MODIFICATION OF THE PATHOGENICITY OF PSEUDORABIES VIRUS BY ANIMAL PASSAGE

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INTRODUCTION

The identity of "mad itch" with the disease known in Europe as pseudorabies was suggested in an earlier paper (1) and more recently confirmed immunologically by the method of cross-neutralization (2). The use of the picturesque term "mad itch," while highly descriptive of the disease to which it has been applied in this country, must therefore give way to the earlier and somewhat misleading term pseudorabies. The etiological agent, which differs in certain ways (1) from the original Hungarian strain of pseudorabies virus (3) obtained from Aujeszky, with which it was compared, will hereafter be referred to as pseudorabies virus (Iowa strain). Only the Iowa strain has been employed in the present work.

EXPERIMENTAL

In our experimental work with pseudorabies virus it has been consistently observed that virus obtained from the brain of an intracerebrally infected rabbit differed in infectivity from that similarly obtained from the brain of an intracerebrally infected guinea pig. When a standard subcutaneous injection of 1 cc. of a 10 per cent suspension of infected brain (100 mg.) was administered, the virus from rabbits was regularly fatal to both rabbits and guinea pigs, while that from guinea pigs was pathogenic for rabbits only. Intracerebral inoculations, however, of 10 mg. doses of either type of virus were regularly fatal to both species. Further experiments, to be reported here, were conducted to determine the nature of the change induced in the pseudorabies virus by guinea pig passage.

The Effect of Serial Passage through Rabbits and Guinea Pigs upon the Pathogenicity of Pseudorabies Virus

The observation that virus from the brain of a guinea pig was innocuous when administered subcutaneously to guinea pigs was made repeatedly with virus that was only 1 or 2 passages removed from rabbits. It seemed of interest, therefore, to determine certainly whether the variations in pathogenicity exhibited by pseudorabies virus of guinea pig and rabbit origin were of a transient nature and induced only for the short time during which it was being adapted to a new host. Consequently, the virus was passed serially through the

TABLE I

Effect of Serial Passage through Rabbits and Guinea Pigs upon the Pathogenicity of Pseudorabies Virus

No. of intracerebral serial passages	No. of animal inoculated subcutaneously with 100 mg. of infected brain, and result
12th rabbit passage—brain Rabbit 118	Rabbit 121—Died 80 hrs. " 122— " 86 " Guinea Pig 216—Died 66 hrs. " " 217— " 78 "
9th guinea pig passage—brain Guinea Pig 213	Rabbit 119—Died 82 hrs. " 120— " 92 " Guinea Pig 214—No illness " " 215— " "

brains of 9 guinea pigs to establish a guinea pig type virus and through the brains of 12 rabbits to establish a rabbit type virus. All of the passage inoculations terminated fatally. The two sets of passages were conducted simultaneously and covered the same total elapsed time. The fact that the disease was more rapidly fatal in rabbits accounted for the greater number of rabbit passages accomplished during the period. The results of tests of the pathogenicity of the two types of virus are outlined in Table I.

The data recorded indicate that prolonged serial passage does not affect the differing pathogenicity of the virus from rabbit and guinea pig brain as determined by subcutaneous injection into guinea pigs in 100 mg. amounts.

The Alteration of the Pathogenicity of Pseudorabies Virus in a Single Passage

To determine whether the change from rabbit type to guinea pig type virus could be accomplished in a single passage, experiments like those recorded in Table II were conducted.

In this experiment virus from a rabbit, pathogenic for guinea pigs when administered subcutaneously, was, by 1 cerebral guinea pig passage, rendered non-pathogenic for guinea pigs on subcutaneous

TABLE II

Alteration of the Pathogenicity of Pseudorabies Virus in Single Passage Intervals

Passage	Source of virus	No. of animal inoculated, route and result
1st	Brain Rabbit 140	Guinea Pig 227 Sc—Died 58 hrs. " " 228 "— " 58 " " " 223 Ic— " 44 " " " 224 "— " 49 "
2nd	Combined brains of Guinea Pigs 223 and 224	Guinea Pig 231 Sc—No illness " " 232 "— " " " " 229 Ic— Died 54 hrs. " " 230 "— " 54 "
3rd	Brain Guinea Pig 230	Rabbit 132 "—Died 46 "
4th	Brain Rabbit 132	Guinea Pig 237 Sc—Died 67 " " " 238 "— " 67 "

Sc = subcutaneously. Ic = intracerebrally.

inoculation. 1 subsequent passage of this guinea pig virus through the brain of a rabbit restored its full pathogenicity for guinea pigs by subcutaneous inoculation.

That the alteration in pathogenicity of pseudorabies virus was not contingent upon a particular route of inoculation was indicated by the fact that the lung and brain of an intranasally inoculated guinea pig and the local subcutaneous lesion of a subcutaneously inoculated guinea pig contained virus which, while fully pathogenic for rabbits when administered subcutaneously, was completely innocuous for guinea pigs by the same route.

Titration of the Pseudorabies Virus

The experiments recorded above suggested strongly that passage of pseudorabies virus through guinea pigs attenuated it for guinea pigs without apparently affecting its pathogenicity for rabbits. To determine whether factors other than attenuation entered to account for the alteration in pathogenicity of the virus by guinea pig passage, a series of virus titrations was conducted. In all, 4 titration experiments were run, using 4 different samples of rabbit brain virus and 4 of guinea pig brain virus. Since the end-points of the titrations coincided approximately, the results of the experiments have been combined and recorded in Table III. In addition, animals receiving the standard 10 mg. intracerebral or 100 mg. subcutaneous dose of either type of virus during the period of the titration experiments have been included in the table to indicate the uniform nature of the results obtained at these dosages.

In the titration experiments dilutions were arranged in intervals of 10 and no effort was made to obtain more definite end-points. Fresh infected brain was used as the source of virus and the quantity administered was calculated on the basis of wet brain weight. The amounts of virus given intracerebrally were administered in 0.1 cc. quantities of physiological saline, while the subcutaneous doses were given in 1 cc. of physiological saline except in the case of the large amounts of guinea pig brain virus that it was necessary to use in infecting guinea pigs. In this last case infected brain was prepared in a 20 per cent suspension and the amount of this suspension administered determined by the size of dose desired. In a single experiment the type of virus under titration was injected into both guinea pigs and rabbits, so that between the species, the results are comparable. The results of the titrations are recorded collectively in Table III.

As shown in Table III, the minimal fatal dose of intracerebrally administered rabbit brain virus was, within a dilution factor of 10, the same for both rabbits and guinea pigs. The same was true for the guinea pig brain virus except that the fatal dose for both species was 0.1 mg. instead of 0.01 mg. Assuming that the difference in titration end-point was a matter of concentration of virus and not of attenuation for both species by guinea pig passage, it may be concluded that the brain of the rabbit contains roughly ten times as much active virus as does that of the guinea pig. This view is further supported by the fact that while 0.1 mg. of the rabbit brain virus proved fatal for rabbits

when administered subcutaneously, 1 mg. of the guinea pig brain virus was required to induce a similar effect.

Consideration of the data in Table III indicates further that the guinea pig possesses roughly a hundred times greater natural resistance to subcutaneously administered pseudorabies virus than does the rabbit, because, in titrating rabbit brain virus, 0.1 mg. was found to

TABLE III

Titration of Rabbit and Guinea Pig Types of Pseudorabies Virus

Dosage	Animals receiving rabbit brain virus								Animals receiving guinea pig brain virus							
	Guinea pigs Ic*		Rabbits Ic		Guinea pigs Sc*		Rabbits Sc		Guinea pigs Ic		Rabbits Ic		Guinea pigs Sc		Rabbits Sc	
	No. inoculated	No. died	No. inoculated	No. died	No. inoculated	No. died	No. inoculated	No. died	No. inoculated	No. died	No. inoculated	No. died	No. inoculated	No. died	No. inoculated	No. died
mg.																
0.0001	4	0	4	0												
0.001	6	2	6	2			1	0	3	0	3	0				
0.01	6	6	6	6	2	0	5	0	4	0	4	2			2	0
0.1	2	2	2	2	6	0	5	5	4	3	4	4			4	1
1	1	1	1	1	7	3	3	3	1	1	1	1			4	4
10	11	11	3	3	7	5			13	13					1	1
100					43	43	22	22					53	3	3	3
200													5	0		
400													8	2		
600													6	2		
800													6	4		
1000													5	4		

* Ic = intracerebrally. Sc = subcutaneously.

be fatal to rabbits subcutaneously while 10 mg. of the same virus was required to kill guinea pigs, at all regularly, by the same route.

DISCUSSION

In attempting to determine the factors underlying an alteration in the pathogenic properties of pseudorabies virus by guinea pig passage, we have resorted to titration of rabbit and guinea pig types of virus. These titration experiments indicate that, in addition to some apparent

attenuation of the virus on passage through guinea pigs, two factors besides attenuation enter to make the alteration in pathogenicity appear greater than it is. The first of these has to do with the presence in rabbit brain of approximately ten times more titratable virus than in guinea pig brain. The second concerns the observation that the guinea pig is approximately 100 times more naturally resistant to pseudorabies virus than is the rabbit.

Since both of these species differences are in the same direction, the question arises as to whether they alone are sufficient to account for what, in the beginning, appeared to be an attenuation of virus by the guinea pig. A tenfold greater concentration of virus in rabbit brain together with a hundredfold greater natural resistance on the part of guinea pigs to subcutaneous infection makes a thousandfold difference with regard to the two species that cannot be attributed to attenuation as it is generally understood. Taking this difference into consideration and calculating on the basis of the known minimal fatal doses of both types of virus for rabbits subcutaneously, the subcutaneous minimal fatal dose of guinea pig brain virus for guinea pigs should have been in the neighborhood of 100 mg. Since titration indicates that it was considerably higher than this, it is apparent that guinea pig passage exerts some attenuating influence on the pseudorabies virus for that species. The fact that the attenuation was evident upon subcutaneous infection and not when the guinea pigs were intracerebrally infected suggests that the explanation for the phenomenon is physical or mechanical.

That the attenuation was not due to an inhibiting substance in either normal or infected guinea pig brain was indicated by a series of experiments that may be briefly stated here. Normal guinea pig brain mixed in 200 mg. amounts with rabbit brain virus in amounts ranging from 10 to 200 mg. failed to prevent a fatal infection when the mixture was inoculated subcutaneously into guinea pigs nor did it lengthen the period of survival of the animals. In like manner, guinea pig brain virus rendered non-infective by heating to 55° and 60°C. for 1/2 hour, when mixed in 200 mg. amounts with rabbit brain virus in 100 mg. amounts, failed to prevent a fatal infection or to lengthen the period of survival when inoculated subcutaneously into guinea pigs.

SUMMARY

Pseudorabies virus, Iowa strain ("mad itch"), after passage through guinea pig brain, fails to produce infection in guinea pigs when injected subcutaneously unless enormous doses are employed. Such virus is still pathogenic for rabbits when given subcutaneously and for rabbits and guinea pigs intracerebrally. Comparison of the amounts of virus present in the brains of rabbits and guinea pigs following fatal cerebral infection shows that the latter contain, per gram, only approximately one-tenth the amount of virus in the former. Comparing the resistance of the two species to subcutaneously administered pseudorabies virus it has been found that rabbits are approximately 100 times more susceptible than guinea pigs. Over and above the working of these two factors, guinea pig passage appears to achieve some actual attenuation of virus when tested by subcutaneous inoculation into guinea pigs.

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