AN EXPERIMENTAL STUDY OF "MAD ITCH" WITH ESPECIAL REFERENCE TO ITS RELATIONSHIP TO PSEUDORABIES

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(Received for publication, May 6, 1931)

INTRODUCTION

In August of 1930 an outbreak of "mad itch" was observed in a herd of dairy cattle in Johnson County, Iowa.[1] In order to evaluate the experimental disease, an account of the clinical picture as observed in this herd will be given. The disease is so rare as to constitute a veterinary curiosity.

There were twelve animals in the herd and of these nine succumbed to the disease. No evidence of illness was observed in the remaining three. The clinical course in the nine fatal cases was rapid and in all but one an extreme pruritus was the cardinal feature of the disease. A persistent and continuous licking of an area of skin somewhere on the hind quarters, most commonly between the udder and leg, and a sudden decrease in milk production constituted the first evidence of illness. After 2 or 3 hours of continual licking the area of skin would be completely denuded of hair and bright red in color. The condition was steadily progressive and the area of abraded skin became larger. The pruritus evidently increased in intensity for the animals would rub violently against posts, barbed wire, gates, or any other convenient object, and would bite and gnaw frenziedly at themselves. By the end of 24 hours the buttocks and thighs were denuded, swollen and discolored, oozing a serosanguineous fluid, and the animals were usually down although still able to rise and walk unsteadily about. There was no temperature elevation at this time. Salivation and marked grinding of the teeth were observed in some cases. The animals became progressively weaker and died within 36 to 48 hours after the first evidence of illness. Death was preceded by clonic convulsions, violent tossing of the head, crying, and rapid shallow respiration. One

[1] Dr. Fred J. Crow of Iowa City called my attention to this outbreak of "mad itch," made the material available to me, and cooperated in the preliminary field work. I thank him heartily.

of the nine animals had failed to show any evidence of pruritus. This animal was ill for 6 days and lay listlessly in the barn lot during this period. On the last day she developed clonic convulsions, salivated, ground her teeth, and died in much the same manner as the others.

Attempts to Reproduce "Mad Itch" Experimentally

Cerebrospinal fluid in a sterile container from one cow and portions of brain from three others in 50 per cent glycerine were brought back to the laboratory on ice. A period of 2 weeks elapsed between the time of the collection of this material and its inoculation. Samples of various specimens were injected subcutaneously and intracerebrally into rabbits, guinea pigs, rats, and mice. Only one was found capable of inducing recognizable disease, this being the brain from the cow that had failed to exhibit any evidence of pruritus and had survived longer than any of the other affected animals.

Physiological salt solution suspensions of the effective brain when injected subcutaneously into rabbits reproduced the clinical picture of "mad itch" as seen in the cattle.

After incubation periods usually ranging from 50 to 75 hours the rabbits appeared uneasy and began biting and scratching mildly at the site of inoculation. There was no temperature elevation at this time. The condition became progressively worse and the biting and scratching more persistent and savage. The skin over the site of inoculation and over a large surrounding area was soon denuded of hair and abraded, and it oozed a serosanguineous fluid. As the animals became weaker, their attempts at biting and scratching were more feeble. They lay on their sides unable to rise, their respirations became rapid and in some cases noisy, and as a rule there was a marked temperature elevation. Death ensued from 6 to 24 hours after the first evidence of pruritus and it was preceded by loss of consciousness and mild clonic convulsions.

This clinical picture has been produced regularly and in quite a large series of rabbits by the subcutaneous injection of either suspensions of the original cow brain or of brains of intracerebrally infected rabbits or guinea pigs. Four out of five attempts to infect guinea pigs by subcutaneous inoculation with the original cow brain were unsuccessful and the material was also innocuous when administered subcutaneously to white rats and white mice.

Injected intracerebrally, the material produced a uniformly fatal disease in rabbits, guinea pigs, and white rats and mice.

Rabbits died in from 24 to 50 hours following inoculation and the period of actual illness was short. After an incubation period of from 20 to 40 hours the animals appeared nervous and excited and soon were observed to be blind. They became more excited and would sometimes run wildly about their cages butting their heads violently against the door. At other times they would retract their heads, lifting a front foot as though fearing attack. They frequently continued the retraction of their heads until they would rise on their hind legs and fall over backwards. No temperature elevation was to be observed until just before death when a marked rise occurred. Death was usually preceded by coma and rapid and sometimes noisy respiration. Salivation and grinding of the teeth were fairly constantly to be observed.

The above picture has been produced regularly in rabbits by the intracerebral injection of the original cow brain or by the injection of suspensions of brains from intracerebrally infected rabbits or guinea pigs. No pruritus resulted following intracerebral inoculation. The symptoms observed in guinea pigs, rats, and mice following intracerebral inoculation were similar to those seen in rabbits but they were of less violent character and the periods of incubation and survival were greater.

The disease induced in rabbits by the intranasal instillation of "mad itch" virus deserves special mention in that it differed from that induced by cerebral or subcutaneous inoculation.

For a period of 4 days following infection the animals appeared perfectly normal, ate well, and exhibited no temperature elevation. On the beginning of the 5th day the respiration of some of the animals was slightly accelerated and in one frequent sneezing was observed. The animals did not appear ill, however, and there was no temperature elevation. Death occurred suddenly on the 5th day and without warning. At autopsy the most significant pathology was found in the lungs. These were of a glistening purplish red color, heavy, and filled with a serosanguineous fluid. The bronchi and trachea usually contained an abundance of a blood-tinged frothy exudate and there were many submucous hemorrhages.

Both the clinical and the postmortem picture of the disease produced by the intranasal instillation of the virus are strikingly suggestive of a rather common bovine disease which, like "mad itch," is generally classed as hemorrhagic septicemia.

Gross Pathology

The postmortem picture of cattle dead of the spontaneous disease was always the same. The skin over the thighs and buttocks was denuded of hair, dark,

leather-like, and smeared with a serosanguineous fluid. When the skin in these regions was incised a dark red, serous, and occasionally gelatinous material was encountered in the greatly thickened subcutaneous tissue. The underlying muscle was not involved. The abdominal viscera, aside from a moderate congestion, appeared normal. The stomachs were well filled. The lungs of some of the cows exhibited areas of an edematous type of congestion; the hearts of all were spotted with numerous petechial hemorrhages, and in all cases there was an excess of pericardial fluid. Aside from moderate injection of the meningeal vessels nothing pathological in the gross was encountered in the central nervous system. There was thus no gross anatomical cause of death apparent in the spontaneous cases.

In rabbits inoculated subcutaneously, the skin in the region of the site of inoculation was denuded of hair, abraded, and moistened by a serosanguineous fluid. The underlying subcutaneous tissue was thickened, edematous, and bloody. The regional lymph nodes were enlarged and congested. The stomach, intestines, and colon were found to be well filled. The urinary bladder was, as a rule, greatly distended. The liver was strikingly of a "nutmeg" appearance. The spleen and kidneys were at the most no more than moderately congested. The heart was injected and at times spotted with petechial hemorrhages. The thymus was large, sometimes almost completely covering the heart, and was dotted with variously sized areas of hemorrhage. The lungs were a glistening purplish red, heavy, and on cut section exuded a serosanguineous fluid. The bronchi often contained a thin frothy mucus. Aside from a mild to moderate injection of the meningeal vessels nothing abnormal was encountered in the central nervous system. In contrast to the absence of gross lesions in cattle, the intensely hemorrhagic pulmonary edema could be readily held accountable for the death of experimentally infected rabbits. The gross pathological picture presented by intracerebrally inoculated rabbits was the same as that outlined for subcutaneously inoculated animals except that the lesions of the skin and subcutaneous tissue were absent. The postmortem picture presented by guinea pigs dead of experimental "mad itch" was the same as that seen in rabbits except that the lesions in the skin and subcutaneous tissue were more extreme and the pulmonary lesions less marked.

The histopathology will be reported later.

Etiology

As has been reported briefly elsewhere (1), no ordinary bacterial organism has been discovered in "mad itch" materials of proven infectivity, by methods of culture and direct microscopic examination. On the other hand, suspensions of emulsified brain of intracerebrally inoculated rabbits when passed through Chamberland L^3 and Berke-

TABLE I

Filtration Experiments

Filter		Material filtered, approximately 10% suspension of rabbit brain		Time of filtration	Amount of filtrate	Maximum negative pressure	Dosage of filtrate administered and route	Rabbit inoculated. No.	Result
Type	No.	Suspension	Rabbit No.						
				min.	*cc.*	*cm. of mercury*	*cc.*		
1. Seitz		A	84 and 85	2		Positive pressure	1 sc.*	95	No illness
							0.15 ic.	76	" "
2. "		B	76, 94, and 95	2	20	" "	1 sc.	114	Died—105 hrs.
3. "		B	76, 94, and 95	2	25	" "	1 sc.	108	No illness
4. Berkefeld V	10	A	84 and 85	0.5		28	1 sc.	92	Died—96 hrs.
5. " V	10	B	76, 94, and 95	1	26	48	1 sc.	106	" —76 "
6. " N	6	A	84 and 85	0.5		37	1 sc.	90	" —99 "
							0.15 ic.	75	" —46 "
7. " N	1	B	76, 94, and 95	2	20	49	1 sc.	107	" —76 "
8. " N	7	B	76, 94, and 95	2	10	30	1 sc.	111	" —90 "
9. " N	8	B	76, 94, and 95	2	20	57	1 sc.	105	" —98 "
10. " N	1	C	110	3	40	45	1 sc.	131	" —89 "
11. " W	9	B	76, 94, and 95	2	6	42	1 sc.	110	No illness
12. " W	11	B	76, 94, and 95	2	8	60	1 sc.	109	" "
13. " W	11	D	108	12	5.5	60	0.15 ic.	109	Died—50 hrs.
14. " W	11	C	110	2	3	43	1.5 sc.	126	No illness
15. " W	11	C	110	12	10	60	1.5 sc.	125	Died—89 hrs.
16. Chamberland L^3	1	B	76, 94, and 95	2	5	55	1 sc.	112	" —125 "
17. " L^3	2	B	76, 94, and 95	2	3.5	45	1 sc.	113	No illness
18. " L^3	4	C	110	10	10	63	1.5 sc.	129	Died—101 hrs.

* sc. = subcutaneously.
ic. = intracerebrally.

feld V, N, and W filters were effective in inducing the disease in rabbits. A summary of the results is given in Table I.

In all but one of the filtrations (No. 7) infusion broth pH 7.3 was used as the vehicle in preparing the brain suspensions, but in this one case in which physiological salt solution was used no observable difference in the filtrability could be noted. In some of the experiments the brain suspensions were frozen and thawed before filtration. This procedure was unessential, for unfrozen suspensions filtered with equal facility. All suspensions were cleared of gross particles by centrifugation for 30 minutes before filtration. The moderately turbid supernatant fluid was then removed for use in the filtrations. To this was added 24 hour broth cultures of *B. prodigiosus* in the ratio of 1 cc. of culture to each 10 to 15 cc. of suspension to be filtered. 1.5 cc. amounts of the resulting filtrates were tested for sterility, as regards *B. prodigiosus*, in broth culture. All filtrates recorded in the protocol except Seitz Filtrates 2 and 3 were bacteriologically sterile. As can be seen from the table, time was an important factor in the passage of the virus through the finer pored filters. Out of five attempts at filtration through Berkefeld W filters, three filtrates in which the filtration was continued for only 2 minutes did not contain virus demonstrable in the dosage employed, while two filtrates in which the filtration was continued for 12 minutes contained quantities of virus lethal for rabbits in ordinary dosage. Difficulty was experienced in the Seitz filtrations because of the facility with which these filters passed *B. prodigiosus* in infusion broth. In one Seitz filtration in which *B. prodigiosus* was retained by the filter the "mad itch" virus was not present in demonstrable quantities in the filtrate. Two other Seitz filtrations are difficult to interpret because while both permitted the passage of *B. prodigiosus* only one permitted the passage of virus in demonstrable quantity. Animals inoculated with filtered material were adequately isolated from animals receiving unfiltered material.

The recording of the same animal in more than one experiment requires explanation. Rabbits negative in certain experiments have, after a period of observation of 2 weeks, been used over again in later experiments. The procedure may be questioned because of the possible development of some immunity following the administration of sublethal doses of virus. However, in the experiments recorded in Table I no animal, negative in an earlier experiment, failed to develop "mad itch" and die when later inoculated. This eliminates from consideration the possibility that in these experiments a confusing immunity had been set up as a result of the earlier use of the animals.

Neutralization of Virus with Immune Serum

It has been possible to induce an immunity in a guinea pig sufficient to protect that animal completely against a subcutaneous dose of rabbit brain virus always lethal for unprotected guinea pigs.

The procedure, in brief, consisted in the preliminary subcutaneous administration of the original cow brain virus which was capable of producing a fatal infection in only one out of five guinea pigs to which it was administered subcutaneously. The four guinea pigs that had been refractory to inoculation with cow brain virus were subsequently inoculated subcutaneously with rabbit brain virus known to be capable of inducing a fatal infection when administered subcutaneously to guinea pigs. Three of the animals died and the fourth (Guinea Pig 168) exhibited no evidence of illness. This fourth guinea pig has since been inoculated subcutaneously seven times with various suspensions of rabbit brain virus of proven pathogenicity for guinea pigs, and has shown no evidence of illness following any of the inoculations. The animal has been bled repeatedly from the heart at suitable intervals between inoculations with virus and the serum has been tested for its ability to neutralize virus. Typical experiments are recorded in Table II.

In the virus neutralization experiments the mixtures were usually kept at room temperature for 2 hours and at refrigerator temperature overnight before inoculation into the test animals. This preliminary period of incubation was unnecessary, for virus mixed with immune serum just before injection, into guinea pigs, was apparently as completely neutralized. Neither was it necessary to have a relatively cell-free virus suspension to attain complete neutralization, for in a number of instances the coarse uncentrifuged brain suspensions were completely neutralized by small amounts of immune serum. With the use of smaller doses of virus and larger amounts of immune serum than are recorded in Table II, it has recently been possible to attain mixtures that were completely neutral for rabbits. It has also been found that mixtures neutral for guinea pigs when administered subcutaneously are also neutral when injected intraperitoneally.

Routes of Infection

Uniformly fatal infections in rabbits have resulted following subcutaneous, intracerebral, intravenous, intratesticular, intraperitoneal, and intranasal inoculation with the "mad itch" virus. Fatal infections result from small doses of virus (0.2 cc.) injected into the tip of the ear and also from scarification through a drop of virus placed on a shaven area of skin. 0.001 cc. of the supernatant fluid of a centrifuged 10 per cent suspension of rabbit brain virus was sufficient to induce typical fatal "mad itch" in a rabbit when injected subcutaneously, whereas 0.0001 of the same virus suspension failed to infect.

TABLE II

Neutralization Tests with Immune Guinea Pig Sera

Source of virus	Amount of virus	Serum	Animal*	Result
	cc.			
			Rabbit	
Supernatant fluid of centrifuged suspension of brains of Rabbits 87, 96, and 97	1	1 cc. immune serum Guinea Pig 168	99	Died—122 hrs.
	1	0.5 cc. immune serum Guinea Pig 168	100	" —112 "
	1	0.1 cc. immune serum Guinea Pig 168	101	" —103 "
	1	1 cc. normal serum Guinea Pig 211	102	" — 74 "— Control
Berkefeld N filtrate of suspension of brain of Rabbit 110	1	2 cc. immune serum Guinea Pig 168	128	Died—174 hrs.
	1	0.5 cc. immune serum Guinea Pig 168	130	" —113 "
	1	1.5 cc. normal serum Guinea Pig 211	131	" — 89 " — Control
			Guinea Pig	
Supernatant fluid of centrifuged suspension of brain of Rabbit 140	1	1 cc. immune serum Guinea Pig 168	235	No illness
	1	0.1 cc. immune serum Guinea Pig 168	234	" "
	1	1 cc. normal serum Guinea Pig 233	236	Died— 83 hrs. Control
10% suspension of brain of Rabbit 165	1	1 cc. immune serum Guinea Pig 168	263	No illness
	1	0.1 cc. immune serum Guinea Pig 168	262	" "
	1	1 cc. normal serum Guinea Pigs 233 and 238	261	Died— 45 hrs.— Control

* All animals inoculated subcutaneously.

Guinea pigs develop fatal infections uniformly following subcutaneous, intraperitoneal, intratesticular, intracerebral, or intranasal inoculation with rabbit brain "mad itch" virus.

White rats and mice develop fatal infections irregularly following intraperitoneal inoculation, regularly following intracerebral inoculation, and are refractory to subcutaneous inoculation with the "mad itch" virus.

Distribution of Virus in the Animal Body

The "mad itch" virus tends to localize largely in the region of the site of inoculation and the lung. The brain of an animal inoculated intracerebrally will be regularly rich in virus, and the lung will also contain it. However, virus cannot be demonstrated in the heart blood, liver, and spleen. In animals inoculated subcutaneously virus is demonstrable in the bloody fluid of the subcutaneous tissue at the site of inoculation and in the lung, but it has not been demonstrable in the heart blood or spleen. Neither is it demonstrable in the brains of subcutaneously inoculated animals unless these animals have survived unusually long as the result of partial protection by immune serum. It was present in the liver of one out of three animals examined. In animals infected intranasally the virus is found in both the lung and the brain but not in the liver or blood. As dependable sources of virus the brains of intracerebrally inoculated animals or the testicles of intratesticularly inoculated animals can be relied upon. In testing for the presence of virus in various tissues 1 cc. of a 10 per cent suspension was uniformly the inoculum employed.

Animals Susceptible to the "Mad Itch" Virus

While most of our experience has been with rabbits, guinea pigs, and white rats and mice, we have tested some other species for susceptibility to the "mad itch" virus. The virus, after fourteen serial intracerebral passages in rabbits was still capable of producing a fatal infection when injected subcutaneously into its natural animal host. A calf inoculated subcutaneously with 1.5 cc. of a 6.6 per cent suspension of rabbit brain virus, after an incubation period of 4 days developed typical "mad itch" simulating in all respects the spontaneous disease as seen in the original cows in Iowa. The animal died after about 45 hours

of illness. The virus produced a fatal "itching" disease when inoculated subcutaneously into a cat. Gray field mice succumbed following intraperitoneal inoculation. Two ducks inoculated intracerebrally with the virus died after 4 and 18 days, and a suspension prepared from the brain of the first of these birds and inoculated in relatively small dosage subcutaneously into a rabbit resulted in a typical fatal "mad itch." One duck inoculated subcutaneously failed to develop any evidence of illness. The virus was innocuous for chickens when administered subcutaneously or intratracheally but following intracerebral inoculation fatal infections resulted in two out of three cases in 6 and 11 days. A suspension prepared from the brain of the first of these chickens and injected in relatively small dosage subcutaneously into a rabbit resulted in typical fatal "mad itch," proving the etiological nature of the disease to which the chickens had succumbed. Two swine inoculated subcutaneously with the virus became ill. No pruritus was observed in either animal. One animal developed what appeared to be an acute encephalitis. It lay on its side unable to rise, had clonic convulsions, and salivated. After a day of this it began to improve and could rise again although its hind quarters were weak and its gait unsteady. Recovery was finally complete. The course of the disease had been entirely afebrile. The other swine exhibited no evidence of active central nervous system involvement. It lay listlessly in its pen, did not eat, and was febrile for 6 days. Its recovery was uneventful and complete. From the clinical picture alone it would have been impossible to state definitely that the disease which developed in either hog was the result of infection with the "mad itch" virus. To obtain information on this point, blood serum from both animals after they had completely recovered was tested for virucidal properties, using as the control serum a sample of blood serum obtained from one of the swine prior to inoculation with the "mad itch" virus.

The results of this virus neutralization are given in Table III.

The virus-serum mixtures recorded in Table III were allowed to stand 2 hours at room temperature and 20 hours at refrigerator temperature before inoculation into animals. Consideration of the data indicates that, in the dilutions used, both samples of convalescent swine serum completely neutralized the "mad itch" virus whereas the

serum from one of the animals obtained before its inoculation with "mad itch" virus was devoid of virus-neutralizing properties. It therefore seems clear that the disease which developed in the swine was actually due to the injected "mad itch" virus and that swine are susceptible to the virus although more resistant than other species tested. Such a fact suggests the possible existence of a swine disease due to "mad itch" virus. It seems probable that a potent virucidal serum can be got from swine.

No definite information concerning the susceptibility of man to infection with the "mad itch" virus has been obtained during the

TABLE III

Neutralization Experiments with Convalescent Swine Sera

Virus — 10% suspension brain Rabbit 148	Serum	Guinea Pig No.*	Result
cc.			
1	0	249	Died—65½ hrs.
1	1 cc. normal serum Swine 810	248	" —70 "
1	1 " " " " 810	238	" —78 "
1	1 cc. convalescent serum Swine 810**	239	No symptoms
1	1 " " " " 810**	246	" "
1	1 " " " " 772***	237	" "
1	1 " " " " 772***	247	" "

* All animals inoculated subcutaneously.

** Serum drawn 17 days after inoculation.

*** Serum drawn 28 days after inoculation.

investigation. The writer and his helper have used ordinary precautions in handling infectious material but as autopsies have occasionally been done without gloves the bare but relatively intact skin of the hands has come in intimate contact with material of known infectivity without any untoward result. The violence of the disease, its uniformly fatal outcome, and the wide variety of species susceptible to it provide sufficient reasons for caution during work with it.

Contagiousness of Experimental "Mad Itch"

In a test of the contagiousness of experimental "mad itch" in rabbits, a doe and her litter of four were used, representing as intimate a form

of contact as was attainable under experimental conditions. All were in the same cage. The young were 22 days old at the time of their mother's infection and not completely weaned although they ate freely of the rations furnished the mother. The doe received 0.2 cc. of a 10 per cent suspension of rabbit brain virus subcutaneously in the tip of one ear and after an incubation period of 58 hours developed typical "mad itch." Pruritus and self-mutilation were limited to the inoculated ear and the side of the head. The animal died after an illness of 8 hours. No disease developed in any of the young rabbits during a period of observation of 2 weeks following their mother's death. They were all then tested for susceptibility by inoculation subcutaneously and all succumbed to the "mad itch" virus. Three other experiments have been conducted in which normal rabbits have been kept in cages with intranasally infected rabbits and in no instance has disease developed as a result of contact.

No attempt has been made as yet to determine how the disease is contracted in the field. It may be of significance that on the farm where the outbreak of "mad itch" was seen in the dairy cattle there had been a highly fatal epizootic of some sort among the rat population the preceding week. However, no rat carcasses were available for examination and no live rats could be found on the place at the time that the cattle were sick.

Viability of Virus when Glycerinated or Dried

Infectious rabbit brain stored in 50 per cent glycerol at refrigerator temperature still contains active and only slightly attenuated virus after 154 days. Similar infectious material frozen and dried by Swift's method (2) still contains active virus after 106 days' storage. Dried or glycerinated material has not been tested after longer periods of storage.

The Relationship of "Mad Itch" and Pseudorabies

The clinical picture of "mad itch" is very suggestive of pseudorabies, first described by Aujeszky (3). In fact, only after some experimentation did any doubt arise that we were dealing with the latter. White (4) in a text-book of veterinary medicine has classed "mad itch" as synonymous with pseudorabies but on no experimental basis. By

veterinary practitioners it is frequently considered a form of bovine hemorrhagic septicemia and, as such, treated unsuccessfully.

Professor Alădar Aujeszky of the Ungarische Tierärztliche Hochschule, Budapest, has kindly furnished me with a Hungarian strain of pseudorabies virus for the purpose of comparing the disease with "mad itch."

Pruritus localized to the region of the site of inoculation is the cardinal symptom after the subcutaneous inoculation of rabbits with either the "mad itch" or the pseudorabies virus. The incubation period and the period of survival following the onset of symptoms are the same with both viruses. Animals inoculated with pseudorabies virus exhibit more symptoms referable to central nervous system involvement and have a more "rabid" appearance than animals infected with "mad itch." Otherwise there are no observable differences in the clinical pictures. The findings at autopsy in an animal dead of "mad itch" are identical with those due to pseudorabies.

The distribution of virus in the body of an animal dead of "mad itch" differs widely from that in pseudorabies. Aujeszky (3), Schmiedhoffer (5), Zwick and Zeller (6), Sangiorgi (7), Carini and Maciel (8), and Isabolinsky and Patzewitsch (9) agree that the pseudorabies virus can be demonstrated not only in the local lesion but also in the brain, blood, and all highly vascular organs; it is a septicemic virus. The observation has been confirmed in this laboratory. In "mad itch," however, the virus is relatively localized, as indicated earlier in the paper.

Data concerning the filtrability of the etiological agent of pseudorabies are contradictory, and consideration of the literature would indicate that the virus is filtrable only with difficulty and irregularity at the best. Schmiedhoffer (5) found that the etiological agent was capable of passage through coarse pored filters which, however, retained *B. coli*, rotlauf, and fowl cholera bacilli. Sangiorgi (10), using a Brazilian strain of the disease, was irregularly successful in passing the virus through Berkefeld filters N and V. Aujeszky (3), Zwick and Zeller (6), Isabolinsky and Patzewitsch (9), and Bertarelli and Melli (11) were unsuccessful in filtering it. In a single filtration experiment conducted in this laboratory with pseudorabies, the virus has been found capable of passage through a Berkefeld filter of N porosity.

For this filtration brain tissue from an intracerebrally inoculated rabbit was suspended in infusion broth of pH 7.3 and frozen and thawed twice before filtration. All of the recorded attempts to filter the pseudorabies virus were carried out prior to 1914, that is to say prior to the recent advances in methods and technic in filtration.

Among the laboratory animals, rabbits are stated to be more susceptible to pseudorabies than guinea pigs, and guinea pigs in turn are more susceptible than white rats and mice (3, 5, 6). This same order of degree of susceptibility is true of "mad itch." However, Zwick and Zeller (6) found that rats were refractory to intraperitoneal inoculation with the pseudorabies virus, whereas in my experience the intraperitoneal injection of the "mad itch" virus almost always results in a fatal infection. Also chickens are considered insusceptible to infection with pseudorabies (3, 5, 6), but they succumb quite regularly to intracerebral inoculation with "mad itch" virus. Swine are stated (5) to be insusceptible to pseudorabies. They develop a non-fatal disease following inoculation with "mad itch" virus. Von Rátz (12), however, has observed pseudorabies in wild swine.

Aujeszky (3), Zwick and Zeller (6), and Schmiedhoffer (5) agree that pseudorabies is not contagious. "Mad itch" has proved not contagious even on intimate exposure, as the present paper shows.

Experimental infection with "mad itch" and pseudorabies can be induced by the same routes. However, Schmiedhoffer (5) has stated that intranasal inoculation of the pseudorabies virus proved innocuous unless the nasal mucous membrane was injured. The "mad itch" virus, on the other hand, regularly induces fatal infections when placed upon the intact mucous membrane of the nose. Using Aujeszky's strain of pseudorabies we have been successful in infecting a rabbit intranasally in the absence of any injury to the nasal mucosa.

It has been possible to obtain virucidal sera from both guinea pig and swine by the injection of the "mad itch" virus, and these sera are capable of completely neutralizing virulent "mad itch" virus as shown by the inoculation of mixtures of virus and serum into guinea pigs or rabbits. The virucidal swine serum is true convalescent serum while that from the guinea pig has been obtained without inducing any observable illness in the source animal. With a view to determining the relationship of the "mad itch" and pseudorabies viruses cross-

neutralization tests were conducted with these sera. It was found that the sera virucidal for "mad itch" were capable of completely neutralizing the pseudorabies virus. The results of a typical experiment are given in Table IV.

There is no record in the literature of the preparation of a pseudorabies virucidal serum, and we have been unsuccessful up to now in immunizing animals.

TABLE IV

*Neutralization of Pseudorabies Virus by Anti-"Mad Itch" Sera**

Pseudorabies virus, 10% suspension of brain of Rabbit 168	Serum	Guinea Pig No.**	Result
cc.			
1	0.5 cc. normal serum Guinea Pigs 233 and 238	270	Died—81 hrs.
1	0.5 cc. anti-"mad itch" serum Guinea Pig 168	272	No illness
1	0.1 cc. anti-"mad itch" serum Guinea Pig 168	271	" "
1	1 cc. normal serum Swine 810	273	Died—93 hrs.
1	1 cc. "mad itch" convalescent serum Swine 772	274	No illness
1	1 cc. "mad itch" convalescent serum Swine 810	275	" "

* The virus-serum mixtures were allowed to stand 2 hours at room temperature and 14 hours at refrigerator temperature prior to inoculation into animals.

** All animals inoculated subcutaneously.

From the data presented in this paper the writer is led to the tentative conclusion that the virus of "mad itch" and that of pseudorabies are the same; the differences observed may be accounted for on the basis of slight differences in strains of the same virus. Further information as to their relationship must await completion of cross-neutralization tests with "mad itch" virus and pseudorabies virucidal serum.

SUMMARY AND CONCLUSIONS

The clinical picture and gross pathology of spontaneous and experimental "mad itch" have been described and the inciting agent has been

shown to be a filtrable virus. It has been possible to prepare virucidal serum capable of neutralizing the virus. Fatal infections are regularly produced in rabbits when the virus is administered subcutaneously, intracerebrally, intravenously, intratesticularly, intraperitoneally, intranasally, or when it is dropped on a scarified area of skin. Its infectivity for other species by various routes is reported upon. The rabbit, guinea pig, white rat, white mouse, gray field mouse, cow, cat, duck, chicken, and hog are susceptible to experimental infection. The disease is not contagious under laboratory conditions and the virus is restricted in the animal body largely to the region of inoculation and the lung. The virus can be stored for relatively long periods in 50 per cent glycerol or in the dried state.

A comparison of "mad itch" with pseudorabies leads to the tentative conclusion that the inciting agents of both are the same, although the strains of the two viruses that are under study possess readily demonstrable differences.

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