Mixed Infections with Coxsackie and Lansing Poliomyelitis Viruses in Mice.* (20585)

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It is already known that under certain circumstances 2 viruses may propagate simultaneously in one host and in the same organ or tissue, each presumably utilizing individual metabolic pathways(1-4). Numerous studies have also shown that under certain experimental conditions one virus may exert an interfering or sparing effect on the other(5-7), or one agent may exalt the virulence of another(8-10). This preliminary report is concerned with certain reciprocal effects observed in mice infected with both Coxsackie and poliomyelitis viruses.

Materials and methods. The 2 group B C virus strains,‡ Ohio-R (type 2) and Nancy (type 3), used in these experiments, have been maintained by passage in 2-day-old Swiss albino mice and the Lansing poliomyelitis virus,§ has been maintained by passage in adult white Swiss mice. The infectivity titer of the stock Lansing virus prepared from cords and medullae from animals which succumbed to the infection within 5 days was $10^{-3.8}$ LD_{50}. Each stock C virus suspension consisted of 20% brain or carcass from infant mice which had become paralyzed following subcutaneous inoculation. The quantity of virus present was determined by subcutaneous inoculation of randomized 2-day-old suckling mice and the 50% end-point (LD_{50}) was calculated according to the method of Reed and Muench (11). When the intraspinal route of inoculation was used the technic of Habel and Li(12) was employed. Animals which died or were found dead within 24 hours after inoculation of the Lansing virus were excluded from the analyses and were considered to be deaths from trauma.

All animals were observed twice and sometimes 3 times daily during the observation period of 30 days for extent of paralysis and deaths from poliomyelitis or C virus infection. Swiss albino mice (CFW) were used throughout these studies.

Experiments with the Ohio-R C virus and the Lansing Virus. Each animal in one group of seventy-nine 4-week-old mice received one intracerebral inoculation of 10,000 LD_{50} of C virus 5 days prior to an injection of the Lansing virus (150 LD_{50}). The 5-day interval used in this experiment is presumed to represent the time required for the blocking virus (Ohio-R) to propagate to the necessary level in the tissue of predilection of the virus which is to be excluded. Normal infant mouse carcass tissue was substituted for C virus in a second group of 81 mice. Each animal in a third group of 30 mice received an injection of C virus followed 5 days later with an injection of normal adult mouse brain tissue. Brain-cord suspensions from animals which died without previous evidence of paralysis and which had received both viruses were tested for C virus by intraperitoneal inoculation of 2-day-old mice and for Lansing virus by intracerebral inoculation of 3-week-old mice.

The results summarized in Table I show that this strain of C virus influenced significantly both the course and outcome of the experimental Lansing infection. The sparing effect became evident within 4 days after injection of the Lansing virus and persisted throughout the course of the experiment. When the experiment was terminated after an observation period of 30 days less than 10% of the controls which had received Lansing virus alone had survived as compared with 36.7% of those which had been inoculated with both viruses. This difference is highly

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TABLE I. Mixed Infections with Coxsackie Virus (Ohio-R Strain) and Lansing Poliomyelitis Virus in Adult Mice.*

No. of mice		79				81		
Procedure		C virus (Ohio-R) and Lansing virus†				Normal infant mouse carcass tissue and Lansing virus‡		
		Polio deaths		Deaths without paralysis§		Polio deaths		
Results		No.	Following paralysis	Polio deaths	C virus deaths	No.	Following paralysis	Without paralysis
Animals infected and dying from poliomyelitis or C virus infection (days after inj. of Lansing virus)	3	2 (2.5)‖		2	1	1 (1.2)		1
	4	8 (12.6)	5	3	1¶	19 (24.7)	17	2
	5	7 (21.5)	6	1		18 (46.9)	18	
	6	3 (25.3)	2	1	1	6 (54.3)	5	1
	7	7 (34.2)	6	1	1**	4 (58.3)	4	
	8	6 (41.8)	4	2	1	0 (58.3)		
	9	3 (45.5)	3			4 (64.2)	2	2
	10	2 (49.9)	1	1		4 (69.2)	3	1
	11-13	5 (53.1)	4	1		10 (81.4)	9	1
	14-16	4 (59.5)	3	1		5 (88.6)	5	
	17-20	2 (62.0)	1	1		2 (90.1)	2	
	21-25	1 (63.3)	1			0 (90.1)		
	26-30	0 (63.3)				0 (90.1)		
Survivors		36.7%				9.9%		

* Unless otherwise indicated all inoculations were made i.c.; inoculum 0.03 ml.
† One inj. of C virus (10000 LD_{50}) 5 days prior to an inj. of Lansing virus (150 LD_{50}).
‡ " " " normal infant mouse carcass (0.03 ml of a 1.0% suspension) instead of C virus.
§ Brain and cord tested for C virus in suckling mice (i.p.) and Lansing virus in 3-wk-old mice (i.c.).
‖ Figures in parentheses indicate cumulative % deaths.
¶ Both Lansing and C virus demonstrated in this brain-cord suspension.
** C virus LD_{50} titer $10^{-4.8}$.

significant ($p = <.0001$). Similar results with the Conn.-5 and Nancy strains of group B C viruses have been observed by Dalldorf(6). More recently, Stanley(6) reported that certain group B C viruses isolated in Sydney, Australia prolonged the incubation period of MEF_1 poliomyelitis virus infection in adult mice.

On the basis of tests for both viruses there was evidence that Ohio-R strain proliferated in the CNS of some of the animals which had also received Lansing virus. At least 5 of the deaths without previous evidence of paralysis were considered to be due to C virus because a 10^{-3} brain-cord suspension produced characteristic C virus infection in suckling mice following intraperitoneal inoculation and failed to produce infection in adult mice following intracerebral inoculation. In one instance the C virus LD_{50} titer was $10^{-4.8}$ and in another both Lansing and C viruses were demonstrated in the CNS. In many instances it was possible to distinguish between the two experimental infections; the symptoms resulting from C virus infection consist of roughened coat, hunching, tremors and spasticity, while in the case of Lansing infections, aside from flaccid paralysis of one or more limbs, the animals have a smooth coat and appear healthy. None of the 30 adult mice which had received massive doses of C virus followed by injections of normal mouse brain showed evidence of virus proliferation in the CNS. Aside from the evidence, then, that the Ohio-R strain of C virus exerts a sparing effect in adult mice infected with Lansing virus, the latter seems to enhance the virulence of this C virus for these otherwise insusceptible animals. Experiments to determine the extent to which Lansing and other infections in adult mice increases the susceptibility in such anmals to C virus infections are still in progress.

Experiments with the Nancy virus and the Lansing virus. All inoculations in this experiment were made intracerebrally. Each animal in one group of forty-four 4-week-old mice received one injection of 500,000 LD_{50} of Nancy virus 5 days prior to an injection of approximately 10 LD_{50} of Lansing virus. The animals in a second group received normal

TABLE II. Effect of Coxsackie Virus (Nancy Strain) on Course and Incidence of Disease in Adult Mice Infected with Lansing Poliomyelitis Virus.*

Procedure / Results		C virus (Nancy) and Lansing virus†				Normal infant mouse carcass tissue and Lansing virus‡			
			Limb paralyzed first				Limb paralyzed first		
		No.	Fore-limb	Hind-limb	Without paralysis	No.	Fore-limb	Hind-limb	Without paralysis
Exp. 1			44 mice				70 mice		
Animals infected and dying from poliomyelitis (days after inj. of Lansing virus)	3					2 (2.8)	1		1
	4					6 (11.4)	5		1
	5	2 (4.5)§		2		7 (21.4)	6	1	
	6	2 (9.1)	1	1		1 (22.8)	1		
	7	2 (16.6)	1	1		2 (25.6)	2		
	8	3 (20.5)	2	1		2 (28.5)	2		
	9	1 (22.5)		1		4 (34.2)	3	1	
	10-13	1 (25.0)		1		2 (37.1)	1		1
	14-17	2 (29.5)	1		1	6 (45.7)	5		1
	18-30	0 (29.5)				0 (45.7)			
Survivors			70.5%				54.3%		
Limb paralyzed first¶									
Forelimb			41.7%				92.9%		
Hindlimb			58.3%				7.1%		
Ratio Forelimb/Hindlimb			0.7				13		
Exp. 2			56 mice				51 mice		
Animals infected and dying from poliomyelitis (days after inj. of Lansing virus)	3					1 (1.9)			1
	4	5 (8.9)	4	1		13 (27.4)	9	3	1
	5	8 (23.2)	3	5		8 (43.1)	8		
	6	6 (33.9)	1	5		4 (50.9)	3		1
	7	9 (50.0)	6	3		1 (52.9)	1		
	8	4 (57.1)	3	1		0 (52.9)			
	9	5 (66.1)	4	1		2 (56.8)			2
	10-13	1 (67.9)	1			11 (78.4)	9		2
	14-17	1 (69.6)	1			4 (86.2)	3	1	
	18-30	0 (69.6)				0 (86.2)			
Survivors			30.4%				13.8%		
Limb paralyzed first¶									
Forelimb			58.9%				90.0%		
Hindlimb			41.1%				10.0%		
Ratio Forelimb/Hindlimb			1.4				8.2		

* All inoculations were made i.c.; inoculum 0.03 ml.
† One inj. of C virus (500000 LD_{50}) 5 days prior to an inj. of Lansing virus (10 LD_{50}).
‡ One inj. of normal infant mouse carcass tissue (0.03 ml of a 1.0% suspension) instead of C virus.
§ Figures in parentheses indicate cumulative % deaths.
‖ 150 instead of 10 LD_{50} of Lansing virus.
¶ Percentages calculated on the basis of those that died following paralysis.

infant mouse carcass tissue instead of C virus and served as controls. Each of 25 mice in still another group received massive doses of C virus followed by an injection of normal adult mouse brain tissue. The results of this experiment summarized in Table II (Exp. 1) indicate that the sparing effect which was evident early in the course of this experiment became less conspicuous as the experiment progressed. When the study was completed the difference in survival rates among controls as compared with those which had received both viruses was not statistically significant. None of the animals which had received large doses of C virus followed by normal mouse brain showed evidence of infection, and the Lansing virus failed to alter the susceptibility of the adult mice to the Nancy infection. The Nancy virus, however, seemed to have a noticeable effect on the pathogenesis of the Lansing virus infection. Generally, forelimb paralysis occurs first in a large proportion of animals inoculated with Lansing virus(13,14). In these studies 93% of the animals receiving

TABLE III. Influence of Route of Inoculation of Coxsackie Virus (Nancy Strain) on Course and Incidence of Disease in Adult Mice Infected with Lansing Poliomyelitis Virus.

	No. of mice	68				56			
	Procedure	C virus (Nancy) and Lansing virus*				Normal infant mouse carcass tissue and Lansing virus†			
			Limb paralyzed first				Limb paralyzed first		
	Results	No.	Fore-limb	Hind-limb	Without paralysis	No.	Fore-limb	Hind-limb	Without paralysis
Animals infected and dying from poliomyelitis (days after inj. of Lansing virus)	3	4 (5.9)‡			4	2 (3.6)			2
	4	2 (8.9)			2	2 (7.2)	2		
	5	8 (20.6)	4	2	2	6 (18.0)	4	2	
	6	8 (32.3)	8			4 (25.2)	4		
	7	2 (35.3)	2§			2 (28.6)	2		
	8	0 (35.3)				2 (32.2)	2		
	9	2 (38.2)		2		2 (35.9)	2		
	10-13	10 (52.9)	10			10 (53.6)	10		
	14-20	8 (64.7)	2	2	4	16 (82.2)	16		
	21-30	8 (76.4)	8			10 (100.0)	10		
Survivors		23.6%				0			
Limb paralyzed first‖									
Forelimb		85.0%				96.3%			
Hindlimb		15.0%				3.7%			
Ratio Forelimb/Hindlimb		5.7				26			

* One i.s. inj. of C virus (500000 LD_{50}) 6 days prior to i.c. inj. of 150 LD_{50} of Lansing virus.
† One i.c. inj. of 150 LD_{50} Lansing virus 6 days after i.s. inj. of normal infant mouse carcass tissue (0.03 ml of 1.0% suspension).
‡ Figures in parentheses indicate cumulative % deaths.
§ One of these animals survived 15 days after first evidence of paralysis.
‖ Percentages calculated on the basis of those that died following paralysis.

Lansing virus alone developed forelimb paralysis first, while this occurred in only 41.7% of those which had received both viruses. In the group of animals which had received both viruses the frequency with which the forelimbs became paralyzed first as compared with the frequency of hindlimb paralysis appearing first showed a ratio of 0.7 to 1. In the control group this ratio was 13 to 1. It is conceivable that the C virus exerts its effect in the higher level of the CNS by "forcing" the Lansing virus to descend the spinal cord to those areas where innervation of hindlimb muscles arises. When this experiment was repeated at a later date with a somewhat larger challenge dose of Lansing virus (150 LD_{50}) essentially the same results were obtained (Table II, Exp. 2).

Another experiment was designed to determine the influence of route of inoculation of the Nancy virus on the pathogenesis of the Lansing virus infection. Each adult mouse in one group received one intraspinal injection of Nancy virus (500,000 LD_{50}) 6 days prior to an intracerebral injection of 150 LD_{50} of Lansing virus. Normal mouse carcass tissue was substituted for C virus in another group of mice, while still another group of mice received an intraspinal injection of C virus followed by an intracerebral injection of normal mouse brain tissue. The results of this experiment are summarized in Table III. No blocking effect was evident for at least 15 days following the injection of Lansing virus. When the experiment was terminated, however, none of the controls survived while 23.6% of those animals which had received both viruses survived; a difference which is statistically significant ($p = .006$). Calculated on the basis of those which died following the development of paralysis, over 96% of the animals receiving Lansing virus alone developed forelimb paralysis first, while this occurred in 85% of those which had received both viruses. In the control group the frequency with which the forelimbs became paralyzed first as compared with the first appearance of hindlimb paralysis showed a ratio of 26 to 1. In the group which had received both viruses this ratio was 6 to 1. Not detailed in the tabulation is the fact that the progress of the infection in the control group followed the usual course with development of hindlimb paralysis in a large

proportion of animals prior to death. Many of the animals which had received a previous intraspinal injection of C virus failed to develop hindlimb paralysis prior to death. This would suggest that the presence of C virus in the lumbar region of the spinal cord limits extension of the Lansing virus thus altering the normal progress of this experimental infection. Experiments designed to determine the validity of this concept are in progress. None of the adult mice which had received an intraspinal inoculation of C virus followed by an intracerebral injection of normal brain tissue showed evidence of virus proliferation. There was no evidence of exaltation of the Nancy strain as demonstrated previously with the Ohio-R virus. In addition to influencing the survival rate of Lansing infection in mice, the intraspinal inoculation of the Nancy virus had a definite effect on the survival time after paralysis was first observed. Animals which had received both viruses survived, on the average, twice as long as those which had received Lansing virus alone. In one instance an animal which had received both viruses developed forelimb paralysis 7 days after the injection of the Lansing virus and died 15 days later. Another animal which had received both viruses developed forelimb paralysis 22 days after the injection of the Lansing virus and was sacrificed over 6 months later.

In addition to the extreme variability in the interval between the intracerebral inoculation of the Lansing poliomyelitis virus and the onset of paralysis, a biphasic distribution of incubation periods occurs with some regularity (14-16). In the present experiments‖ the biphasic distribution of incubation periods was evident only among those animals which had received Lansing virus alone. Neither of the groups of mice which had received intracerebral injections of the Ohio-R or Nancy viruses prior to the Lansing inoculation showed evidence of biphasic distribution of incubation periods.

Summary. The following reciprocal effects have been observed in mice infected with viruses which do not possess the same degree of pathogenicity for the host. 1. The Ohio-R strain of C virus influenced significantly both the course and outcome of experimental Lansing poliomyelitis infections in 4-week-old white Swiss mice. 2. In the presence of the Lansing infection the virulence of the Ohio-R virus seemed to be enhanced for these otherwise insusceptible animals. 3. The Nancy strain of C virus had a noticeable effect on the pathogenesis of the Lansing infection in 4-week-old mice. The frequency with which the forelimbs became paralyzed first as compared with the first appearance of hindlimb paralysis was influenced by the presence of Nancy virus in the higher levels of the CNS. The presence of Nancy virus in the lumbar region of the spinal cord, following intraspinal inoculation, seemed to limit extension of the Lansing virus thus altering the normal progress of this experimental infection. 4. There was no evidence of enhancement of the virulence of the Nancy virus in the presence of the Lansing infection. 5. In addition to exerting a sparing effect on the Lansing infection, animals which had received a previous intraspinal inoculation of Nancy virus survived twice as long as those which had received Lansing virus alone. 6. The biphasic distribution of incubation periods generally observed among animals infected with the Lansing virus was not evident among those animals which had received both viruses.

‖ CFW mice were used in the experiments because they show less variability than other Swiss mice in their reaction to the Lansing virus(17).

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Comparative Protective Effect of Cysteine against Fast Neutron and Gamma Irradiation in Mice. (20586)

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There are numerous examples of protective effects by hypoxia and chemicals in radiation biology(1) and, for the most part, one is prone to interpret these phenomena in terms of the postulated energy transformations in irradiated water. Perhaps the most cogent evidence for this sort of mechanism is derived from comparison of the modifying influence of oxygen and of various substrates on the radiation-induced reactions in simple aqueous solutions and in living systems. The observations of Thoday and Read(2,3) and, more recently, of Giles *et al.*(4) are particularly significant. The former found a negligible effect of oxygen deprivation on growth reduction and chromosome aberrations in *Vicia faba* roots irradiated with alpha particles, while the latter observed an intermediate protective effect of hypoxia on aberration frequency in *Tradescantia* inflorescences exposed to fast neutrons. In each case, oxygen deprivation afforded considerable protection against the effects of gamma or X-irradiation. These findings parallel certain of the radiochemical reactions involving oxygen in aqueous solution and will be discussed in connection with the data presented here. The present experiments are concerned with a comparison of the protective efficiency of cysteine against the acute lethal effects of fast neutron and gamma irradiation in mice. Fast neutrons have been estimated to be some 4.5 times as effective as gamma rays for acute killing of mice(5). In view of the radiobiological importance of ionization density and of its contribution to chemical effects in water, useful information regarding radiation mechanisms may be gained from such comparisons of protective efficiency.

Methods. Female mice (CF1) 10 to 12 weeks of age and weighing 18-25 g were maintained on a diet of Rockland checkers and water *ad libitum*. Control and experimental animals were irradiated simultaneously, caged together in groups of 10 to 15 and otherwise handled similarly. Cysteine was administered as a 12.5% solution of the hydrochloride neutralized to pH 7 with 10 N sodium hydroxide. Only freshly prepared solutions were employed. The cysteine dose was 1200 mg/kg of body weight and was given intravenously. Control mice were injected with an equivalent volume of 5% sodium chloride. In general, cysteine was injected between 5 and 15 minutes before exposure. The exposure time varied from 80 to 90 minutes for gamma rays and 60 to 90 minutes for neutrons. The mice were exposed in the gamma-neutron radiation chamber recently described(6). The dose rate of gamma rays from the Co^{60} source was about 11-12 r per minute. Fast neutrons were produced by allowing the neutrons from the thermal column of the Argonne CP-3 reactor to impinge on uranium. Appropriate shielding devices prevented slow neutrons from reaching the animal exposure position. Several methods of dosimetry indicate that the gamma