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Spread of Virus in an Unvaccinated Case of Human Rabies.

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A necropsy on an unvaccinated case of human rabies presented an opportunity to investigate several questions regarding the spread of the virus in human beings and the development of immune bodies during the course of the disease. Since rabies is noted for the centrifugal spread of the virus it was desirable to determine (particularly for correlation with poliomyelitis in man) to what extent the virus spread outwards along the olfactory pathway and whether or not it appeared in the feces in a case in which the portal of entry was known to be the hand.

The patient was a 55-year-old man who was bitten on the right hand by a dog. There was no treatment other than superficial cleansing of the wound. He died 2 months later after an illness of 2 to 3 days with a clinical diagnosis of rabies. Necropsy was limited to the head, and the following structures were investigated: (1) The skin and subcutaneous tissue at the site of the bite (which was still marked by hyperemic scar tissue) after superficial sterilization with iodine and alcohol, (2) upper cervical cord and medulla, (3) cornu ammonis, (4) olfactory bulbs, (5) nasal mucosa (mostly olfactory) removed after an intracranial exposure of the roof of the nasal cavity, (6) tonsillar and pharyngeal tissues, (7) saliva obtained by swabbing out the mouth and throat with sterile absorbent cotton, (8) feces removed from the rectum by spatula, (9) subarachnoid fluid over the exposed cerebral cortex removed with a needle and syringe, and (10) heart blood. The feces was prepared for inoculation in two different ways: (a) one 2 g sample was shaken with glass beads in 10 cc of broth and after horizontal centrifugation at about 2000 rpm for 15 minutes the supernatant liquid was passed through a Berkefeld "V" filter which had previously been saturated with 20 cc of broth; (b) a 2.5 g sample was shaken in 15 cc of distilled water and after centrifugation as above, the supernatant liquid was mixed with 2 cc of anaesthetic ether and thoroughly shaken for 10 minutes; after 5 hours in the refrigerator at approximately 5°C the mixture was again centrifuged and the very opalescent fluid portion removed. Preparation (a) was bacteria-free while preparation (b) was not. The saliva and mucus

were freed from the absorbent cotton with the aid of 30 cc of physiological salt solution. After horizontal centrifugation one part was injected into mice without any further treatment, another part was treated with 15% of anaesthetic ether in the same manner as the feces, and still another part was mixed with one-tenth its volume of rabbit serum and passed through a Berkefeld "V" filter which had been saturated with 10 cc of 10% rabbit serum in physiological salt solution. The other tissues were prepared as indicated in Table I. With the exception of the heart blood, subarachnoid fluid, and the centrifuged, untreated suspensions of the nasal mucosa and of the tonsillar and pharyngeal tissues, each specimen was injeced into 6, two- to three-week-old white mice and one rabbit; where this number of animals does not appear in the table it means that they died within one or 2 days as a result of the inoculation.

The results shown in Table I indicate that while there was no demonstrable virus at the site of inoculation, *i.e.* the bitten area, there was enough present in the cervical cord and medulla, the cornu ammonis, and the olfactory bulbs to produce rabies in all the inoculated animals after a relatively short incubation period. The diagnosis of rabies was made by the demonstration of large numbers of typical Negri bodies in films of the brains of succumbing animals, by positive passage, and finally by neutralization with rabies immune serum which was kindly supplied by Dr. L. T. Webster of the Rockefeller Institute. A very small amount of virus, only enough to produce rabies in one of 6 mice, was present in the untreated, centrifuged suspensions of the nasal mucosa and of the tonsillar and pharyngeal tissues, while none was found in the Berkefeld "V" filtrates of the same preparations. Virus was also not demonstrated in the heart blood, subarachnoid fluid, saliva, and feces. The unaffected mice and rabbits were observed for 8 weeks and were then tested for immunity by an intracerebral injection of a 10^{-3} dilution of the virus (derived from the cord and medulla of this case and passaged in mice) which represented approximately 10 minimal mouse cerebral lethal doses. All of 62 test mice and 15 control mice of the same age developed rabies; 5 test rabbits and one control also succumbed.

With regard to the centrifugal spread of the virus along the olfactory pathways in human rabies, it would appear therefore that after entry by way of the nerves supplying the hand the virus can spread to and be present in appreciable amounts in the olfactory bulbs; the small amount which was demonstrated in the nasal mucosa indicates how little the extracranial part of the olfactory system can

TABLE I.
Distribution of Virus in an Unvaccinated Case of Human Rabies.

Material tested	Preparation for inoculation	Amt cc	Site	Animals inoculated	Result	Negri bodies	Passage
Skin lesion (site of bite)	Centrifuged suspension	.03	i. cer.	6 mice	0,0,0,0,0,0		
		.5	''	1 rabbit	0		
		.6	masseter				
Heart blood	.....	.03	i.cer.	6 mice	0,0,0,0,0,0		
Subarachnoid fluid over cortex	.....	.03	''	'' ''	0,0,0,0,0,0		
Upper cervical cord and medulla	10% susp.	.03	''	'' ''	8*,10,10,11,11,11	+	+
		1.0	i.a.				
		.5	i.cer.	1 rabbit	CNS 8, D9†	+	+
		2.5	i.a.				
Cornu ammonis	'' ''	.03	i.cer.	4 mice	13,13,14,14	+	+
		1.0	i.a.				
Olfactory bulbs	Ground in 1 cc saline	.03	i.cer.	6 ''	12,12,12,13,13,14	+	+
		.5	''	1 rabbit	CNS 10, D11	+	+
Olfactory mucosa 10% suspension in 10% rabb. ser.	Centrif. susp.	.03	''	6 mice	14,0,0,0,0,0	+	+
	Berkefeld "V" filtrate	.03	''	6 ''	0,0,0,0,0,0		
		1.0	i.a.				
		.5	i.cer.	1 rabbit	0		
		.5	masseter				

Tonsillar and pharyngeal tissues 10% suspension in 10% rabb. ser.	Centrif. susp.	.03	i.cer.	6 mice	21,0,0,0,0,0	+	+
	Berkefeld "V" filt.	.03	"	6 "	0,0,0,0,0,0		
		1.0	i.a.				
		.5	i.cer.	1 rabbit	0		
		.5	masseter				
Saliva	Centrif. susp.	.03	i.cer.	6 mice	(3 bact.); 1d?,0,0		
	Berkefeld "V" filt.	.03	"	6 "	0,0,0,0,0,0		
		1.0	i.a.				
		.5	i.cer.	1 rabbit	0		
		4.0	i.a.				
	Ether-treated	.03	i.cer.	6 mice	0,0,0,0,0,0		
		1.0	i.a.				
Feces	Berkefeld "V" filt.	.03	i.cer.	6 "	0,0,0,0,0,0		
		1.0	i.a.				
	Ether-treated	.03	i.cer.	6 "	0,0,0,0,(2 bact.)		

*Numerals refer to day on which signs of nervous system involvement were first observed in mice. One of the earliest signs (which we have never observed in mice injected with other viruses) consisted of a momentary flaring of the ears when the mice were picked up with a forceps and dropped back into the cage.

†CNS 8, D9—Signs of nervous system involvement 8th day and dead 9th day.

Other abbreviations: i.cer. = intracerebral; i.a. = intraäbdominal; 3 bact. = 3 mice died of bacterial infection; 1d? = mouse died on 19th day but because it had been chewed by its mates neither passage nor examination for Negri bodies or bacterial infection was possible.

be affected. While the failure to demonstrate virus in the saliva and feces may partly be due to inadequate methods, it may also be that there was insufficient centrifugal spread of the virus. The present status of the presence of virus in the salivary glands or saliva in human rabies is rather indefinite. Thus, Williams,[1] stated that "glands from human beings are seldom infective for test animals," and Leach[2] recently reported isolation of the virus from the salivary glands of only one of 3 human cases. Kraus, Gerlach, and Schweinburg[3] pointed out the discordance in the results and opinions concerning infectivity of saliva, and added that no case of rabies has been known to be produced by the bite of a human being. Palawandow and Serebrennaja[4] reported that they produced rabies in guinea pigs [the evidence is not unequivocal, however] by intramuscular injection of saliva from a 12-year-old girl with rabies, and according to Pawan[5] the above authors obtained similar results with saliva from 5 rabid persons. Pawan[5] tested the saliva of 6 persons with signs of paralytic rabies (Trinidad) by rubbing swabs moistened with saliva into the scarified abdominal wall of 7 rabbits, all of which became paralyzed and exhibited Negri bodies; by a similar method he demonstrated the virus in the saliva of bovines, horses, and vampire bats.

The other question to be investigated was whether or not immune

TABLE II.
Test for Neutralizing Antibodies Against Rabies Virus in Patient's Post-mortem Serum.

Dilution of virus	Result with		
	Broth control	Patient's serum	Mouse immune serum
10–2	5,5,5,6*	4,5,5,6	8,10,10,0
10–3	6,6,7,8	5,7,7,8	12,0,0,0
10–4	7,8,10,0	6,7,7,15	10,0,0,0
10–5	0,0,0,0	7,7,0,0	0,0,0,0
10–6	0,0,0,0	n.t.*	n.t.

*0.03 cc of the various dilutions of each mixture was injected intracerebrally into each of 4 mice. The numerals refer to the day on which signs of involvement of the nervous system (rabies) were first observed. n.t. = not tested.

1 Williams, A. W., *Abt's Pediatrics,* 1925, **6**, 251.

2 Leach, C. N., personal communication; see also Leach, C. N., and Johnson, H. N., Abstracts of communications, Third International Congress for Microbiology, 1939, p. 108.

3 Kraus, R., Gerlach, F., and Schweinburg, *Lyssa bei Mensch und Tier,* Vienna, 1926, pp. 80, 128-131.

4 Palawandow, H., and Serebrennaja, A. I., *Z. f. Immunitätsforsch.,* 1930, **68**, 236.

5 Pawan, J. L., *Ann. Trop. Med.,* 1937, **31**, 267.

bodies would be present in an individual who died 2 months after the introduction of rabies virus into the body. The question was of some interest first, because it is known that neutralizing antibodies appear in animals and human beings within 2 to 3 weeks after the injection of effective rabies vaccines, and second, because in diseases like equine encephalomyelitis and yellow fever in which the viruses are viscerotropic as well as neurotropic, neutralizing antibodies are often found in animals and human beings succumbing to the infection. The patient's serum obtained post-mortem was tested against the virus which was isolated from his spinal cord and medulla and passaged in mice 8 times; an immune serum from mice inoculated with a fixed strain of rabies virus (supplied by Dr. Webster) was tested simultaneously. Mixtures of equal parts of the test sera and various dilutions of the virus were injected intracerebrally in mice. The results, shown in Table II, indicate that the patient's serum had no neutralizing antibodies against the virus, while the mouse immune serum exhibited definite protection.

The results obtained in the present investigation throw some additional light on the behavior of rabies virus in man, but it will, of course, be necessary to carry out similar studies in other unvaccinated cases to establish whether or not the present findings are the exception or the rule.

Summary. In a 55-year-old man who died of rabies two months after a bite on the hand, virus was not demonstrated at the site of the bite but was present in appreciable amounts in the cervical cord and medulla, the cornu ammonis, and the olfactory bulbs; only a trace was found in the nasal mucosa and the tonsillar and pharyngeal tissue, and none was found in the feces, saliva, subarachnoid fluid, and heart blood. The patient's serum obtained post-mortem had no neutralizing antibodies for the virus.