THE SPARING EFFECT OF CANINE DISTEMPER ON POLIOMYELITIS IN MACACA MULATTA

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The present report is a detailed description of experiments in which monkeys (*Macaca mulatta*) suffering from canine distemper were found resistant to intracerebrally inoculated poliomyelitis virus, a phenomenon briefly reported previously (1).

Methods

Distemper was induced in monkeys by intracerebral (20 animals) or subcutaneous or subcutaneous and intraperitoneal inoculation of the supernatant fluid of a 20 per cent emulsion of ferret spleen taken from animals moribund with distemper. The preparation of the inoculum, the doses used and the responses in the animals were identical with the methods and observations described in the previous report (2).

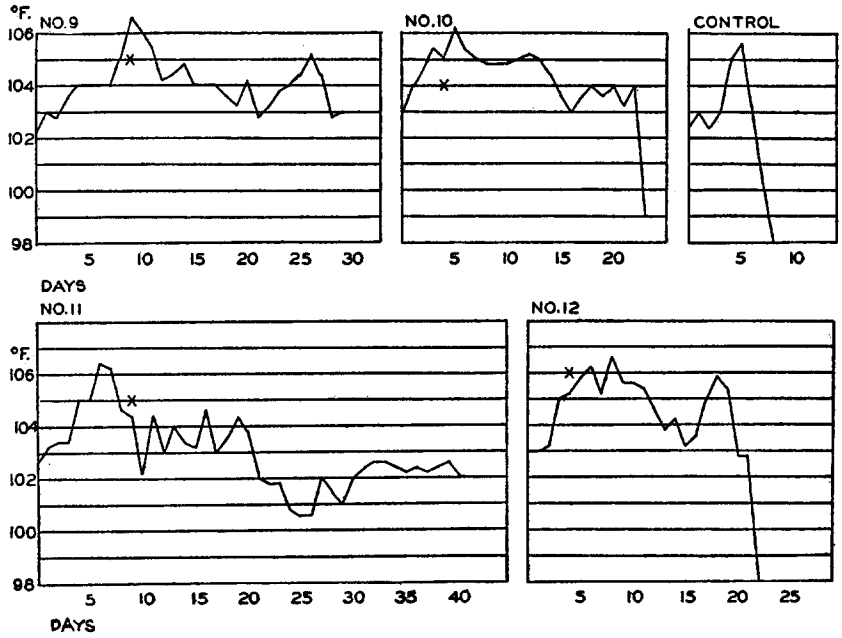
The poliomyelitis virus used in the present experiments was MV virus present in pooled, glycerinated monkey cord. Samples from various cords were taken, prepared in a 10 per cent emulsion which was centrifuged slowly for 10 minutes and given under light ether anesthesia into the cerebral tissue. The dose employed in the present experiments was 0.2 cc. or approximately 10 minimal lethal doses as based on our own titrations. When distemper was given intracerebrally the opposite hemisphere was used for the injection of poliomyelitis virus.

The virus of equine encephalomyelitis (eastern) was given as the supernatant fluid of a centrifuged emulsion (10 per cent) of monkey brain. The animals had been infected with guinea pig brain and both the monkeys and guinea pigs had behaved in typical fashion (3).[1]

The distemper vaccine used consisted of formolized dog spleen tissue. This was given subcutaneously over the flank. A second dose was injected after 14 days and this followed in another 2 weeks by an intradermal injection of splenic tissue dried in vacuum over phosphorous pentoxid and redissolved in saline before

[1] Virus supplied through the courtesy of Dr. Carl TenBroeck.

using. The distemper antiserum used was prepared from dogs immunized with distemper virus. It was given intraperitoneally in doses of 15 to 30 cc.[2]



TEXT-FIG. 1. The temperature records (degrees Fahrenheit) of four monkeys inoculated with distemper and, 9 days later, with poliomyelitis virus. The numbers will identify the animals in Table I. Nos. 9, 10 and 11 show the type of febrile response produced by distemper alone, No. 12 shows, 9 days after the inoculation of poliomyelitis virus, an abrupt rise in temperature which was accompanied by symptoms of poliomyelitis and led to death from poliomyelitis. Whether the rise in temperature in No. 9 on the 26th day is due to the same cause is unknown. No symptoms of poliomyelitis were recognized in this animal or Nos. 10 and 11. Death in No. 10 was due to pneumonia. The record of No. 11 has been continued to show the typical period of subnormal temperatures which occurs late in distemper in monkeys. The periods shown commence with the day on which the animals were inoculated with distemper. The day of inoculation with poliomyelitis virus is indicated by a cross.

The animals used weighed approximately 6 pounds and were maintained in clean and airy cages and carefully fed an excellent diet. Their temperatures were

[2] All of these preparations were furnished through the courtesy of Lederle Laboratories, Pearl River, N. Y.

taken twice each day as routine, although the afternoon temperatures alone have been used in Text-fig. 1. The monkeys used in the present experiments were in excellent condition and the other element of critical importance, the poliomyelitis virus, was regularly and invariably producing a brief, fatal form of the disease at the time.

EXPERIMENTAL

Results of Intracerebral Inoculation of Poliomyelitis Virus in Monkeys Suffering from Distemper Produced by Intracerebral, Subcutaneous, or Subcutaneous and Intraperitoneal Inoculations of Distemper Ferret Spleen.—Six groups of monkeys inoculated with poliomyelitis virus at various intervals following inoculations of distemper virus, together with their controls, are represented by Table I.

As the table shows the concurrence of the two infections produced a greatly modified outcome of the poliomyelitis inoculation. Thus 12 of the 21 experimental animals recovered and half this number recovered without paralysis. The results also indicate that the most favorable interval is 9 days following the inoculation with distemper virus, a time when the lesions of distemper are at their peak of activity (2), when the fever has reached or just passed its maximum and when inclusion bodies are extremely numerous; in other words the period when the distemper is at a maximum of intensity. Of the four animals inoculated with poliomyelitis at this time two recovered without evidence of poliomyelitis, a third died of an extensive lobar pneumonia, having shown no evidence of poliomyelitis, and one died of the latter disease. Study of the temperature records in these cases clearly indicates the maximal sparing effect. Thus animals which die of poliomyelitis or develop extensive paralyses have always shown an abrupt rise in temperature after a suitable period of incubation, which may easily be identified even when superimposed on the natural febrile reaction of distemper. The records of the four animals in this group have been redrawn (Text-fig. 1) to illustrate this point.

As is also evident from Table I, but even more so from the individual records the presence of distemper greatly modified the course of poliomyelitis even when death was not prevented. Thus death was delayed on an average 8 days among the experimental animals and the febrile response to poliomyelitis usually modified in that the sud-

TABLE I

Effect of Inoculation of the Virus of Poliomyelitis in Monkeys Ill with Canine Distemper and the Resistance of the Survivors to Reinoculation

Animal	Time after distemper inoculation that poliomyelitis was given	Duration of incubation of poliomyelitis before paralysis	Extent of paralysis	Outcome		Results of reinoculation	
				Recovered	Died	Resistant	Susceptible
	days	*days*			*days*		
1	4	No paralysis	0	x			x
2	4	13	+	x			
3	4	13	++++		13		
4	4	13	++	x			
Control		7	++++		10		
5	7	13	++	x		x	
6	7	13	+++	x		x	
7	7	No paralysis	0	x			
8	7	12	++++		24		
Control		8	++++		20		
9	9	No paralysis	0	x		x*	
10	9	" "	0		16†		
11	9	" "	0	x			x
12	9	12	++++		21		
Control		8	++++		11		
13	13	15	++	x		x	
14	13	No paralysis	0	x			x
15	13	7	++++		9		
16	13	No paralysis	0	x			x
Control		7	++++		10		
17	20	11	++++		20		
18	20	12	++	x		x	
19	70-20	13	++++		32		
20	70-20	8	++++		16		
21	70-20	9	++++		32		
Control		8	++++		13		

* Recovered with paralysis.

† Death due to lobar pneumonia.

den drop which presages death in control animals was delayed and less sudden.

The results also indicate that the sparing effect had largely subsided

by the 20th day, that is at the end of the febrile period of distemper.

What the table does not show, but which the records in Text-fig. 1 are representative of, is that while the distemper modifies the poliomyelitis the reverse is not true, the duration and degree of fever, the subsequent period of subnormal temperature are not shortened or modified in the least by the poliomyelitis.

Course of Experimental Poliomyelitis in Monkeys Convalescent from Canine Distemper, Having Had Distemper Vaccine or Receiving Distemper Antiserum.—The course of poliomyelitis produced by intracerebral inoculation of MV virus in monkeys convalescent from distemper is shown in Table II. In it are included the salient features of poliomyelitis occurring in monkeys which had had distemper at various times, monkeys which had distemper vaccine and attenuated virus and four animals which received distemper antiserum.

The results indicate no significant resistance to poliomyelitis as a result of these various experiences with distemper or as a result of antiserum unless the two animals which had complete courses of distemper vaccination do indeed indicate an altered resistance. Unfortunately the group was small. That both animals behaved in almost identical fashion and that their controls developed typical poliomyelitis justifies some consideration.

The only other group which behaved in atypical fashion comprised three monkeys which had distemper as a result of intracerebral inoculation, which 53 days later were reinoculated with distemper and which 20 days later were inoculated with poliomyelitis. The modified course of the poliomyelitis in these animals could well be due to the second distemper episode, since partial protection may be observed in Table I in animals inoculated 20 days following distemper.

The ineffectiveness of distemper antiserum is quite evident from Table II. Serum from distemper convalescent monkeys has likewise been found to have no neutralizing properties when mixed with equal parts of a suspension of poliomyelitis virus and stored overnight.

Results of Reinoculation with Poliomyelitis in Monkeys Convalescent from Concurrent Distemper and Poliomyelitis.—Nine of the survivors of the experiments included in Table I were subsequently reinoculated with poliomyelitis by the same route and with a similar preparation of virus as that first used. Four of the animals showed no febrile

or symptomatic response to this inoculation, four developed typical attacks of poliomyelitis and succumbed. One animal developed a febrile response but recovered with paralysis. The resistant animals

TABLE II

The Course of Experimental Poliomyelitis in Monkeys Which Had Recovered from Distemper, Had Been Inoculated with Distemper Vaccine or Were Treated with Distemper Antiserum

Animal	Previous experience	Duration	Comment
		days	
1	Antiserum at time of inoculation	7	Typical
2	" " " " "	9	"
3	Antiserum during preparalytic stage	8	"
4	" " " "	8	"
Control		8	"
5	Complete course of vaccine with febrile response. Poliomyelitis 1 mo. later	30	Atypical
6	" "	30	"
Control		9	Typical
"		5	"
7	Dried virus. Brief febrile response. Poliomyelitis 1 mo. later	12	"
8	" "	6	"
9	" "	13	"
Control		13	
"		5	"
"		7	"
10	Distemper. 10 wks. later poliomyelitis	11	"
11	" " " " "	11	"
12	Distemper. 4 mos. later poliomyelitis	6	"
13	" 5 " " "	13	"
14	" " " " "	12	"
Control		7	"

Vaccine and antiserum were furnished by Lederle Laboratories.

were all cases which had been paralyzed by the first experience with poliomyelitis, the susceptible animals were in each case animals which had been completely spared, in which the temperature records gave no clue to a response to poliomyelitis and in which no paralysis was

observed. The exception was an animal which recovered from the primary attack without paralysis but in whose records it was at one time noted that some weakness was present in a leg. This disappeared shortly afterward and no residual paralysis was observed. These records are likewise incorporated in Table I.

Course of Experimental Poliomyelitis in Monkeys Suffering from Vaccinia Encephalitis and the Concurrence of Poliomyelitis and Equine Encephalomyelitis.—Six monkeys inoculated intracerebrally with MV virus were subsequently inoculated with 0.2 cc. of the supernatant fluid of a 10 per cent emulsion of monkey brain taken from a fatal case of equine encephalomyelitis. Three animals received the second disease 2 days after the poliomyelitis inoculation and the other three after 3 days. Death occurred in the first group 6, 6 and 7 days following the original inoculation or 4, 4 and 5 days after the inoculation with encephalomyelitis. In the second group death occurred 3, 5 and 6 days after the encephalomyelitis inoculations. Two control cases of poliomyelitis died on the 6th and 13th days and two control cases of equine encephalomyelitis on the 4th day following inoculation. It was evident from the foregoing that the poliomyelitis did not curb the course of the encephalomyelitis and that the latter did not modify the development of the poliomyelitis in a significant degree.

Two animals suffering from vaccinia encephalitis were likewise inoculated with poliomyelitis virus and promptly succumbed with poliomyelitis, indicating that the former did not exclude the development of the latter.

The Results of Injection of Normal Ferret Spleen.—As a further control of the present studies 0.2 cc. of the 20 per cent supernatant fluid of a centrifuged, 20 per cent emulsion of normal ferret spleen was injected intracerebrally into each of five monkeys. In no case did a febrile response develop and in each instance (three animals) in which poliomyelitis was inoculated after an interval of 7 days typical and fatal quadriplegia developed.

DISCUSSION

The evidence presented indicates that during the course of distemper in the monkey, that animal is highly resistant to the develop-

ment of experimental poliomyelitis, that the resistance reaches a maximum during the 2nd week of the distemper and that the characteristic feature of the resistance is that many of the animals are spared an experience with poliomyelitis intimate enough or massive enough to produce the typical disease, to produce any clinical manifestations at all, or to lead to a fixed immunity to poliomyelitis. With this interpretation in mind it has seemed wise to speak of the phenomenon as a sparing effect until the precise mechanism is better understood.

The protective effect of distemper is intimately associated with the disease itself and disappears during convalescence. The response of distemper immune monkeys to poliomyelitis is not known since to date no solid, fixed immunity to the disease has been observed in our animals. However, it is possible that an immunity would influence the course of poliomyelitis, since two animals which had had protracted contact with attenuated distemper virus appeared to show some resistance to poliomyelitis similar to the effects of pseudorabies in animals immune to B virus (4).

Since the original observation (1) that such a sparing effect did exist between these two diseases, Findlay and MacCallum have reported a similar phenomenon (5). They observed that if Rift Valley fever virus were injected intraperitoneally in monkeys and followed in 2 hours by inoculation of pantropic yellow fever virus, approximately 60 per cent of the animals survived the yellow fever. This was an extension of the work of Hoskins (6) who reported the year previous that a neurotropic strain of yellow fever virus protected 60 per cent of monkeys from the pantropic virus. In this case the interval had to be less than 20 hours to be effective. Judging by the protocols of these experiments the phenomenon would seem to be like that we have observed. Also of possible interest to the discussion are the studies of Magrassi (7) and Doerr and Seidenberg (8) on the effects of double inoculations of rabbits with encephalitic strains of herpes virus. Magrassi observed that if such a virus be injected peripherally and followed on the 7th or 8th day by an intracerebral inoculation of the same virus the two nullified each other, whereas each was capable of producing fatal encephalitis if given alone. This was confirmed by Doerr and Seidenberg who also showed that the first inoculation might be intracorneal and produce the same result.

Possibly related studies are those of Collier (9) who observed an increased rate of disappearance of fowl plague virus in rats which had, 14 to 54 days previously, been injected with fowl plague, rabbit myxoma or Rous sarcoma virus. The evidence in these experiments is not extensive enough to justify a close comparison.

In discussing their results Findlay and MacCallum point out that it has been repeatedly observed that certain closely related virus diseases of plants, yellow mosaic and tobacco mosaic, two different strains of X virus of potatoes, etc., are mutually exclusive, that the presence of one prevents the growth of the other.

The consensus of opinion appears to be that in the case of the plant viruses the strains must be generically related to produce this effect. This relationship was of course present in Hoskins' work in which strains of yellow fever virus were employed. As Findlay and MacCallum point out there are many points of similarity between Rift Valley and yellow fever, clinical symptoms and morbid anatomy being quite similar, the same species of monkeys being susceptible to both, both being transmitted by aedes mosquitoes and both having neurotropic strains. However no cross immunity has been demonstrable by serological tests, or *in vivo* immunity in man or other tested animals, and the pathogenic range is quite dissimilar. Findlay and MacCallum found that inactivated neurotropic virus, normal brain tissue and fowl pest virus were ineffectual.

Closely related diseases or strains of the same disease have therefore been the rule in the experiments in which a sparing effect has been noted. This point is of interest in the light of the present report, suggesting, as it does, a relationship between poliomyelitis and canine distemper. Such has indeed been suggested on several occasions but no significant evidence has been collected to support the view. Of our own observations there is little to indicate a similarity. The clinical responses and sequelae are different, as are the distribution of virus and presumably the lesions of the central nervous system although the evidence on this point is still too limited to be of much weight. No cross immunity has been demonstrated to date. Indeed the sole point of similarity which we have observed has been the reaction of the reticulo-endothelial structures which seems quite similar in both diseases.

The possibility that the effect is due to temperature alone seems precluded by the reported failure of hyperpyrexia (10) as a treatment of experimental poliomyelitis as well as isolated cases in which animals recovered during a period when the febrile reaction was insignificant.

Findlay and MacCallum suggested that the explanation of their experiments was a cellular blockade against the second virus. Reticulo-endothelial blockade with India ink proved ineffective. Reticulo-endothelial blockade may well exist in animals suffering from distemper: the changes in the splenic sinuses are very suggestive.

Another possibility is that both viruses require, for their propagation, a common cell protein or other substance which the conjugation of the first virus exhausts and thereby prevents the multiplication of poliomyelitis virus.

The designation of this phenomenon as a sparing effect seems justified by the observation that recovered animals have evidently been spared an intimate contact with poliomyelitis virus, and hence are still susceptible to it, and because of the desirability of clearly distinguishing this immunity mechanism from those due to the development of tissue resistance or serological immune substances, neither of which occur in the present instance.

CONCLUSIONS

1. *Rhesus* monkeys inoculated with canine distemper are relatively or completely immune to experimental poliomyelitis during the first 2 weeks of the distemper.

2. Monkeys convalescent from distemper are not resistant to experimental poliomyelitis.

3. Two monkeys vaccinated with distemper virus responded to poliomyelitis in a modified manner.

4. Distemper antiserum did not influence the course of experimental poliomyelitis in *rhesus* monkeys.

5. Equine encephalomyelitis and vaccinia encephalitis showed no sparing effect on the course of experimental poliomyelitis.

6. The concurrence of distemper and poliomyelitis in monkeys seems to represent a new immunity mechanism in the virus field.

BIBLIOGRAPHY

1. Dalldorf, G., Douglass, M., and Robinson, H. E., *Science*, 1937, **85**, 184.
2. Dalldorf, G., Douglass, M., Robinson, H. E., *J. Exp. Med.*, 1938, **67**, 323.
3. Hurst, E. W., *J. Path. and Bact.*, 1936, **42**, 271.
4. Sabin, A. B., and Wright, A. M., *J. Exp. Med.*, 1934, **59**, 115. Hurst, E. W., *J. Exp. Med.*, 1936, **63**, 449.
5. Findlay, G. M., and MacCallum, F. O., *J. Path. and Bact.*, 1937, **44**, 405.
6. Hoskins, M., *Am. J. Trop. Med.*, 1935, **15**, 675.
7. Magrassi, F., *Z. Hyg. u. Infektionskrankh.*, 1935, **117**, 573.
8. Doerr, R., and Seidenberg, S., *Z. Hyg. u. Infektionskrankh.*, 1937, **119**, 135.
9. Collier, W. A., *Z. Hyg. u. Infektionskrankh.*, 1932, **113**, 758. Collier, W. A., and Levy, R., *Deutsch. tierärztl. Woch.*, 1932, **40**, 764.
10. Jungeblut, C. W., and Kopeloff, N., *J. Infect. Dis.*, 1931, **49**, 348.