CHARACTERISTICS OF A STRAIN OF LYMPHOCYTIC CHORIOMENINGITIS VIRUS ENCOUNTERED AS A CONTAMINANT IN TISSUE CULTURES OF RABIES VIRUS

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During the course of propagating rabies virus in tissue culture (1), a strain of lymphocytic choriomeningitis was encountered as a contaminant. The method of isolation and identification of the strain will be described in the present paper.

Reasons for Suspecting Contamination of the Culture

The rabies culture virus was suspected as being contaminated because of certain changes in its behavior.

Following intracerebral inoculation into mice, it no longer induced paralysis on the 7th to 9th days, followed by a 2 day course of illness, but instead brought about convulsions without paralysis on the 6th day, followed within a few hours by death. When injected intraperitoneally into mice as a vaccine, it no longer protected them against a subsequent test injection of known rabies virus. Finally, this tissue virus, when mixed with known rabies hyperimmune serum and injected intracerebrally into mice, remained pathogenic under those conditions in which known rabies virus plus the same immune serum becomes non-pathogenic.

Isolation of the Contaminant

The contaminant was isolated by filtering the culture virus repeatedly through Seitz pads.

The filtrate, unlike that from known rabies virus, whether in culture or brain tissue, proved infective for mice following intracerebral inoculation. Subsequent serial passage of this filtrate through mice, intracerebrally, established its virulence at the level of 0.03 cc. 10^{-5} to 10^{-6}. It was then filtered again, passed through mice, and titrated. This procedure was carried out successfully several times with the contaminated rabies culture virus.

Identification of the Contaminant

A twice filtered strain passed through mice and designated II S.F. was identified as lymphocytic choriomeningitis by the following tests.

Mice.—Intracerebral injections of virus through the 10^{-5} dilution gave rise regularly to convulsions on the 5th or 6th day followed by death within 24 hours, with muscles spastic and limbs extended. Blood and spleen, shortly before death, contained virus in large amounts. Intranasal, intramuscular, or intraperitoneal injections of virus were not harmful to adult mice. 150,000 or more intracerebral lethal doses injected intraperitoneally into 2 weeks old mice, however, proved fatal. Mice succumbing to the infection showed at autopsy widespread, though moderate, accumulations of mononuclear leucocytes beneath the pia.

Guinea Pigs.—Intracerebral injections of II S.F. resulted either in no ill effects or in a mild, febrile response on the 3rd to 8th days sometimes accompanied by mild weakness. Generally the animals recovered, even when a massive dose was employed.

Macacus rhesus Monkeys.—The virus proved not especially virulent for these monkeys, even when injected intracerebrally in large doses. Aside from temperature rises lasting from 5 to 7 days, the animals remained well.

TABLE I

Neutralization Tests of Strain II S. F. in Rabies, Equine Encephalomyelitis, and Lymphocytic Choriomeningitis Immune Sera

Test sera	Duration of life of mice injected intracerebrally with 0.03 cc. of virus in dilutions				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Guinea pig, normal	—	6,* 7, 7, 8	6, 6, 7, 7	7, 8, 8, 8	8, S, S, S
Rabbit, lymphocytic choriomeningitis immune	6, S, S, S	S, S, S, S	S, S, S, S	S, S, S, S	S, S, S, S
Rabbit, normal	7, 7, 7, 8	7, 7, 8, 8	7, 7, 7, 8	8, 8, 8, 10	8, S, S, S
Rabbit, rabies immune	6, 6, 6, 7	6, 7, 7, 7	7, 7, 8, 8	8, 8, S, S	S, S, S, S
Rabbit, Eastern equine encephalomyelitis, immune	8, 8, 8, 8	7, 7, 7, 17	8, 8, 8, 9	9, S, S, S	S, S, S, S
Rabbit, Western equine encephalomyelitis, immune	8, 8, 9, 10	7, 8, 8, 8	8, 9, 9	S, S, S, S	S, S, S, S

* 6 = mouse died 6 days following injection.

S = mouse remained well 21 days. — = dilution not tested.

The II S.F. virus, according to the above findings, gives rise in mice to reactions which are typical of those described for lymphocytic choriomeningitis virus, whereas in guinea pigs and monkeys the reactions are much less severe than those ordinarily observed following injection of lymphocytic choriomeningitis virus. The identity of the II S.F. strain was readily established, however, by cross-immunity and neutralization tests.

Guinea pigs and mice immunized against II S.F. proved immune to a known strain of lymphocytic choriomeningitis.[1] Mice immunized against lymphocytic choriomeningitis likewise proved immune to II S.F.

[1] We are indebted to Dr. J. E. Smadel for assistance in carrying out the cross-immunity and neutralization tests. He immunized mice for us against lymphocytic

Neutralization tests were run with II S.F. plus antirabies, Eastern and Western equine encephalomyelitis, and lymphocytic choriomeningitis immune sera. The II S.F. virus from the brain of a prostrate mouse was emulsified and made up in serial tenfold dilutions. 0.3 cc. of diluted virus was added to 0.3 cc. of undiluted serum. The mixtures were incubated at 37°C. for 2 hours and then left standing 2 hours at room temperature. 0.03 cc. of each mixture was then injected intracerebrally into four W-Swiss mice 18 to 21 days of age. Duration of life was recorded in days and the protective action of the various sera expressed in number of intracerebral lethal doses resisted. Table I shows that the lymphocytic choriomeningitis serum protected against 1,000 lethal doses of II S.F. but that all other sera failed to show significant protection.

From the above data it was apparent that the rabies tissue culture contaminant was in fact a strain of lymphocytic choriomeningitis virus.

Possible Modes of Access of Lymphocytic Choriomeningitis Virus to the Rabies Tissue Culture Flasks

The two most likely ways by which II S.F. lymphocytic choriomeningitis virus might have gained access into the rabies tissue cultures were either by way of the embryo mouse brain tissue or the monkey serum. The mouse origin was a definite possibility, although in this case an unlikely one. Lymphocytic choriomeningitis virus is known (2) as a native disease in mice and yet our stock, the W-Swiss, is highly and uniformly susceptible to it, both old and young.

The contamination occurred, in our opinion, by way of the monkey serum. In the first place, a spontaneous epidemic of lymphocytic choriomeningitis broke out among a stock of monkeys in The Rockefeller Institute at about the time that the contamination must have occurred (3). Secondly, animals with this disease carry the virus in their blood in large quantities. Thirdly, monkeys at The Rockefeller Institute, tested for susceptibility to this virus, were surprisingly resistant, suggesting that they might have been immunized. Fourthly, blood from several of the normal monkeys in our colony contained neutralizing antibodies against the virus.

Table II shows the results of neutralization tests carried out as described above with strain II S.F. and sera from supposedly normal rabbits, guinea pigs, mice, and monkeys. Virus mixed with sera from normal rabbits, guinea pigs, and mice titred 10^{-5} to 10^{-6}, 10^{-5}, and 10^{-6} respectively. Virus mixed with sera from supposedly normal monkeys, on the other hand, titred

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choriomeningitis, tested our animals immune to II S.F. for their resistance to lymphocytic choriomeningitis virus, tested II S.F. immune serum for neutralizing and complement fixation potency against lymphocytic choriomeningitis virus, and finally, supplied us with lymphocytic choriomeningitis immune serum for our own tests with II S.F. virus.

10^{-4}, 10^{-5}, 10^{-5}, 10^{-5}, 10^{-4}, and 10^{-3}. These low titres with monkey sera suggest that the animals themselves may have experienced a mild infection with the virus.

Special Characteristics of Strain II S.F. Lymphocytic Choriomeningitis Virus

Filterability.—Strain II S.F. traversed Seitz pads readily. According to Rivers and Scott (4), lymphocytic choriomeningitis virus will occasionally traverse an Elford membrane with average pore diameter as small as 150 mμ. More recently, Scott and Elford (5) reported that the virus on one

TABLE II

Neutralization Tests of Strain II S. F. in Sera from Supposedly Normal Rabbits, Guinea Pigs, Mice, and Monkeys

Test sera	Duration of life of mice injected intracerebrally with 0.03 cc. of virus in dilutions					
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Rabbit 1	6,* 6, 7, 7	7, 7, 7, 7	7, 7, 7, 7	8, 8, 8, 9	7, S, S, S	—
Rabbit 2	—	—	7, 7, 7, 7	7, 7, 7, 7	7, 8, 8, S	10, S, S, S
Rabbit 3	—	—	7, 7, 7, 8	7, 7, 8, 8	8, 9, S, S	S, S, S, S
Guinea pig	—	6, 7, 7, 8	6, 6, 7, 7	7, 8, 8, 8	8, S, S, S	—
Mice (pooled)	—	6, 6, 7, 8	6, 6, 7, 7	7, 7, 7, 7	8, 8, 8, S	—
Monkey 1	7, 7, 8	7, 7, 8, 8	9, 9, S, S	S, S, S, S	S, S, S, S	—
Monkey 2	6, 6, 7, 7	7, 7, 7, 7	7, 7, 7, 8	9, 9, S, S	8, S, S, S	—
Monkey 3	—	5, 6, 7, 7	7, 7, 7, 7	8, 8, 8, 8	9, S, S, S	—
Monkey 4	—	6, 7, 7, 8	7, 7, 7, 7	7, 8, 8, S	8, S, S, S	—
Monkey 5	—	7, 7, 7, 7	8, 8, S, S	S, S, S, S	S, S, S, S	—
Monkey 6	—	7, 7, 7, 8	S, S, S, S	S, S, S, S	S, S, S, S	—

* 6 = mouse died 6 days following injection.

S = mouse remained well 21 days. — = dilution not tested.

occasion traversed a 120 mμ average pore diameter membrane but not one with smaller dimensions. This finding agreed with their results of centrifugation tests and indicated that according to formula the size of the virus must be in the neighborhood of 40 to 60 mμ.

The following tests on the II S.F. strain, carried out with the aid of Dr. J. Bauer,[2] gave somewhat lower values and are reported in some detail because of the uniformity of response of the mice and the clear cut results obtained. Each experiment was performed in the following manner.

[2] We are indebted to Dr. J. H. Bauer for assistance in carrying out the filtration tests. He filtered the material supplied to him through membranes prepared and calibrated by him. The filtered preparations were then returned to us for testing in mice.

The brains of twenty 3 weeks old W-Swiss mice prostrate following an intracerebral injection of 0.03 cc. of a 10^{-2} suspension of II S.F. mouse brain virus were removed, emulsified, and suspended in 72 cc. of diluent. As diluent in Experiment 1, a mixture of 3 parts of 10 per cent horse serum in distilled water and 1 part hormone broth was employed; in Experiments 2 and 3, plain hormone broth was used. The material was then centrifuged at 3,000 R.P.M. for 15 minutes and the supernatant collected in a sterile flask. After titrating its virulence in 3 weeks old W-Swiss mice, the suspension of virus was passed through a Berkefeld N candle previously satisfied with 20 cc. of the same diluent. The Berkefeld filtrate was titred in mice as described above and then filtered through Elford membranes by Dr. Bauer. Dr. Bauer's technique for passing this material through graded collodion membranes was similar to that previously described (6).

TABLE III

Intracerebral Virulence for Mice of Strain II S. F. before and after Filtration through Graded Collodion Membranes

Experiment No.	Dilution of virus injected intracerebrally								Mortality of mice following injection of undiluted filtrates through membranes with average pore diameters in mμ of:									
	Before filtration				Berkefeld N filtrates													
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	150	125	113	105	100	96	83	73–75	60	50	40
1	3/3*	3/3	2/4	—	4/4	4/4	4/4	—	10/10	10/10	—	—	0/10	—	—	0/10	0/10	0/10	0/10
2	4/4	4/4	3/4	—	—	4/4	1/4	1/4	—	10/10	—	—	5/10	—	—	0/10	0/10	0/10	0/10
3	3/3	2/3	0/3	0/3	3/3	3/3	3/3	0/3	—	10/10	4/10	5/10	—	0/10	0/10	0/10	0/10	—	—

* 3/3 = three mice died out of three inoculated.
— = not tested.

The various filtrates were tested by us for the presence of virulent virus by injecting each in 0.03 cc. amounts intracerebrally into ten 3 weeks old mice respectively. The mice were observed for 4 weeks.

Table III shows the results of three tests. In the first test, the unfiltered virus titred 10^{-6} (in 0.03 cc. amounts) and the Berkefeld filtrate, 10^{-5} or better. This material traversed the membranes with 150 mμ and 125 mμ average pore diameter, but not those with average pore diameters of 100 mμ or smaller. In the second test the titres of the unfiltered and Berkefeld filtered materials were the same as those in the first test, namely, 10^{-6} and 10^{-5} respectively. This material passed the 125 mμ and 100 mμ membranes but not those with smaller pore diameters. Finally, in the third test, the unfiltered material titred 10^{-5}, the Berkefeld filtrate, 10^{-5}, and this latter suspension traversed the 125 mμ, the 113 mμ, the 105 mμ, but not the 96 mμ or smaller membranes.

The smallest membrane permitting passage of the virus was that with an average pore diameter of 100 mμ. The size of the virus is taken, therefore, according to formula, as 33 to 50 mμ.

Virulence of II S.F. for Mice.—The virulence of different strains of

TABLE IV

Susceptibility of 21 Day Old W-Swiss Mice to Intracerebral Inoculation of Lymphocytic Choriomeningitis Virus, Strains II S.F. and C

Strain of virus	Duration of life of mice injected intracerebrally with 0.03 cc. of virus in dilutions						
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
C	10,* 10, S, S	8, 8, 8, 9	7, 9, 9, 10	6, 8, 8, 8	9, S, S, S	—	—
II S.F.	—	—	—	7, 7, 7, 7	7, 7, 8, 8	7, 8, S, S	S, S, S, S

* 10 = mouse died 10 days following injection. S = mouse remained well 21 days. — = dilution not tested.

TABLE V

Susceptibility of 21 and 60 Day Old Mice to Intracerebral and Intraperitoneal Inoculation of Lymphocytic Choriomeningitis Virus, Strain II S.F.

Route of inoculation and volume injected	Dilution of virus	Duration of life of mice in days			
		21 day old mice		60 day old mice	
		Test 1	Test 2	Test 1	Test 2
Intracerebral 0.03 cc.	10^{-4}	—	—	7,* 7, 7, 7	6, 6
	10^{-5}	7, 7, 7, 7	6, 7, 7, 7	7, 7, 8, 8	7, 7, 7, 7
	10^{-6}	7, 7, 8, 8	6, 7, 7, S	7, 7, 8, 8	7, 8, 8, S
	10^{-7}	7, 8, S, S	7, 8, 8, S	9, S, S, S	8, S, S, S
	10^{-8}	S, S, S, S	8, S, S, S	—	S, S, S, S
Intraperitoneal 0.5 cc.	10^{-2}	7, 7, 7, 7, 7, 7, 7, 7, 7, 7, 8, 8, 8, 8, 8, 8, 8, S, S, S, S, S, S, S, S, S, S, S		24 mice tested: all survived	
	10^{-3}	7, 7, 8, 8, S, S, S, S, S, S, S, S, S, S, S, S, S, S		20 " " " "	
	10^{-4}	7, 7, 7, S, S, S, S, S, S, S, S, S, S, S, S, S, S, S		20 " " " "	
	10^{-5}	20 mice tested: all survived		19 " " " "	
	10^{-6}	20 " " " "		21 " " " "	

*7 = mouse died 7 days following injection. S = mouse remained well 21 days. — = dilution not tested.

TABLE VI

Immunity of 21 Day Old and 60 Day Old W-Swiss Mice 17 Days after Vaccination with Lymphocytic Choriomeningitis Virus, Strain II S.F.

Age of mice tested	Vaccine given intraperitoneally	Number of intracerebral M.L.D. in vaccine†	Mice dead following vaccination	Fate of vaccinated mice injected intracerebrally with 0.03 cc. of virus in dilutions						Amount of immunity in M.L.D.
				10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	
60 days	0.5 cc. 1:100	1,600,000	0/24	S, S, S, S, S, S	S, S, S, S, S, S	S, S, S, S, S, S	S, S, S, S, S, S	—	—	10,000+
	0.5 " 1:1,000	160,000	0/20	S, S, S, S	D, S, S, S	D, S, S, S	S, S, S, S	S, S, S, S	—	10,000+
	0.5 " 1:10,000	16,000	0/20	S, S, S, S	S, S, S, S	S, S, S, S	D, S, S, S	S, S, S, S	—	10,000+
	0.5 " 1:100,000	1,600	0/19	S, S, S	S, S, S, S	S, S, S, S	S, S, S, S	S, S, S, S	—	10,000+
	0.5 " 1:1,000,000	160	0/21	S, S, S, S, S, S	S, S, S, S, S	S, S, S, S, S	S, S, S, S, S	—	—	10,000+
	None	None		—	—	7,* 7, 7, 7	7, 8, 8, 8	8, 8, S, S	S, S, S, S	
21 days	0.5 cc. 1:100	1,600,000	17/29	S, S, S, S	S, S, S, S	S, S, S, S	—	—	—	10,000+
	0.5 " 1:1,000	160,000	4/20	S, S, S, S	D, S, S, S	S, S, S, S	S, S, S, S	—	—	10,000+
	0.5 " 1:10,000	16,000	3/20	S, S, S, S	S, S, S, S	S, S, S, S	D, S, S, S, S	—	—	10,000+
	0.5 " 1:100,000	1,600	0/20	S, S, S, S	S, S, S, S	S, S, S, S	D, S, S, S	S, S, S, S	—	10,000+
	0.5 " 1:1,000,000	160	0/20	S, S, S, S, S	S, S, S, S, S	S, S, S, S, S	S, S, S, S, S	—	—	10,000+
	None	None		—	—	6, 6, 7, 7	7, 7, 8, 8	8, S, S, S	10, S, S, S	

† The titer of the virus suspension used for the vaccination of mice was 10^{-7}.

* 7 = mouse died 7 days following injection. S = mouse remained well 30 days. — = dilution not tested. D = mouse died immediately following injection.

lymphocytic choriomeningitis virus for mice on intracerebral inoculation varies considerably. It may be low, 10^{-2} to 10^{-3} (Scott and Elford, 5; Coggeshall, 7), or irregular, with mice surviving in the lower dilutions (Rivers and Scott, 4), or high and regular (Armstrong and Lillie, 8). Strain II S.F. has been constantly high (10^{-6} or even 10^{-7}, when younger mice were used) and uniform (Tables I, II, and V).

Whether this difference rests primarily on the test mice or on the strains themselves is not definite, although both factors may be involved.

Strain C,[3] reputed to be non-virulent for mice above the 10^{-2} dilution, was passed three times intracerebrally through 3 weeks old W-Swiss mice and then titrated intracerebrally. Table IV shows the C strain to be virulent through the 10^{-5} dilution but less regular in its effects than the II S.F. strain run at the same time as a control.

Differences in Virulence of Strain II S.F. in 21 and 60 Day Old Mice.—Lymphocytic choriomeningitis contact infection occurs more readily in newborn than in adult mice (9). Sabin and Olitsky (10), moreover, have shown that equine encephalomyelitis virus is more virulent for young than for old mice when injected intraperitoneally. Strain II S.F., injected in 0.03 cc. doses intracerebrally, often shows a tenfold greater virulence in 60 day old than in 21 day old mice and, when injected intraperitoneally in 0.5 cc. amounts, it is non-virulent for the 60 day old but fatal to most of the 21 day old mice in a dilution of 10^{-2} and to some extent in dilutions of 10^{-3} and 10^{-4} (Table V).

Capacity of II S.F. to Induce Immunity in Mice.—Rivers and Scott have shown that mice surviving intraperitoneal or intranasal inoculation of lymphocytic choriomeningitis virus are immune to a subsequent intracerebral injection. Tests on the II S.F. strain, besides confirming these findings, afforded a rough measure of the high grade immunity attained.

II S.F. virus was titrated intracerebrally in 21 and 60 day old mice. Batches of each were then vaccinated intraperitoneally with 0.5 cc. of the same suspensions used for the virulence titrations, in dilutions from 10^{-2} to 10^{-6} inclusive. 17 days later the vaccinated and unvaccinated mice were injected intracerebrally with 0.03 cc. of test II S.F. virus in dilutions of 10^{-2} to 10^{-7}.

Table VI shows that as little as 160 cerebral lethal doses, given intraperitoneally as a vaccine, protected the mice against a later intracerebral injection of 10,000 lethal doses.

[3] This strain was kindly furnished by Dr. L. T. Coggeshall.

SUMMARY

A strain of lymphocytic choriomeningitis virus has been encountered, which grows readily in mouse embryo, serum, Tyrode culture media. Its origin is not definitely known but appears to be either the mouse brain tissue or, more probably, the monkey serum.

This strain gives clear cut results on filtration tests through Elford membranes, establishing the size of the virus, according to formula, as 33 to 50 mμ.

The strain shows a high and uniform virulence in W-Swiss mice. This appears to be due in part, at least, to the age and strain of mice employed for passage and titration.

The strain has been found to be more virulent in young than in old mice, especially following intraperitoneal inoculation.

Finally, the strain, when given as a vaccine intraperitoneally in amounts as small as 160 intracerebral lethal doses, induces an immunity against subsequent intracerebral inoculations of as much as 10,000 lethal doses.

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