THE SPARING EFFECT OF COXSACKIE VIRUS INFECTION ON EXPERIMENTAL POLIOMYELITIS

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Coxsackie viruses are frequently found in the throat washings or feces of patients having the symptoms of poliomyelitis (1–4). On numerous occasions both poliomyelitis and Coxsackie viruses have been found in the same specimens (4–6) and the simultaneous appearance of humoral antibodies for both has been reported (7). It is not known whether the two influence one another, whether the Coxsackie virus infection mitigates or perhaps intensifies the effects of poliomyelitis. The present report shows that combined infections, in the mouse, have a sparing effect on the course and outcome of poliomyelitis.

Materials and Methods

The Lansing strain of poliomyelitis virus was originally supplied by Dr. Charles Armstrong. That of the 104th mouse generation was used in the present experiments. Pooled mouse brains were suspended in salt solution plus 10 per cent infusion broth, clarified by centrifugation, dispensed in screw-top containers, and held at −70°C. until used. The "Nancy" strain of Coxsackie virus was used in the experiments reported in detail. It was isolated and described by Melnick and Kraft (8). By our criteria it is a typical Group B virus but differs in neutralization tests from our other Group B strains and has been tentatively classified as Type 3. The Nancy strain was chosen because of its relatively low virulence when received as virus of the 4th mouse generation. Later experiments showed that Type 1, Group B virus also exerts a sparing effect on poliomyelitis and could be freely substituted for the Nancy strain.

The mice were of the Albany standard strain. The colony is known to be latently infected with TO mouse encephalomyelitis virus. All the animals required for a given experiment were pooled and redistributed by random sampling.

Several factors are of decisive importance in interference experiments involving poliomyelitis and Coxsackie viruses. The susceptibility of mice to both viruses varies with age. Mature mice are completely resistant to Coxsackie virus infection and suckling mice are somewhat less susceptible than adults to poliomyelitis (9). The incubation period of poliomyelitis in immature mice is prolonged (10). Furthermore, time is a critical factor in most interference experiments involving living animals. The experiments were therefore designed to provide different intervals between the administration of the two viruses in mice of several ages and to provide a group of poliomyelitis controls of the same age as each experimental group when challenged with poliomyelitis virus.

The Coxsackie viruses were given intraperitoneally or subcutaneously; the poliomyelitis virus, intracerebrally. The dose of the former was 0.03 ml. of 0.1 or 10 per cent infected mouse

brain suspension. The poliomyelitis virus dose was 0.02 ml. of a 1 or 10 per cent infected mouse brain suspension. The animals were observed for 21 days following the last inoculation.

There has been no difficulty in distinguishing between the illnesses caused by the two agents, and signs suggesting combined disease have seldom been seen. The criterion of poliomyelitis has been the characteristically abrupt appearance of flaccid paralysis in an otherwise sleek, healthy appearing mouse. The signs of Group B Coxsackie virus infection are a roughened coat, stunted growth, fine tremors, or spasticity.

Occasionally a mouse inoculated with poliomyelitis virus was found to have died without signs of poliomyelitis having been recognized on the previous day. This was more common in the experimental groups. In these cases signs of Coxsackie virus infection had usually been noted at some time and, when histologic examination was possible, lesions of Coxsackie virus infection were found.

RESULTS

The results of all the experiments have been similar. Two have been summarized in Tables I and II. The individual records of the younger group shown in Table I are shown graphically in Fig. 1. It is evident that previous infection with Coxsackie virus, Group B, exerts a significant sparing effect on poliomyelitis induced from 4 to 8 days following the inoculation of the Coxsackie virus. The effect is evident in the survival rate, the prolongation of life among the mice that do succumb to poliomyelitis, and the number that develop paralysis. The effect is mutual and the experimental groups inoculated at the most favorable times fare better than either group of controls.

Several experiments have been summarized in Table III to illustrate the apparent effect of age and the duration of the interval between inoculations. Mice 5 to 8 days old at the beginning of an experiment, which were inoculated with poliomyelitis virus from 4 to 10 days later, survived in 45 per cent of the cases. Only one-fifth manifested paralysis in contrast to a paralysis attack rate of 97 per cent among the controls. The deaths due to Coxsackie virus infection were fewer than half those among the Coxsackie virus controls. The second group consisted of mice of the same age but that had received poliomyelitis virus simultaneously or before the 4th day. Of these only 17 per cent survived; more than half were paralyzed. The third group consisted of older mice; that is, mice from 9 to 13 days of age when first inoculated. Mice of this age are less responsive to Coxsackie virus infection, the disease is less uniform, the signs of infection irregular. None of the animals survived; 60 per cent became paralyzed. It is probable that several of the non-paralytic deaths listed were actually due to poliomyelitis, which runs a brief course in older animals, paralysis and death often occurring within 24 hours.

TABLE I

Interference of Coxsackie Virus Infection with the Course and Outcome of Experimental Poliomyelitis

Age of mice at onset	Interval between inoculations	No. of mice in group	Died following paralysis			Died with signs of Coxsackie virus infection	Survived	
			No.	Per cent	Average duration of life		No.	Per cent
days	*days*				*days*			
5	0	8	6	75.0	8.5	1	1	16.6
	Polio controls	8	8	100.0	4.6			
	4	8	3	37.5	14.0	3	2	25.0
	8	8	2	25.0	19.5	2	4	50.0
	Polio controls	6	6	100.0	8.6			
	12	5	3	60.0	12.3	1	1	20.0
	Polio controls	8	7*	87.5	4.4			
	Coxsackie controls	15				15	0	
9	0	8	8	100.0	8.6			
	4	8	5	62.5	9.6	3	0	
	Polio controls	6	6	100.0	8.6			
	8	8	3	37.5	12.0	5	0	
	Polio controls	8	7*	87.5	4.4			
	12	6	2	33.3	9.0	1	3	50.0
	Polio controls	10	9*	90.0	7.2			
13	0	7	5	71.4	10.2	2	0	
	Polio controls	6	6	100.0	8.6			
	4	8	3	37.5	5.3	5	0	
	Polio controls	8	7*	87.5	4.4			
	8	7	5	71.4	9.2	2	0	
	Polio controls	10	9*	90.0	7.2			
	12	5	3	60.0	6.6	2	0	
	Polio controls	10	3‡	30.0	6.0			

* In each of these groups one mouse died without evidence of paralysis.

‡ Seven mice died of unknown causes.

The average duration of life among the mice of the experimental groups that eventually died following paralysis proved to be 12 days for the 5 day

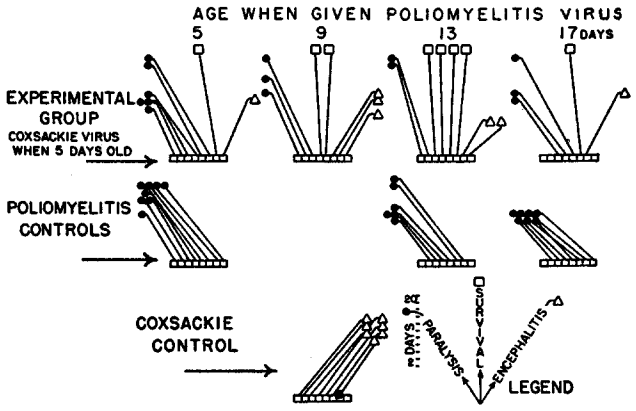

FIG. 1. Interference of Coxsackie virus infection with experimental poliomyelitis.

TABLE II

Interference of Coxsackie Virus Infection with the Course and Outcome of Experimental Poliomyelitis

Interval between inoculations	No. of mice in group	Died following paralysis			Died with signs of Coxsackie virus infection*	Survived	
		No.	Per cent	Average duration of life		No.	Per cent
days				*days*			
0	7	5	71.4	9.4	2	0	
Polio controls	7	7	100.0	8.4			
2	7	4	57.2	15.5	2	1	14.3
Polio controls	7	7	100.0	9.4			
4	7	3	42.8	17.6	1	3	42.8
Polio controls	7	7	100.0	7.7			
6	6	0			1	5	83.6
Polio controls	8‡	7	100.0				
8	6	0			4	2	33.3
Polio controls	6	5	83.4			1	16.6
Coxsackie controls	14				8	6	42.8

The Coxsackie virus (Group B, Type 3) was given subcutaneously in 0.03 ml. amounts. Lansing poliomyelitis virus was inoculated intracerebrally. The dose was 0.02 ml. of 10 per cent mouse brain suspension. The mice were 5 days old when the experiment was begun.

* May include deaths due to other causes.

‡ One death in this group without observed paralysis.

group, 9.5 days for the 9 day group, and 8.3 days for the 13 day old group. In each of the three groups duration was longer than among the poliomyelitis controls of the same ages. The ratio of duration among experimental and control mice was 2.1 for the 5 day mice, 1.4 for the 9 day old mice, and 1.2 for the 13 day old animals.

TABLE III

The Influence of Age and Interval on the Coxsackie-Poliomyelitis Interference

Age of mice when inoculated with Coxsackie virus	Interval between the two inoculations	No. of mice	Outcome					
			Died				Survived	
			Paralyzed		Not paralyzed			
			No.	Per cent	No.	Per cent	No.	Per cent
days	*days*							
5–8	4–10	72	14	19	23	32	35	49
5–8	0–4	36	20	55	10	28	6	17
9–13	4–10	37	22	60	15	40	0	
4–7	Coxsackie controls	52			37	71	15	29
Corresponding	Poliomyelitis controls	141	137	97			4	3

DISCUSSION

The experiments closely resemble those in which lymphocytic choriomeningitis protected *rhesus* monkeys against poliomyelitis (11, 12). In both, the interval between inoculations proved to be important and, in both, the effect was mutual. In the experiments using monkeys, many animals became paralyzed but recovered. Recovery has not been seen in our mice. All paralyzed mice have died although some have survived for as long as 2 weeks. In the first lymphocytic choriomeningitis-poliomyelitis experiments few deaths were caused by the choriomeningitis virus. Later the strain became more virulent. The effect on poliomyelitis in both cases was essentially the same. In the present work a comparison between benign and malignant forms of Coxsackie virus infection has not been possible.

During 1947–1949 our isolations of Coxsackie viruses have been more frequent during times and in places where paralytic poliomyelitis has been less frequent (1). This may mean that certain outbreaks have consisted of cases of Coxsackie virus infection as well as cases of poliomyelitis. It may mean that the two interfere and that poliomyelitis is less often paralytic when Coxsackie virus infection is superimposed. Since few tests for poliomyelitis virus have been made, a conclusion is not warranted.

Search for evidence of interference in man may prove to be a formidable

problem. If an interference occurs, the difficulties in isolating the viruses could well be extreme, for their infectivity may be diminished. Another hazard was suggested by Rhodes and Chapman (13) in discussing the protection against MM virus infection provided by lymphocytic choriomeningitis. They pointed out that the effect persisted to a measurable degree for 30 days, longer than the presence of virus could be demonstrated. This was true of the lymphocytic choriomeningitis-poliomyelitis interference in monkeys as well. In the present experiments no effort was made to determine whether infectivity disappeared before the sparing effect wore off, but in view of the persistence of a measurable, though slight, effect in the older age groups after the longest intervals, it seems likely. Unfortunately, neither poliomyelitis nor Coxsackie virus infection is well adapted to serologic diagnosis and a serologic study also seems impractical.

Howitt and Nichols reported that mixtures of Coxsackie and poliomyelitis viruses were given to *rhesus* monkeys without evidence of interference (14). This may have been due to the resistance of *rhesus* monkeys to Coxsackie virus infection. Melnick also found no evidence of interference between the two viruses (15). Chimpanzees infected orally with Coxsackie virus were fully susceptible to poliomyelitis virus given a short time later. Three week old mice that had recovered from Coxsackie virus infection were not resistant to poliomyelitis. This agrees with our own experience. Melnick's experiments in which immature mice were used involved challenge with poliomyelitis virus after 1 hour, 1 or 18 days. Sulkin and Manire reported that the inoculation of Lansing poliomyelitis virus into 3 day old mice protected some against Coxsackie virus infection (16). The reverse was not demonstrated. A Group A strain of virus was used.

SUMMARY

Young mice infected with Group B Coxsackie virus were rendered markedly resistant to poliomyelitis virus given from 4 to 10 days later. A sparing effect was detectable in somewhat older mice and in young mice inoculated with poliomyelitis after shorter intervals, but in both cases few mice survived.

Interference was manifest by survival, by prolongation of the course of poliomyelitis, and by a decreased frequency of poliomyelitis.

BIBLIOGRAPHY

1. Dalldorf, G., and Sickles, G. M., *Science*, 1948, **108,** 61. Dalldorf, G., and Gifford, R., *New England J. Med.*, 1951, **244,** 868.
2. Melnick, J. L., Shaw, E. W., and Curnen, E. C., *Proc. Soc. Exp. Biol. and Med.*, 1949, **71,** 344.
3. Howitt, B. F., *Proc. Soc. Exp. Biol. and Med.*, 1950, **73,** 443.
4. Armstrong, M. P., *et al.*, *Canad. J. Pub. Health*, 1950, **41,** 51.
5. Melnick, J. L., Ledinko, N., Kaplan, A. S., and Kraft, L. M., *J. Exp. Med.*, 1950, **91,** 185.
6. Dalldorf, G., *Bull. New York Acad. Med.*, 1950, **26,** 329.

7. Melnick, J. L., and Kaplan, A. S., *Proc. Soc. Exp. Biol. and Med.*, 1950, **74**, 812.
8. Melnick, J. L., and Kraft, L. M., *Fed. Proc.*, 1950, **9**, 585.
9. Sabin, A. B., *Proc. Soc. Exp. Biol. and Med.*, 1950, **73**, 394.
10. Dalldorf, G., *in* The Pathogenesis and Pathology of Viral Diseases, (J. G. Kidd, editor), New York Academy of Medicine, Section on Microbiology, Symposium No. 3, December 14 and 15, 1948, New York, Columbia University Press, 1950, 31.
11. Dalldorf, G., Douglass, M., and Robinson, H. E., *Science*, 1937, **85**, 184.
12. Dalldorf, G., *J. Immunol.*, 1939, **37**, 245.
13. Rhodes, A. J., and Chapman, M., *Canad. J. Research*, 1950, **28**, 245.
14. Howitt, B. F., and Nichols, V. J., Inoculation of *cynomolgus* monkeys with Coxsackie viruses alone, combined, or with poliomyelitis virus; presented before the Laboratory Section, American Public Health Association, St. Louis, Missouri, November 1, 1950.
15. Melnick, J. L., *Bull. New York Acad. Med.*, 1950, **26**, 342.
16. Sulkin, S. E., and Manire, G. P., *Texas Rep. Biol. and Med.*, 1950, **8**, 368.