showed that the cells cultivated in Medium III invariably remained in better condition and also outlived those cultivated in Medium II. When transferred after 56 days of cultivation to a growth-promoting medium, all the colonies cultivated in Medium III proliferated again, while only 25% of those cultivated in the simpler medium were found to be capable of renewed growth. In one experiment, some colonies that had been cultivated for a month by the procedure outlined above were left for another month without change of fluid. At the end of this time, the cells were still found to be in good condition and able to proliferate.

But Medium III is not serumless. In order to incorporate some vitamin A in it, 0.07% of serum had to be used. Vitamin A was added to this medium because it is always present in serum and has also been found a necessary constituent of artificial growth-promoting media. But no evidence that it was essential for maintenance has been obtained. Hence, to ascertain if this vitamin and the serum used to dissolve it could be eliminated, sister colonies of fibroblasts were cultivated in Medium III, with and without vitamin A. All the differences observed were in favor of the serumless medium. The cells in this medium seemed to be a little clearer and in better condition throughout the entire period of their cultivation. After 62 days, the colonies that had been carried in the serumless medium were transferred to a new coagulum and given growth-promoting nutrients. They responded by growing actively.

To summarize: Four media have been described in which fibroblasts in pure strain have been maintained in vital condition and with little or no proliferation for periods varying from 42 to 56 days. The first of these media is simple, inexpensive, and easy to prepare. The last is serumless.

10179 P

Simultaneous Distemper and Lymphocytic Choriomeningitis in Dog Spleen and the Sparing Effect on Poliomyelitis.

GILBERT DALLDORF AND MARGARET DOUGLASS. (Introduced by R. E. Shope.)

From Grasslands Hospital, Valhalla, N. Y.

While continuing the study of canine distemper in monkeys, evidence accumulated which indicated that our virus-source material

was a mixture. This has been verified by the recovery of the virus of lymphocytic choriomeningitis from 4 different samples of dog spleen containing the virus of canine distemper and by its presumptive demonstration in 7 other instances. The present report submits evidence that the virus of lymphocytic choriomeningitis occurs in the dog and to correct our earlier description of canine distemper in the monkey and the sparing effect of distemper on experimental poliomyelitis.[1, 2]

Three bacteriologically sterile 20% suspensions of pooled dog spleens taken from separate harvests of canine-distemper virus of the same strain were inoculated intracerebrally in 0.03 cc amounts into groups of mice. A uniform clinical response resembling Traub's description of lymphocytic choriomeningitis followed.[3] Part of one suspension was inoculated subcutaneously into 2 puppies. Fifteen days later these were moribund with canine distemper and were sacrificed. Six mice inoculated with splenic emulsion of one of these puppies also sickened and died. Fourteen of 18 examined mice had lymphocytic chorioiditis.

At the same time a considerable number of mice from the same colony were inoculated intracerebrally with dog-spleen suspensions

TABLE I.
Tests for the Recovery of Virus of Lymphocytic Choriomeningitis.

Mice injected with distemper dog spleens from various serial passages of the original strain.		
No. of Mice	Material Pool No.	Day of death of mice
12	1	5, 5—, 7—, 7+, 8, 8+, 9, 9+, 10+, 12, *12*, *12*
10	1 (intraperitoneal)	6, 8, 8, 9, 10, 11, 14, S, S, S
6	1*	8, 8+, 8+, 8+, 11+, 11
8	2	3, 8+, 8+, 9—, 9+, *9*, *9*, *9*
8	3	1, 8, 8+, 8+, 10—, S, S, 10+
Mice injected with distemper dog spleens from other sources and with other materials.		
No. of Mice	Material Pool Strain	Day of death of mice
6	"B"	S, S, S, S, S, S
8	"C"	S, S, S, S, S, S, S, S
6	"D"	S, S, S, S, S, S
14	Sterile broth	S, S, S, S, S, S, S, S, S, S, S, S, S, S

All injections given intracerebrally. Pool No. 1 also given intraperitoneally. * indicates the spleen of a puppy which had been inoculated with Pool No. 1 15 days previous and which was followed by severe distemper. The + and — signs indicate that lymphocytic chorioiditis was or was not present. The *italics* indicate mice sacrificed when moribund for supply of virus.

1 Dalldorf, G., Douglass, M., Robinson, H. E., *J. Exp. Med.*, 1938, **67**, 333.

2 Dalldorf, G., Douglass, M., Robinson, H. E., *J. Exp. Med.*, 1938, **67**, 323.

3 Traub, E., *J. Exp. Med.*, 1936, **63**, 533.

secured from other sources and containing canine-distemper virus, with various other suspected materials and with sterile broth. All remained well. The data of representative groups have been incorporated in Table I as evidence both of the freedom of our stock animals from spontaneous lymphocytic choriomeningitis as well as the presence of a virus, capable of producing the clinical response of lymphocytic choriomeningitis, in the original materials.

Three guinea pigs were inoculated subcutaneously (0.5 cc) with one of the pooled dog-spleen suspensions and developed the symptoms of lymphocytic choriomeningitis, emaciation, dyspnea, and conjunctivitis. All 3 died. Two of these were demonstrated to have the lesions of lymphocytic chorioiditis (heavy round-cell infiltrate of the chorioid plexus and liver).

A fifth sample of pooled dog-spleen from the first series produced a severe disease in monkeys having the features described by Armstrong[4] as occurring in lymphocytic choriomeningitis. Splenic samples of 3 of these monkeys were injected into mice and produced the symptoms and lesions of lymphocytic choriomeningitis. Virus was also demonstrated in the blood of 2 of the monkeys. One blood sample was injected intravenously into 6 mice of which 4 survived. These were reinoculated intracerebrally 41 days later with known lymphocytic choriomeningitis virus received from Dr. T. M. Rivers and were found resistant while controls all died.

A sixth sample of pooled dog-spleen was injected subcutaneously into a young rhesus monkey which sickened but recovered; 105 days later this animal was inoculated intracerebrally (0.5 cc of a 10% suspension) with known lymphocytic-choriomeningitis virus and showed no response.

A number of other observations, in particular certain adrenal inclusion-bodies, lead us to believe that all of the material we have been using as a source of canine-distemper virus has been contaminated. This has led to a trial of the sparing effect of lymphocytic-choriomeningitis virus in rhesus monkeys intracerebrally inoculated with poliomyelitis virus. In these experiments the same methods and materials have been used as in our original reports with the exception that suspensions of guinea-pig brain taken from animals infected with a known strain of lymphocytic-choriomeningitis virus or the strain recovered from dog spleen and repeatedly passaged through monkeys were used instead of splenic suspensions from dogs or ferrets with distemper. The results have been summarized in Table II.

Since the virus of lymphocytic choriomeningitis has been repeat-

[4] Armstrong, C., *Pub. Health Rep., U. S. P. H. S.*, 1934, **49**, 1019.

TABLE II.
Sparing Effect of Lymphocytic Choriomeningitis on Experimental Poliomyelitis.

No. of Animals		Symptoms predominantly those of		Lesions predominantly those of		Outcome	
		Polio.	Chorio.	Polio.	Chorio.	Recovered	Died
8	Lymph. chor. virus injected before or simultaneously with inoculation with poliomyelitis	0	8	0	3	4	4
10	Lymph. chor. virus injected during incubationary period of poliomyelitis	5	5	3	4	3	7
12	Lymph. chor. virus injected after the appearance of preparalytic stage	11	1*	6	3	5	7
23	Poliomyelitis, controls	20†		7	0	5	18
6	Lymph. chor. controls	0	6			1	5

*Shortly after the second virus was injected the fever, irritability, and other signs of poliomyelitis disappeared.

†Three controls were cases of "missed infection."

edly found in the passage distemper-material we have used and was presumably present in our original experiments and since it is capable of producing a sparing effect on experimental poliomyelitis it seems advisable to report these results. Unfortunately we are not yet able to state definitely what part, if any, canine distemper played in the original work. This as well as extended study of the significance of lymphocytic choriomeningitis in the dog, its distribution and possible significance in the dissemination of the disease are all to be studied. Further investigation of the sparing effect of lymphocytic choriomeningitis on experimental poliomyelitis is also in progress.