TABLE II. Partial Reversal of Hemagglutination Inhibition by Uridine Derivatives. Tested with GDVII Virus in Tissue Cultures.

	% reduction of titer compared with control					
	5-Chlorouridine, 1 mg/ml		5-Aminouridine, .5 mg/ml		5-Hydroxyuridine, .3 mg/ml	
Compounds tested	Alone	Combined	Alone	Combined	Alone	Combined
Uridine, 1 mg/ml	100	75	87	60	87	50
3	87	75	—	—	—	—
Uracil, 1	94	87	87	75–87	—	—
3	—	—	75	75	—	—
Cytidine, 1	87	87	87	75	—	—
			1 mg/ml			
Uridine, 3			87	75		

tion of Theiler's GDVII strain of murine encephalomyelitis virus in tissue cultures of one-day mouse brain. Guanine, uridine, uracil, guanylic or cytidylic acids did not inhibit. Various substituted nucleosides including amino-, chloro-, diazo-, formamido-, hydroxy- and methyluridine were inhibitory. Uridine partially reversed this inhibition. 5-Chlorouracil, ribosylthymine and glucosylthymine did not inhibit; 2,6-diaminopurine did. 5-Chlorouridine was not effective against this viral infection in mice.

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Poliomyelitis Infection in Cortisone-Treated Hamsters Induced by the Intraperitoneal Route.* (19451)

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It was previously reported that cortisone enhances greatly the experimental poliomyelitis infection in the Syrian hamster(1,2). The studies embodied in this paper deal with the production of the disease in this animal by the intraperitoneal route with the aid of cortisone(3).

Experimental. Syrian male hamsters weighing 20-28 g, supplied by the Lakeview Hamster Colony and the Breeding and Laboratory Institute, and Swiss mice, CF1 obtained from the Carworth Farms, weighing 15-20 g, were employed. All the animals were observed

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TABLE I. MEF_1 Infection in Hamsters by the Intraperitoneal Route.

Total No.	Treatment with cortisone	MEF_1 Time of inoculation	MEF_1 Inoculum dilution	Paralysis onset Range of days	Mean survival time	Mortality rate, %
121	—	—	1:20 to 1:12000		—	0
45	2 mg 4x 24, 27, 20 hr	—	—	—	14	6
10	2 mg 1x	2 hr after cortisone	1:20	5–6	7.3	30
10	5 mg 1x	Immed. after cortisone	1:20	6–16	12	60
10	2 mg 2x 24 hr	2 hr after 2nd cortisone	1:20	5–8	7.4	100
10	2 mg 3x 19, 27 hr	Immed. after 3rd cortisone	1:1000	4–10	8	66.7
10	2 mg 4x 24, 27, 20 hr	"	1:100	3–5	4.7	100
15		"	1:500	5–10	8	100
10		"	1:1000	5–9	7.6	55.5
10		"	1:8000	5–12	9	60
10		"	1:12000	4–11	—	0
10	3 mg 3x 19, 24 hr	2½ hr after 3rd cortisone	1:20	4–7	6.5	100
10	3 mg 4x 4, 20, 3 hr	Immed. after 3rd cortisone	1:100	3–5	5.5	100

daily for a period of 4 weeks. The intracerebral inoculation dose was 0.03 ml for mice and 0.05 ml for hamsters, and the intraperitoneal dose for hamsters was 0.5 ml. All the studies described were done with strain MEF_1 supplied by Dr. Peter K. Olitsky and maintained in this laboratory by serial intracerebral mouse passages. An emulsion of a large pool of brains was preserved in an electric refrigerator at —30°C. The intracerebral mouse LD_{50} titer was $10^{-3.78}$. Since 0.5 ml was used for the intraperitoneal inoculation and the mouse titrations were based on intracerebral inoculation of 0.03 ml the doses used for the intraperitoneal inoculation are best expressed in terms of dilutions rather than in equivalent mouse LD_{50} titers until the ratios of the intracerebral mouse and intraperitoneal hamster LD_{50} titers are determined experimentally. The anti-Lansing monkey convalescent serum obtained from Dr. Hilary Koprowski and another similar serum prepared in this laboratory were used for the neutralization experiments described. The author is thankful for these investigators for making the materials available. Cortisone, Cortone acetate, Merck and Co., containing 25 mg per ml was injected intramuscularly in a volume not exceeding 0.2 ml. When smaller concentrations of the hormone were needed the dilutions were made in saline. Lyophilized ACTH, ACTHAR, Armour Laboratories, was dissolved to a suitable concentration in saline and injected in a volume of 0.25 ml intramuscularly.

Results. As may be seen from Table I, it was possible to infect hamsters consistently by the intraperitoneal route following cortisone treatment. The animals developed a rapidly progressing, highly fatal disease, the severity of which equaled and possibly exceeded that obtained on intracerebral inoculation of the virus into cortisone-treated hamsters. The results were unequivocal inasmuch as no clinical disease was elicited following the inoculation of the virus into hamsters receiving no cortisone. There was a clear-cut reciprocal relationship between the amount of cortisone and the concentration of the virus necessary for the production of the disease. The mean survival time and the mortality rate were readily influenced by these relationships, resulting under optimum conditions in an incubation period as short as 3-5 days and 100% mor-

TABLE II. Recovery of Virus from Hamsters Infected Intraperitoneally.

Treatment with cortisone	MEF_1 inoculum dilution	Organ tested: Name	Organ tested: Hr after inoculation	Organ tested: Dilution	Result in mice†
—	1:20	Brain	96	1:20	0/8
5 mg 1x	,,		20	,,	0/7
	,,		70	,,	0/8
	,,		192	,,	5/8
—	,,	Serum	96	1:2	0/8
5 mg 1x	,,		70	1:10	0/6
	,,		192	,,	0/6
2 mg 2x 24 hr	,,		23	,,	1/16
	,,		23	1:100	0/16
	,,		96	1:10	13/16
	,,		96	1:100	2/16
3 mg 3x 19, 24 hr	,,	Brain	96	,, *	6/6
	,,	Serum	96	1:10	11/16
	,,		96	1:100	4/16
3 mg 4x 4, 20, 3 hr	1:100	Brain	120	,,	6/6
	,,		120	1:1000	5/6
	,,		120	1:5000*	5/6
	,,	Serum	120	1:10	6/6
	,,		120	1:100	2/6
	,,		120	1:1000	1/6
5 mg 4x 19, 24, 24 hr	1:1000		72	1:10	6/6
	,,		72	1:100	3/6
	,,		72	1:1000	0/6
	,,		120	1:10	6/6
	,,		120	1:100	6/6
	,,		120	1:1000	5/6
	,,		120	1:4000	1/6

* Higher dilutions not tested.
† Numerator = No. of dead animals. Denominator = Total No. of animals tested.

tality rate. It is of interest that a single injection of 5 mg was less effective than 2 injections of 2 mg given at an interval of 24 hours. Four injections of 2 mg gave rise to a fatal disease following the inoculation of 0.5 ml of the virus diluted as high as 1:8000. Even after taking into consideration the fact that a volume 10 times greater was employed for the intraperitoneal than for the intracerebral inoculation, it may still be assumed that the intraperitoneal route compares quite favorably with the intracerebral route in cortisone-treated hamsters.

The identity of the disease was established as follows: 1. There was obtained a typical clinical disease in a great majority of animals frequently resulting in tetraplegia in animals surviving for at least 4 days. Characteristic histological changes were invariably seen in the spinal cord in the animals showing the disease during life. 2. The virus recovered from the brain of the intraperitoneally infected hamsters produced typical symptoms in mice. It was neutralized by the anti-Lansing convalescent monkey serum on intracerebral testing in mice. In these titrations 200 mouse LD_{50} were completely neutralized by the serum diluted 1:10 following incubation of the mixture for one hour in a water bath at 37°C. 3. It was also possible to neutralize the virus in the hamsters. In these experiments the hamsters received 4 injections of 2 mg of cortisone at intervals of 3, 20, and 3 hours. One-half ml of suitable dilutions of virus and serum incubated in a water bath at 37°C for one hour were injected intraperitoneally immediately following the fourth injection of cortisone. There was obtained consistent neutralization of the virus diluted 1:500 with the serum diluted 1:40, while in

the absence of the serum the virus similarly diluted and incubated gave 100% mortality with a mean survival time of 5 days.

As may be seen from Table II, the virus multiplied in the central nervous system incidentally to the development of the disease. In addition the animals showed a marked viremia, the extent of which depended largely on the total dose of cortisone, the frequency of its administration and the concentration of the virus. The proof that the viremia obtained was not due to the discharge of the virus into the vascular system from the central nervous system through breaking down of some barrier by means of cortisone was found in the observation that hamsters similarly treated with cortisone and inoculated with the virus intracerebrally failed to show any evidence of viremia at different stages of the disease.

It was previously noted that in contrast to cortisone, ACTH in large doses failed to modify the poliomyelitis infection in the hamster inoculated with the virus intracerebrally(1,2). Similarly 15-20 mg of ACTH injected intramuscularly in 3 and 4 divided doses over a period of several days failed to elicit the infection by the intraperitoneal route in 40 hamsters inoculated with 0.5 ml of strain MEF_1 diluted 1:20. All the animals thus tested remained well during the entire period of observation.

Discussion. The results reported in this paper indicate an extraneural multiplication of the poliomyelitis virus following the intraperitoneal inoculation into cortisone-treated hamsters, since the viremia observed fails to appear following the intracerebral inoculation of the virus into hamsters treated similarly with cortisone. Unpublished work in collaboration with Dr. S. M. Aronson indicates a good correlation exists between histological changes and concentration of the virus in certain organs and tissues outside of the central nervous system. Among these are changes in periadrenal adipose tissue and necrotizing calcified lesions in paravertebral muscles incidentally to a high concentration of the virus. The results are of special interest in connection with the pathogenesis of the disease because they suggest that extraneural multiplication of the virus may be one of the phases of the infection on parenteral introduction of the virus. The demonstration by Horstmann(4) and Bodian(5) of a viremia in the chimpanzee following feeding of the virus, points in the same direction.

The possibility of producing the infection by the intraperitoneal route offers a new method for intraperitoneal testing of neutralizing potency of sera and for investigations of the effect of chemotherapeutic agents before fixation of the virus by the central nervous system. Other distinct advantages of this method result from the fact that by adjusting the dosage of cortisone and the concentration of the virus it may become possible to obtain various degrees of severity of the disease ranging from a prolonged incubation period and low mortality rate to a uniformly severe disease of short incubation period and high mortality rate, thus affording the opportunity for screening of agents under widely different conditions.

Summary. It was possible to produce a violent and uniformly fatal poliomyelitis infection in the hamster by the intraperitoneal route following treatment with cortisone. The disease was accompanied by a pronounced viremia apparently due to the multiplication of the virus outside of the central nervous system under these conditions. No clinical infection was observed in non-treated hamsters thus inoculated. No disease was produced following the intraperitoneal inoculation of the virus in the hamsters receiving ACTH.

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