabinet until needed. Virus suspensions were titrated intracerebrally in 3- to 4-week-old Swiss mice after storage for different intervals in the CO_2 cabinet. Two suspensions of each of the 2 viruses were used. One suspension of SLE virus was titrated after 5 days storage at about —70°C and the second after 11 days. The LD/50 titer(5) in each case was $1 \times 10^{-8.5}$. One suspension of WEE virus was titrated 4 days after storage and the second

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TABLE I. Interval Required for Interference between St. Louis Encephalitis Virus and Western Equine Encephalomyelitis Virus. 18 rats in each series.

Material instilled into nose		Interval between SLE and WEE, hr	Interval between WEE and death (days) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Results	% mortality
WEE (controls)		—	0‡	0	13	4	0	0	1											18/18§	100
SLE*	WEE†	2	0	0	17	1														"	"
"	"	6	0	0	17	1														"	"
"	"	12	0	0	16	2														"	"
"	"	24	0	1	13	2	1													17/18	94
"	"	48	0	0	0	4	3	0	2	0	4	2								15/18	83
"	"	72	0	0	1	0	0	0	1	4	2	1	0	0	0	0	0	0	1	10/18	56
"	"	96	0	0	0	0	0	0	0	3	2	1	1	1						8/18	44

* SLE = St. Louis Encephalitis virus. † WEE = Western Equine Encephalomyelitis virus.
‡ Zero indicates no deaths that day. Other number indicates No. of deaths that day.
§ Numerator indicates No. of deaths; denominator, No. of animals tested.

after 9 days. The LD/50 titers were 1 x $10^{-7.8}$ and 1 x $10^{-8.8}$, respectively. Male Sprague-Dawley rats, 24 days of age and weighing 38 to 54 g, were used. All inoculations were made under light ether anesthesia. All rats were observed daily for at least 21 days following inoculation of WEE virus.

Experimental. Interval required for interference. 144 3- to 4-week-old rats were separated into 8 groups of 18 animals each. Seven of the groups received a single intranasal inoculation of 0.05 ml of a 10% suspension of SLE virus (LD/50 8.5). At the intervals indicated in Table I these animals were challenged intranasally with 0.05 ml of a 10% suspension of WEE virus (LD/50 8.8). One group of 18 animals received WEE virus as controls. The experiments were designed so that all 144 rats were inoculated with WEE virus on the same day. The data presented in Table I show that 100% mortality was observed in the control group and also in the groups which had received SLE at either 2, 6, or 12 hours before being inoculated with WEE virus. In the group which received SLE virus 24 hours before WEE virus was inoculated, only one animal survived. It would appear that the above SLE-treated rats were as susceptible to WEE virus as were the controls, since not only was the mortality essentially the same, but in addition, they succumbed to WEE virus as early as did the controls. In the group which received WEE virus 48 hours after the inoculation of SLE virus, while only 3 of 18 animals survived, it should be noted that the survival time was somewhat longer than that in the case of the controls. It has already been pointed out that when 3- to 4-week-old rats, which receive WEE virus intranasally 72 hours after having been inoculated with SLE virus by the same route, do succumb, they survive for a longer period than do control animals(1). It was only in the rats which had been inoculated with WEE virus at 72- and 96-hour intervals that a high degree of protection was noted. The data show that roughly 50% of the animals in these 2 groups were resistant to WEE virus. This finding confirms results already reported(1,2) in which it was noted that 50 to 65% of 3- to 4-week-old rats, which were inoculated intranasally with SLE virus and challenged 72 hours later by the same route with WEE virus, resisted infection with the latter virus.

Duration of interference. The purpose of this aspect of the studies was to determine how long rats which had had SLE virus inoculated intranasally would remain resistant to WEE virus subsequently inoculated by the same route. Twenty-seven 3- to 4-week-old rats were inoculated intranasally on each of 3 consecutive days with 0.05 ml of a 10% suspension of SLE virus (LD/50 8.5). Three inoculations were given since it has been shown that 3 daily intranasal inoculations give a higher degree of protection against nasally instilled WEE virus than that obtained by a single inoculation(2). Controls of the same age received 0.05 ml of a 10% suspension of normal mouse brain (NMB) on each of 3 consecutive days at the time the test

TABLE II. Duration of Interference between St. Louis Encephalitis Virus and Western Equine Encephalomyelitis Virus.

No. of rats	Material instilled into noses of rats	Interval between NMB or SLE and WEE, days	Interval between WEE and death (days) 1	2	3	4	5	6	7	8	9	10	Results	% mortality
11	NMB* × 3 WEE	10	0	0	3	5	1	0	0	1			10/11	91
14	SLE × 3 "	10	0	0	0	0	0	1	0	1			2/14	14
11	NMB × 3 "	21	0	0	8	3							11/11	100
13	SLE × 3 "	21	0	0	0	1	2	2	0	2	2	2	11/13	85

* NMB = Normal mouse brain.

animals received their 3 inoculations of SLE virus. On the 10th day following the third inoculation of SLE virus, 14 of the SLE-treated animals received 0.05 ml of a 10% suspension of WEE virus (LD/50 7.8) intranasally. The remaining 13 SLE-treated rats were inoculated intranasally with 0.05 ml of a 10% suspension of WEE virus (LD/50 7.8) 21 days after the third inoculation of SLE virus. Eleven control animals were used for each group of test animals. The results (Table II) indicate that the interfering effect persisted for at least 10 days, since only 2 of the 14 animals proved to be susceptible to the WEE virus, while 10 of the 11 controls succumbed. The interfering effect did not persist until the 21st day, however, since rats tested 21 days after SLE virus had been inoculated intranasally were highly susceptible to WEE inoculated by the same route in that 11 of the 13 animals tested succumbed.

Discussion. From the data presented it is evident that at least 72 hours must elapse between the inoculation of the SLE virus and the subsequent inoculation of the WEE virus before a significant degree of interference can be observed. When SLE virus is instilled into the noses of normal 3- to 4-week-old rats it invades the CNS by the olfactory pathway and can be detected in the anterior rhinencephalon 24 hours later(1,3,4). However, virus at this time is present in low concentration in this part of the CNS. It would appear for nasally instilled SLE virus to interfere with the passage of WEE virus along the olfactory pathway that it must first pass along this pathway and then undergo multiplication particularly in the anterior rhinencephalon. The evidence suggests that the type of interference described here is not dependent alone upon the presence of the SLE virus in the CNS, since it has been shown that SLE virus, reaching the brain following nasal instillation, did not protect rats against WEE subsequently injected directly into the brain(1).

That the interference between SLE virus and WEE virus is not based on specific immunity is evident from data presented which show that while the interfering effect of SLE persists for at least 10 days, it cannot be demonstrated 21 days following the inoculation of the interfering virus.

Summary. St. Louis encephalitis virus, following nasal instillation, did not protect rats from Western equine encephalomyelitis virus inoculated 2, 6, 12, and 24 hours later by the same route. If the equine encephalomyelitis virus was inoculated 48 hours after the St. Louis encephalitis virus, some interference was noted since these animals survived longer than did controls. A high degree of protection against equine encephalomyelitis virus was observed in rats that had had St. Louis encephalitis virus inoculated intranasally 72 and 96 hours before the equine encephalomyelitis virus was inoculated by the same route. Rats inoculated intranasally with St. Louis encephalitis virus are resistant to nasally instilled equine encephalomyelitis virus for as long as 10 days after the former virus is inoculated, but by the 21st day this resistance disappears.

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