THE EPIDEMIOLOGY OF VESICULAR STOMATITIS

A review of some of the literature
and a proposal for further field studies

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INTRODUCTION

In a review of vesicular stomatitis (VS) published in 1968, Saulmon (90) stated that "the mechanism of infection, the method of transmission and the reservoir of the virus" for this disease are unknown. The situation is still the same today. In spite of a tremendous amount of work with the vesicular stomatitis viruses (VSV) in the laboratory, the basic features of the transmission cycle and survival of the virus in nature are still unclear.

The following is a review of some of the literature on vesicular stomatitis, particularly that dealing with pathogenesis, field studies and epidemiological analyses, which might serve as a basis for consideration of what further studies are needed, to solve the mystery of the means of transmission and the reservoir of the VSV.

Vesicular stomatitis has been recognized as a clinical entity since 1884 (110). Olitsky first described the virus of VS in 1926 (81) and Cotton (16, 17, 18) identified the two main virus serotypes, the New Jersey (NJ) and the Indiana (Ind.), as causes of the disease in 1926 and 1927. Since that time many outbreaks of vesicular stomatitis have been identified and described, particularly in the United States, and to a lesser degree in Mexico, and in Central and South America (1, 2, 8, 40, 43, 48, 51, 52, 71, 74, 92, 93, 104).

CLINICAL CHARACTERISTICS

Vesicular stomatitis (VS) is a viral disease which causes vesicular mouth, foot, and teat lesions in cattle, horses and swine, and less frequently, an influenza-like illness in man. Vesicular stomatitis is grouped with the "vesicular deseases" of livestock, which include foot-and-mouth disease, vesicular exanthema of swine and swine vesicular disease. In addition to being a cause of economic loss in beef and dairy herds, the disease is of paramount importance to animal health authorities because of the clinical similarity of the lesions with those produced by foot-and-mouth disease. The clinical appearance of the disease is well described in standard texts (22, 23, 28).

In cattle with VS the mortality is practically nil and sequela are few. The vesicular mouth lesions heal quickly but a loss of weight and a temporary drop in milk production is seen in most animals affected. Mastitis is sometimes seen as a complication (40). In swine, foot lesions are frequent and lameness is often the first sign observed. U.S. oubreaks caused by the NJ virus with high mortality rates in affected pigs have been observed to occur in Central America (W.J. Turner, personal communication, 1975). Clinical cases in sheep and goats are rare and possibly these animals are not susceptible to natural VS infection (40), although cases in sheep have been reported in Colombia (12). The pathological changes produced by VS in cattle and swine also have been described by Seibold and Sharp (94) and Chow and McNutt (21) respectively.

VIRAL AGENTS

The virus etiology of VS was established by Cotton in 1926 and in the following year he demonstrated that there were two antigenically distinct types of the virus (named after the states where they were first isolated, as NJ and Ind.). In addition to the original Ind. isolate now known as Indiana 1, two additional subtypes have been isolated in recent years, Cocal or Indiana 2, and

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Alagoas or Indiana 3 (29). The vesicular stomatitis virus is now classified as a member of the Rhabdovirus group (50). It is an RNA - containing virus, shaped like a bullet, cylindrical, with one end rounded and the other end flat. The dimensions of this virus are approximately 173 mμ long and 72 mμ wide (70). The virus is not thought to be able to survive for very long outside a vertebrate or invertebrate host (42).

DIFERENCES BETWEEN THE NEW JERSEY AND INDIANA VS SEROTYPES

Although the NJ and Ind. types of VSV are morphologically similar, they are serologically and immunologically distinct, and appear to have different ecological requirements. There are also some clinical differences between the two major types of VS. The NJ type generally produces more severe clinical changes and may have a shorter incubation period. Foot lesions have been seen only among cattle affected with NJ type virus (40, 71). Ind. VSV seems to cause more outbreaks in cattle in which only teat lesions are seen (71).

Most outbreaks have been caused by the NJ virus serotype (71). The Ind. type virus has been isolated from only four epizootics in the United States namely 1925, 1942, 1956 and 1964 (90, 96). NJ VSV has a single serotype, ranges farthest into the temperate areas of North America and appears to be restricted to vertebrate hosts (43). Ind. VSV has been found in arthropods as well as vertebrates and has at least three serotypes, two of which appear to be limited to South America (Indiana 2 and 3).

The Indiana 2 strain, named "Cocal" virus by Jonkers and co-workers (59) was recovered from mites collected from rice rats trapped in Bush Bush Forest, Trinidad, and also near Belém, Pará, Brazil. In neither case was this virus associated with clinical stomatitis, although strain specific antibodies were found in horses in Trinidad (29).

In July 1964 an outbreak of vesicular disease in mules was discovered on a sugar plantation in the state of Alagoas, Brazil. It was then found that similar outbreaks had occurred during the previous two months elsewhere in that state and in the neighboring state of Pernambuco. Mules and horses were affected most frequently, but cases occurred also in cattle and strain-specific antibodies were found in the serum of plantation workers who had complained of fever, headaches and malaise at the time the first outbreak in mules was investigated. This strain was found to be different serologically from both Indiana 1 and the Indiana 2 type found in Trinidad and Argentina and has been designated Indiana 3 (Alagoas) (29, 83).

New Jersey and Indiana 1 VSV are rarely found together in the same herd at the same time, or even in the same area. However, in some recent outbreaks in Mexico, both viruses have been found on the same ranch, in the same animal, and even in the same tissue sample (71).

EPIDEMIOLOGICAL FEATURES OF VESICULAR STOMATITIS

The main epidemiological features of the disease are well known (8, 40, 41, 42, 44, 45, 58, 74, 90). In the United States, vesicular stomatitis outbreaks start suddenly during warm weather and particularly during the rainy season. The outbreaks may appear almost simultaneously over a fairly large area, on widely separated premises. The affected farms or ranches are irregularly distributed and the disease seems to skip across the countryside. Often no cases are observed on premises adjacent to those affected.

The incidence of disease can vary widely among affected herds. Usually about 10-15% of the animals show clinical signs of the disease (71, 74), although herds have been seen with a 100% attack rate. Mouth lesions are produced almost exclusively in some outbreaks, while in others teat lesions predominate (27, 104). Clinical cases are mainly seen in adult animals, and cattle and horses under one year of age are rarely affected (8, 71, 85). However, vesicular stomatitis has been reported in suckling pigs (85).

In the United States outbreaks subside in the fall or after a period of cold, dry weather, and stop abruptly after the first killing frost. For many years, cases in cattle, horses, and pigs were seen every year in the coastal plain area of the Southeaster United States and this area is considered enzootic for VS. In other areas, particularly the

Appalachians, in the Upper Mississippi Valley and the Rocky Mountains, outbreaks in cattle and horses are seen periodically, possibly on a cyclical basis, with intervals of two to ten years or more, and in these instances the disease is considered epizootic.

In Mexico the enzootic area is found on the coastal plain around Veracruz and across the low-lying portions of the Isthmus of Tehuantepec, while scattered sporadic outbreaks are more likely to occur at higher altitudes further inland (71). However, there are many areas in the United States and Mexico where the disease has never been reported. In South America VS has not been reported in Bolivia, Chile, Guyana, Paraguay, or Uruguay (3).

On some premises cattle only are affected, on others only horses, although cattle and horses may be in contact in both situations. Swine on the same premises with affected cattle and horses usually remain unaffected. In some cases only the swine may be affected, while cattle and horses in contact show no clinical signs.

In the United States the enzootic areas for VS seem to be characterized by flat, mucky swamp land crisscrossed by sluggish streams. The growing season is long, the vegetation is lush and the moisture is high. Epizootic areas are associated with natural waterways, brush and trees, and pastures that have been wet several weeks previously. Disease outbreaks that occur in the hilly, pine and brush country in late summer frequently may appear first in the irrigated or flooded bottom lands (90).

In Mexico the area enzootic for VS is characterized primarily by a tropical rainy climate with no cool season, and with either no dry season or with a short dry season with high total rainfall. The area has a mean annual temperature of 25° C, is humid or even perhumid, and from June to November, the months of highest VS incidence, usually has a water surplus. Vesicular stomatitis is seen only sporadically in the arid and semiarid regions of Mexico or in the mountainous areas in the interior where the mean annual temperature is between 15-20° C (6, 71).

Vesicular stomatitis can be considered enzootic in warm climates in certain areas where it reappears annually and where a considerable portion of the susceptible animal population possesses antibodies. The disease is epizootic in colder climates where it appears irregularly and where the susceptible animals usually are free of antibodies. Infections of wildlife, man and swine are characteristic of areas in which the disease is enzootic. In the epizootic areas, the infection is recognized primarily as a clinical disease of horses and cattle.

A number of studies have indicated that VSV infection rates are higher in enzootic areas than epizootic areas. In the southeastern U.S. coastal plain 50% of the cattle tested were positive for VS serumneutralizing (SN) antibodies, while just outside this area only 10% were positive (45, 68). Similarly, in epizootic areas, antibodies were found only in animals of the age group that went through the previous epizootic. In Panama, Shelokov found no evidence of Indiana VSV antibodies in humans in urban areas, but up to 35% positivity in forested areas (96, 97). On the other hand, in outbreaks of VS in epizootic areas, the ratio of clinical cases to inapparent infections is higher than in enzootic areas, presumably because fewer animals have a preexisting immunity.

PATHOGENESIS AND TRANSMISSION OF VESICULAR STOMATITIS

In studies on the pathogenesis of vesicular stomatitis, VS viruses were found incapable of penetrating the intact skin (8). However, typical lesions in cattle were readily produced by inoculation or by rubbing of virus into abrasions on the gums or tongue, and on the skin of the coronary band or teats. Injection at other sites usually resulted in an inapparent, immunizing infection. Rubbing virus on the intact mucosa or the introduction of virus in the feed or water did not result in infection (43).

The incubation period after experimental incubation of the VSV is 2-5 days.

In VS in cattle the lesions appear at the site of inoculation but are very rarely followed by secondary vesicles. The disease remains localized and there is no generalization, as evidence by the formation of vesicles at other sites, except in very occasional cases.

Swine react differently to VS than cattle and the pathogenesis in these animals may be of a different type. Vesicular stomatitis can be spread by contact in swine, and in a large outbreak in a hog cholera serum production plant in 1943, the majority of cases were thought to have been infected in this way (92). When inoculated intravenously in swine, VSV will produce lesions on the feet and on the snout. In enzootic areas in the United States swine become infected with VS earlier in the season, while in epizootic areas cases in swine are rarely seen (46). This is difficult to explain in view of the high susceptibility of this species to infection by the various routes (92). Furthermore, the quantity of virus necessary to produce an infection in swine is not greater than that required in cattle (40).

Although virus-laden meat scraps can cause VS lesions in swine with scarified snouts, the disease is not thought to be related to garbage feeding, and infection by ingestion does not seem to be a very likely means of transmission (84).

The difficulty in reproducing the clinical disease in cattle by any other means than local inoculation has led to the impression that the primary infection must also take place in this manner. Actually, most susceptible animals can be infected by the nasopharyngeal route. In one study an aerosol of vesicular stomatitis virus did not induce mouth lesions in a cow which was exposed to it but this animal developed neutralizing antibodies and was refractive to injection. Vesicular stomatitis with typical lesions of the mouth, salivation and pyrexia was induced only by intracutaneous inoculation of the tongue and gums or by rubbing virus over an abraded mucuos surface (40).

Although most reviews of VS state that infection through contact is difficult to achieve, there is considerable experimental evidence that it does occur. Cotton (16, 17, 18) observed this type of spread but only early in the disease. Patterson *et al.* (84) found that saliva collected from infected pigs prior to development of vesicles contained active virus. However, isolation of VSV from the nasopharynx is rarely reported even though VS virus can be easily spread by this route using aerosols (86). Tesh *et al.* isolated VSV-Indiana from throat specimens in 7 of 8 experimentally infected marmosets but they were not successful with other species of wild animals (109). Vesicular stomatitis virus-New Jersey type has been isolated from mouse mothers after inoculation of suckling mouse litters (30) and transmission of VS among monkeys and other arboreal vertebrates by direct contact is thought to be possible (103).

With infections in laboratory workers the virus is spread from affected animals by their excessive salivation, or by actual contact with virus-laden tissues when animals are examined or treated (86). It is difficult to isolate virus from humans with VS (86), although Fellowes *et al.* (31) isolated virus from the blood of an infected lab worker at the Plum Island Laboratory. Nasopharyngeal washings collected at the same time were negative. Surprisingly enough, no evidence of spread from man to man has been seen, although this would be expected (86). In several instances families of infected lab workers have been tested for complement fixing (CF) or SN antibodies with negative results (86).

It has been suggested that infection in humans or even animals may take place via the conjunctiva, since irritation of the eye preceded general symptoms in a number of human cases of VS (86).

A wide variety of mammals can be infected with VSV. Mature guinea pigs inoculated with VSV in the foot-pad epithelium develop vesicles there in 48 hours. Mature mice inoculated intracerebrally become paralytic in 3 days and die by the fifth day (19, 20). Suckling mice develop a fatal infection irrespective of the route by which the virus is introduced (80, 99).

In experimental infections in Panamanian wild animals, Tesh and his coworkers (109) found that mamals were highly susceptible to VSV infection (all but two of 38 species tested had neutralizing antibodies by the twelfth day after inoculation). The susceptibility of the different species to VSV depended mainly on age. With one exception, adult mammals remained asymptomatic following subcutaneous inoculation of VSV, but VSV-Ind. and VSV-NJ caused death in a variety of suckling mammals within 2-4 days after subcutaneous inoculation. Central nervous system signs were seen in many of the dying animals, but vesicular lesions were not seen.

Sabin and Olitsky (80, 89) carried out some classical experiments on the pathogenesis of VS in mice, to determine why older mice were more resistant than young mice to VSV. Although injection of VSV directly into the brain was fatal for both young or old mice, they found that when the virus was given intranasally only young mice (3 weeks) succumbed, while older mice (1 year) were found to be resistant.

No evidence of a generalized systemic or blood infection could be found in either old or young mice after the nasal instillation of VSV. No virus could be demonstrated in the nasal mucosa one hour after instillation, but two days later it was abundant in both the nasal mucosa and the anterior rhinoencephalon. On the fourth day the virus was present in large amounts in the brain and nasal mucosa of the young mice and they showed definite clinical signs of central nervous system (CNS) involvement. The older mice showed no signs of disease after nasal instillation, even though virus was present between the second and fifth day in the anterior rhinoencephalon. The experiments showed that in young mice the rest of the brain is invaded, with subsequent death of these animals in five days with encephalomyelitis, while in the old mice the progression of the virus is arrested somewhere in the anterior rhinoencephalon. While the VSV reached the brain of the young mice by way of the first cranial nerve, a preexisting localized barrier in the anterior rhinoencephalon, which develops with age, prevented the virus from reaching the brain of the old mice.

A similar phenomenon has been observed when young and adult mice were exposed by the intramuscular and intraocular inoculation routes.

Why are old mice generally resistant to all forms of peripheral inoculation of VSV when intracerebral injection is equally fatal for mice of all ages? Evidently, VSV injected intracerebrally soreads primarily in an "open" system in contiguity with the ventricles, while after peripheral inoculation the virus progresses in a closed system of neurons in young mice, but is unable to do so in old mice because of localized barriers at the site of inoculation.

Why are young mice more susceptible to VSV than old mice and why does the reverse seem to be true for cattle? Clinical cases are seen in adult cattle, while calves under one year are rarely seen with lesions. According to antibody surveys, as soon as the protection provided by maternal antibodies wanes, young cattle are as susceptible to infection with VSV as older cattle. Why, then, are fewer lesion cases found in younger animals? Does tissue injury (abrasions, inoculations) in the presence of VSV produce some effect in older animal that is not seen in younger animals? Would this explain the apparent spread of VS in dairy herds along milking lines ? (18).

ANTIBODY PRODUCTION AND IMMUNITY TO VESICULAR STOMATITIS

In cattle and horses experimentally infected with NJ and Ind. VSV, complement fixing antibody titers appear 6-8 days after inoculation and reach a peak in 9-16 days (37). Thereafter they gradually decline and disappear in 50-110 days. Serum neutralization titers can be demonstrated in 6-8 days post-inoculation and increase to relatively high levels within 4-5 weeks. The titers of neutralizing antibodies remain high but fluctuate over a long period of time. In some instances, VS SN antibodies persisted up to 8 years in cattle (102).

Sorensen and his co-workers (102) suggested that the fluctuating SN antibody titers could be explained by the persistence of the VSV in the animal host, and that the rise and fall in titer could be due to a periodic "contained escape" and subsequent retreat of the virus to its chronic foci. However, it is interesting to note that within 30-60 days after recovery from VS, many animals can be reinfected experimentally with the same strain of virus, with clinical stomatitis resulting. These animals may even possess significant titers of antibody at the time of reinfection. The existing titers rise rapidly after challenge.

In studies in Georgia, Hanson and his group found that 70% of the cattle of milking age, 35% of the heifers and 70% of the young calves less than three months of age possessed antibodies neutralizing VSV (42, 44). The serum antibodies in calves were undoubtedly a reflection of their transfer with colostrum. In hamsters, maternal antibodies persist in the offspring for 2-3 months

(109). In Georgia, 71% of suckling pigs tested were positive for VS antibodies.

The severity of the disease varies in cattle in the enzootic areas, most infections being silent and unrecognized. Mild infections can be induced experimentally by exposure of cattle to the virus by nebulization. The virus multiplies and antibodies are induced but signs of disease are absent. Serological studies show that this can occur naturally. In one herd in Wisconsin all the cattle had VS antibodies one month after the appearance of the disease but only 50% had been clinically affected (8, 40).

VACCINATION AGAINST VESICULAR STOMATITIS

The inoculation of live VSV intramuscularly in cattle does not cause lesions, but will result in the production of neutralizing antibodies in most cases. This observation has led to the experimental use of live VSV intramuscularly as a vaccination procedure for cattle in Panama (69), Georgia (69), Guatemala (15) and Peru (104). However, the vaccine is not in general use at the present time.

Live VS NJ virus vaccine, administered intramuscularly during an epizootic markedly reduced the number of clinical cases of VS in lactating dairy cattle. The period during which VS was present in affected herds was markedly shortened following vaccination. While absolute protection of the vaccinated animals against intralingual challenge was not achieved, a significant degree of protection was stimulated in cattle by the vaccination. Ninety percent of the vaccinated animals developed VSV NJ antibodies. Although VS was active in the area, none of the vaccinated cattle developed clinical VS, while 26% of the cattle in neighboring non-vaccinated herds did develop vesicular lesions.

During a VS vaccine field trial it was observed that the clinical disease appeared infrequently in herds that had 50% or more of the cattle vaccinated. In herds vaccinated during the early stages of an epizootic no active cases of clinical VS were seen seven days after vaccination. The vaccine virus did not spread from animal to animal by milking or by saliva. Fifty percent of the cattle still had VS SN antibodies one year after a single injection of vaccine. Only 5% showed no demonstrable rise in antibodies after a second vaccination. In cattle which already had VS antibodies, 76% showed an increase in titer after the first vaccination. It was found that 60% of the animals had VS antibodies before vaccination. In herds with actual history of VS, this figure rose to 82%. Even on farms with no history of clinical cases of VS, 24% of the cattle had VS antibodies.

VESICULAR STOMATITIS IN WILD ANIMAL POPULATIONS

Many serological surveys for VS have been carried out in wild animal populations, in a search for possible reservoirs for VSV (45, 46, 53, 54, 57, 59, 60, 62, 63, 103, 108, 109).

Serological surveys in Southeastern U.S. revealed antibodies in white-tailed deer (63), raccoons (44), bobcats (62) and wild pigs (46). Forty-eight percent of the raccoons, 60% of the deer, 83% of the feral swine and 33% of the bobcats surveyed possessed neutralizing antibodies for VSV. In surveys in the same area Karstad found that certain species of shore and wading birds also had antibodies for VSV. However, several laboratory studies have failed to demonstrate that birds may play any role in the natural history of VS.

Jenney *et al.* also found evidence of VSV infection in deer, raccoons, opossums and squirrels (53, 54). Trainer and Hanson (111) found 8% of deer sera from Texas positive for VSV antibodies, and forty-three percent of 122 wild Rio Grande turkeys trapped at a wildlife refuge in South Texas during 1964-65 had antibodies for NJ VSV, although no clinical disease was reported in livestock (38).

It was thought unlikely that deer were reservoir hosts of VS since the experimental disease was short-lived and was quickly followed by high levels of virus neutralizing antibodies (63). Raccoons were highly susceptible but the infections were clinically inapparent, and excretion of the virus and latent infection were not detected.

Jonkers found evidence of Cocal virus infection (Indiana 2-VSV) of rodents in the Bush Bush forest in Trinidad during the rainy seasons of 1961 and 1962. The 1961 epizootic was explosive in

character and involved about 65% of the forest floor rodent population (57, 59, 60).

Tesh and his coworkers (107, 108, 109) studied the prevalence of VSV neutralizing antibodies in various Panamaniam human and animal populations. Among wild animals VSV-Indiana antibodies were found mainly in arboreal and semi-arboreal species. Infection rates for VSV-NJ in feral animals were highest in bats, carnivora and certain rodents.

A large scale survey for antibodies to VSV-Ind. in monkeys and other wild vertebrates was performed in different parts of Panama. Approximately 75% of 267 monkeys in Darien Province were positive for neutralization tests whereas only 19% positive results were obtained from 383 monkeys collected near Panama City (103).

Wild vertebrates other than monkeys showed a much higher antibody rate in arboreal mammals than in ground-living animals. Two-toed sloths and tropical porcupines were among the arboreal mammals showing high VSV-Ind. rates, and sentinel monkeys exposed in the area showed increases in VSV-Ind. antibody titers (103).

The complete list of wild vertebrates found with VS antibodies in Panama includes a large variety of *terrestrial species,* like spiny rat, pocket mouse, rice rat, cotton rat, domestic rat, rabbit, 9-banded armadillo and water opossum; *vertebrates mainly arboreal* including five species of chiroptera: leaf-nosed, fruit, long-tongued, round-eared and big-eared bats; various arboreal vertebrates as kinkajou, olingo, 2-toed sloth, 3-toed sloth, pygmy anteater, porcupine, squirrel, climbing rat, night monkey, marmoset, spider monkey, white-faced monkey and howler monkey; and *semiarboreal vertebrates* like anteater, opossum (common, 4-eyed, masked) water opossum and raccoon (108). These animals are found in a wide range of habitats and ecological situations, most with no regular contact with domestic animals or man. Obviously, besides the cycle of infection in domestic livestock, the VS virus somehow was mantaining itself independently in forest areas.

VESICULAR STOMATITIS IN HUMANS

Vesicular stomatitis appears to possess appreciable pathogenicity for man, infection commonly occuring as a laboratory-acquired disease and among rural populations in areas where the disease exists in livestock and wild animals hosts (9, 31, 34, 56, 97, 108).

There have been human cases in laboratory workers and in animal handlers at research stations where VS investigations were being carried out. Before additional safety precautions were included, 95% of the laboratory workers, 100% of the regular animal handlers and 70% of the trainees involved with VS projects at the Beltsville ARS laboratories during a 7-year period showed positive antibody titer for VSV, 15% to the Ind. type alone - 40% to the NJ type alone - and 40% to both serotypes (86). Of 54 cases of VS in man, diagnosed by serological tests at Beltsville, 31 (57%) reported clinical symptoms. Since using plastic face shields to protect the eyes and disposable air filters over the nose during inoculation and observation of infected animals, cases rarely occur among the workers.

The illness in humans is usually influenza-like, occasionally severe and sometimes associated with oral and pharyngeal vesicles. Many of the early cases resulted from getting virus suspension into the eyes while grinding with mortar and pestle, while examining infected animals, and while collecting vesicular material. Presumably the other cases resulted from inhalation, ingestion or inoculation of virulent virus during laboratory or veterinary manipulations, and there was no special reason to implicate biting anthropod vectors.

In 1956, antibodies to VS were found in blood samples collected from 18 persons living on farms in Southeastern Georgia where VS had been diagnosed among livestock (72). Approximately half of the persons tested had significant antibody titers to VS (42). Later, Hanson and Karstad (44) demonstrated neutralizing antibodies in 25% of sera from 200 unselected febrile patients in the enzootic VS area of rural Georgia. Some of the people exposed to sick cattle during an epizootic in New Mexico and Colorado developed febrile illnesses followed by rises in VSV Indiana 1 antibody titers (34). In a study of the clinical cases, it was found that naturally acquired VS infections was similar to those acquired accidentally in the

laboratory.

Studies in Panama showed that domestic animals and humans there had high antibody rates to both Ind. and NJ VSV (108). The antibody prevalence in humans increased with age, suggesting a direct relation between VSV infection rates and length of residence in the endemic area. Geographic and species differences in antibody rates between VSV-Ind. and VSV-NJ implied that the two virus serotypes may have different cycles in nature. Available evidence suggested that VSV-Ind. might be anthropod-transmitted, but the mode of VSV-NJ transmission could not be determined.

An outbreak of VS-NJ type was studied in a dairy herd in Western Panama in 1960-1961 (9). In a survey of humans in the area, the presence of neutralizing antibodies to this virus was considerably higher (34-71%) among people with a history of having worked with cattle than among those who had not (7-15%). The possible mode of spread to humans was considered to be by contact with infected animals or by anthropods. While both types of spread may have occurred, direct contact appeared to be more important.

Utilizing human serum samples randomly collected throughout Central America for a comprehensive survey of various communicable diseases, Johnson and his coworkers reported the astonishing finding that about one-half of the adults in Central America had antibodies to NJ or Ind. VSV. In surveys carried out in some 200 villages throughout Central America, 48% of the persons tested had antibodies for NJ VSV and 18% for Ind. VSV. Evidence of infection with NJ VSV was found in nearly every locality surveyed. Ind. VSV was not quite as prevalent, but had a similar geographic distribution (55).

THE ROLE OF ARTHROPODS IN THE TRANSMISSION OF VESICULAR STOMATITIS

Because cases and outbreaks of VS occur mainly in the warm, rainy months, and in view of the rapid occurrence of cases over a wide area, and the association of the disease with wooded premises and natural waterways, it is commonly believed that VS is an arthropod-borne disease. This is further supported by the isolation of the Ind. type of VSV from *Phlebotomus* sandflies in Panama (96, 107) in areas where the disease is enzootic, and from *Aedes* mosquitoes in New Mexico on the same premises where clinical cases in cattle were seen (105). Cocal VS virus (Indiana 2) has been isolated from mites on rice rats in Trinidad and from *Culex* mosquitoes in Trinidad and in Brazil (57, 59, 60). New Jersey VSV was isolated on one occasion from non-blood sucking eye gnats *(Hippelates pusio)* trapped on a premise in Colorado where clinical VS in cattle was seen (51, 113).

In addition, Ind. VSV multiplication, with subsequent transmission to mice, was seen in *Aedes aegypti* mosquitoes (7, 77). A similar finding was made with Cocal virus, also in mosquitoes *(Culex pipiens quinquefasceatus* and *Trichoprosopon digitatum)* (59). Furthermore, VSV has been propagated *in vitro* in *Antheraea eucalypti* moth cells (116) and in continous cell lines of *Aedes aegypti* and *Aedes albopictus* mosquito tissues (4, 5), and in cells of the fruit fly, *Drosophila melanogaster* (76, 88).

Donaldson (24) described bat infections in laboratory conditions with the Cocal virus and its transmission to suckling mice through *Aedes aegypti* (L) mosquitoes feeding on viremic bats. In bats maintained at 22° C viremia was detected during 10 days while in hibernating bats the viremic period reached 16 days. It was concluded that in VS endemic regions, the possibility of a blood sucking arthropods-bats-arthropods cycle must be considered (25).

Even though the NJ type of VSV is even more enzootic in Panama than the Indiana type NJ VSV has never been isolated from arthropods there, while Ind. VSV has been isolated repeatedly from *Phlebotomus* sandflies. Since leishmaniasis is also transmitted by *Phlebotomus* and is found in much the same area, it has been suggested that this disease and Ind. VS may have similar ecological requirements (108).

The significance of a single isolation of NJ VSV from *Hippelates pusio* is uncertain since these eye gnats feed on mucus and are not blood sucking insects. There is a possibility that this insect plays a role in mechanical transmission rather than

developing a carrier state in which the virus multiplies in its tissues (51, 54). The same may apply to *Phlebotomus* sandflies, a group of insects that sometimes feed on wounds and body secretions. Could virus obtained from lesions on the muzzle and teats of infected cattle be introduced by these flies into wounds or abrasions on uninfected cattle caused by plant spines or stubble?

Mechanical transmission of NJ VSV by various species of biting diptera was shown to be experimentally possible in 1955 (33). Ferris, *et al.* found that a number of species of mosquitoes and tabanids were capable of picking up the virus and transmitting it for short periods to laboratory hosts. Since the insects did not remain infective for more than three days, and since there was no intrinsic incubation and little host specificity it appears that the transmission was mechanical and not biological. It was observed that insects, feeding on areas of an animal's body that do not show clinical signs of the disease, are able to transmit VSV to the animal without causing overt signs and cause a serological response to the virus.

In spite of the accumulating evidence for the transmission of VSV by arthropods, some investigators have raised considerable question about their role as vectors. Repeated failures to isolate VS virus from a wide variety of biting flies and mosquitoes in enzootic and epizootic areas has caused doubt about the hypothesis of biting insects as the major vectors of VS (90). The simultaneous appearance of the disease in an entire herd or many herds, if attributed to the work of a vector, would require access to an infective reservoir of a very large size. Such a reservoir has not yet been found.

In Panama, VSV-Ind. activity was detected in an area during a period when it was not detected in *Phlebotomus* sandflies. If sandflies are indeed the sole vector of VSV-Ind., one would have expected a much higher virus isolation rate from these insects than was actually observed (107). In Trinidad, failure to isolate Cocal virus more frequently from mosquitoes and sentinel mice suggested that mosquitoes probably were not the principal agents responsable for virus transfer during epizootic periods. Also, the single isolation from *Gigantolaelaps* mites in Trinidad was not adequate to confirm these arthropods as a principal vector (57). Collectively, these observations imply that there may be other undetected sources of the virus in nature. Although Tesh and his coworkers (106) demonstrated transovarial transmission of VSV-Ind. in *Phlebotomus* sandflies, which could possibly explain how the virus is maintained in nature without vertebrate hosts, the transovarial transmission rates they found (20-30%) were not thought to be high enough to sustain the virus for long in the insect population in the absence of one or more of the following: 1) selective survival of infected sandflies, 2) virus transmission to many females during insemination by infected males, 3) or existence of another virus source that occasionally replenishes the transovarial cycle.

Jonkers reported that the 1961 Cocal virus epizootic in small rodents in Trinidad bore considerable resemblance to epizootics in livestock caused by the VSV. The sudden appearance of infection in a large proportion of the susceptible population some time after heavy rains in a wooded environment, the failure to incriminate flying biting arthropods, the absence of viremia in naturally infected animals, and finally the affinity of the agents involved for the skin, all suggested that the epizootiology of this group of agents was basically similar (60).

Jonkers (58) has raised a number of objections to the hypothesis that VS is arthropod-borne in a paper published in 1967:

1 - if the typical lesions in cattle and horses are produced only at the sites of abrasions and cuts on the skin and mucosa of the mouth, for example, it is difficult to imagine the biting habits of a vector that would account for this;

2 - if an arthropod vector is responsible, why are animals in certain widely separated pastures affected while those in adjacent pastures are not?;

3 - the sudden appearance of clinical cases in a large proportion of the herd, sometimes on the same day (48), would require a large number of infective vectors and presumably an extensive epizootic in wildlife. If the disease were spread by vectors a more gradual spill-over of the epizootic in wildlife into livestock would appear more probable;

4 - swine often are not affected during epizootics even when in contact with cattle and horses (40, 74). This is difficult to reconcile with a vector-borne disease unless a vector were postulated that bites cattle and horses, but does not bite swine;

5 - similarly, the paucity of cases in stabled cattle or horses is difficult to explain if the disease is vector-borne, since blood-sucking vectors are commonly found in stables;

6 - the density of the livestock population in an area does not appear to be an important factor in the spread of VS (8, 41). This is also not consistent with a vector-borne disease;

7 - if the lesions are caused by the actual introduction of the virus by the vector at the site of the lesions, why are only mouth lesions found in some herds and only teat lesions in others?;

8 - virus isolation attempts with arthropods caught during VS outbreaks in the United States have, with one exception (105), so far been unsuccessful (62). In fact, even where virus isolations were made from possible vectors, as in Trinidad when Cocal virus was found in mites and mosquitoes, in relation to an epizootic in field rodents, neither the mosquitoes nor mites were thought very likely to be responsible for the extensive dissemination of the virus (60). In Panama, it was suggested that the rate of virus isolation from sandflies was too low to explain the high level of VSV-Ind. infection in the animal and human populations (107);

9 - one of the main difficulties in supporting the claim that VS is an arthropod-borne disease is that in experimental studies the viremia produced in cattle, horses and rodents was found to be of low titer and short-lasting (42, 67). In Trinidad, Cocal virus epizootics in rodents were not accompanied by sufficient viremia to infect blood-sucking insects with any regularity (57). So far, an animal host which demonstrates a long-lasting viremia of high enough titer to regularly infect a blood sucking vector has not been found for VS.

There are some experimental findings which may explain this anomaly. Virus clearance studies (10) have demonstrated that the VS virus particles are effectively removed from the blood by the phagocyte cells of the RE system. In man, Ind. VSV is differentially taken up by and multiplies in monocytes, thought to represent the circulating form of the fixed tissue macrophage. Since these cells seem to be important as processors of antigenic information necessary to initiate an immune response, antibody formation following infection is swift and unaccompanied by a period of significant viremia. This pattern has been found following experimental infection of cattle, horses and a variety of small mammals (26, 55).

VESICULAR STOMATITIS VIRUS AS A PLANT VIRUS

In many VS outbreaks lesions are seen predominantly at a single body site, such as in the mouth or on the teats. This observation combined with the fact that the VS virus does not pass through the unbroken skin, has suggested the possibility that nonspecific lesions are necessary for virus entrance, as for example scratches from brambles and briars on the mouth or teats. From this, Jonkers (58) has formulated a hypothesis which suggests that the VS virus already is present in the pasture itself before the appearance of the first cases, and is brought into contact with livestock either by the eating of virus-infected material (the result being specific mouth lesions if virus introduction in the mucosa is effected) or by lying down in or walking through an area in which infective virus is present (with the production of teat or foot lesions).

Jonkers claimed that considering the pasture as the epizootiological unit would explain the spotty distribution of outbreaks (only some pasture are affected), the absence of spread from premise to premise, and the paucity of cases in stabled animals (in cases in dry-lot cattle fed fresh-cut fodder, the virus would supposedly be brought in with the feed). This hypothesis would also explain the absence of spread by contact alone, the simultaneous occurrence over a wide area, the higher incidence during the rainy season (related in some way to growth or availability of some agent in the pasture), and the lower incidence in swine.

Along these lines, McDermid suggested in 1951 that VS virus grows on certain lands as a saprophyte and that infection may be transmitted to livestock via hard stubble (73). Johnson and his coworkers (55) have gone further to propose that the VS virus is basically a plant virus (this is suggested by the morphological similarity of the VS virus with certain bullet-shaped rhabdoviruses which infect plants) and that it is transmitted to vertebrates by *Phlebotomus* sandflies, which are known to suck plant juices, or by some nonbiting insects, such as aphids (known to transmit certain plant viruses) which might pass the virus to certain domestic animals by being ingested with plant meals. They further proposed that in its plant form the virus has a double coat on the virus particle which renders it noninfectious for vertebrates. The virus would be converted to a new singlecoated form by passage through insects, and this form would be infectious for vertebrates.

In spite of the speculation about VSV being a plant virus, no isolations of this virus has been made yet from plants or plant insects such as aphids.

AN ALTERNATIVE MECHANISM OF TRANSMISSION

Although the hypothesis that VSV is a plant virus is somewhat more consistent with field observations than the suggestion that the disease is arthropod-borne, a number of questions still remain. In addition to affecting cattle, horses and swine, VS is widely distributed in the wild animal population in certain areas, and it would be difficult to account for infections in these animals, especially the arboreal and semi-arboreal species, through contact with the virus in pastures. The same question would arise about the source of outbreaks in stabled swine herds not fed fresh-cut fodder. It would be even more difficult to explain the widespread infection of humans if contact with the virus in pastures were required.

One source of confusion may be that epizootics of VS in cattle and horses make their dramatic appearance with the sudden, practically simultaneous onset of hundreds of clinical cases of vesicular disease, dispersed over a wide area. Since the mouth, teat or foot lesions are difficult to produce experimentally except by inoculation of the epithelium or contact of the scarified mucosa with the VS virus, it is assumed that the same process takes place in nature, and that the most logical site for the infection to take place is the pasture.

Actually, cases of VS with lesions are a small minority of the total infections. On the basis of serological surveys of domestic and wild animals and humans, the great majority of the infections appear to be inapparent and asymptomatic. If these infections cannot be explained simply by contact with the virus in forage, for example, or by spread from insect vectors, for the reasons discussed above, what other mechanisms of transmission might be considered? One other explanation is that transmission takes place by direct contact, most likely by the nasopharyngeal route.

According to this hypothesis, the main mechanism of transmission for VS would be by respiratory spread through normal contact, with the great majority of the infections being inapparent, and with persistence of the virus in the animal host for long periods of time, possibly in a masked form. The appearance of the typical vesicular lesions in the infected animal might then occur in some manner analogous to what occurs in humans with long-term herpes simplex infections, where herpetic eruptions appear when local resistance is reduced by various nonspecific factors.

Although this could explain what might happen in individual cases, some further hypothesis is required to show how large numbers of clinical cases occur simultaneously over a wide area. The most reasonable basis for this phenomenon is that some environmental factor or factors, presumably climatic, in some way provokes the virus to produce vesicular lesions in already infected animals. That the distribution of VS affected herds is spotty and some herds are more severely affected than others, and some have mouth lesions only while others have teat or foot lesions, may depend on some type of graded, differential response to the environmental influences or factors by the chronically VS infected cattle.

There is some experimental evidence (39) that environmental factors such as temperature may play a role in the appearance of clinical cases of

VS. Mortality of mice inoculated IC with VSV was significantly lower among groups acclimated to 8° C than among those acclimated to 27° C or 35° C. Also, the incubation period of infection was longer among the mice adapted to the low temperatures, and rates of metabolism were higher among mice maintained at 8° C than among those kept at 25-35° C, as evidenced by increased food consumption and weight gain.

The acclimatization to a low temperature prior to inoculation was essential to influence the survival rate of the mice favorably. Exposure to cold at the time of inoculation or shortly thereafter did not alter the course of the infection. Since it has been shown experimentally that susceptible animals can be infected with VSV as readily during the winter as in the summer, there remains the possibility that, while the environmental temperature may not influence the ability of the virus to initiate infection, it may alter appreciably the response of the host to the infection.

In recent years temperature-sensitive (ts) mutants have been described for many different viruses including VSV. Although most of the ts virus mutants were produced in the laboratory, spontaneous ts mutants are known to occur at a low frequency in many virus populations. Increasing evidence suggests that naturally selected ts mutants may be involved in disease states *in vitro*, and that these mutants may play a role in either establishment or maintenance of persistent virus infections (87).

Virus recovered from many different types of persistent infections has been found to have an impaired ability to replicate at higher temperatures. This has led to the suggestion that investigators seeking to isolate virus from tissue explants from animals with diseases in which latent or persistent virus infections are suspected, should incubate such cell cultures at 31° C or 33° C, in addition to the conventional incubation at 37° C (87).

Could these findings have some applications to the natural history of the VSV? Are ts mutants of VSV responsible for persistent VS infections in cattle, for example? Could further mutation of these virus strains, possibly provoked by environmental changes of some kind, result in virus types that cause the typical vesicular lesions in affected animals?

Field observations during epizootics also suggest some possible influence of environmental factors. Only a few questionable clinical cases of VS have been reported in the U.S. in the winter and early spring months, and the virus has never been isolated during these months of the year (40). Lauerman (68) reported that there appeared to be a marked increase in the number of VS cases occuring after passage of an anticyclone. He also observed spread of the epizootic in the direction of the anticyclone passage. Similarly, Hanson and his coworkers (43) have reported that VS does not always appear simultaneously in an epizootic area. Study of cases in Georgia and Alabama suggested that the disease moved outward from one or two centers, and that it moved along corridors, from herd to herd, rather than being resident in particular pastures. Also, the direction and time of movement appeared to coincide with the passage of storm fronts (41).

One difficulty with the theory that VS is spread by contact among livestock is that the disease is unknown in some areas in spite of heavy populations of susceptible animals. If contact were the only requirement, widespread infection would be expected. However, it is possible that the spread is by contact, but actual infection requires special environmental conditions or altered metabolic states.

INTEREPIZOOTIC SURVIVAL OF THE VESICULAR STOMATITIS VIRUS

No matter what the basic mechanism are for the transmission of VS from animal to animal, it is difficult to explain the interepizootic survival of the VSV. In certain areas of the United States epizootics have occurred at intervals of greater than 10 years. Persistence of virus as an occult infection for such prolonged periods would appear very improbable, compared with introduction from areas where infections are observed every year. Hanson suggested that there could be introduction of an infected animal such as a cow or a pig or the migration of an unidentified host or vector into the area where the epizootic eventually occurs (41).

Since VS can be found in certain areas in Mexico throghout the year, Hanson further suggested that semitropical America might be the origin of most outbreaks of VS, and that the virus might be introduced into the United States by movement of infected cattle along sales routes and the migration of reservoir animals, although no domestic or wild animal has yet been incriminated as a reservoir host. Migratory birds have also been suggested as a possibility (40, 41).

It also has been suggested that certain insects might transmit the virus over long distance. Aphids, leaf hoppers and lepidoptera that attack field and pastures plants migrate on warm southern winds from the Gulf Coast into Canada each spring. The journey may take 2-6 weeks and a succession of migratory waves may follow the initial one (41).

The introduction of VSV into epizootic areas by migrating vertebrates or insects still would not explain the sudden outbreak of clinical cases over a wide area. This would require the migration of enormous numbers of virus carriers, if the occurrence of the clinical cases is to be linked to the onset of primary infections in an area. On the other hand, the virus could be introduced into a "clean" area by migrating carriers, with a gradual build-up of asymptomatic infections, until conditions are appropriate for a sudden appearance or "outbreak" of clinical cases over a wide area.

Another possibility is that VS does persist in animals in inapparent form for long periods of time in epizootic areas, without the appearance of clinical cases, because the environmental conditions for their occurrence do not exist. When these conditions supervene, even though at long intervals, clinical cases and outbreaks are seen. This would suggest that even though the environmental conditions necessary for VS outbreaks do not regularly exist in the epizootic areas, they may occur on rare occasions, as exceptional events.

It would appear that no single theory of transmission serves to explain all the field and laboratory observations with VSV. It is likely that more than one system operates in nature. Although respiratory spread by direct contact of infected animals would appear to be possible and consistent with many field observations, arthropod vectors may also play a significant role under certain conditions, as in the tropical forests areas in Panama, for example. A series of different reservoirs for the virus may exist, and transmission may take place in different ways under different conditions, and may be different for the NJ type of VSV than for the Indiana type, even though both may be found in the same area, are morphologically similar, and produce basically the same clinical picture.

QUESTIONS TO BE RESOLVED REGARDING THE EPIDEMIOLOGY OF VESICULAR STOMATITIS

The many unknowns that exist about VS might be summarized by listing some of the questions that arise about the epidemiology of this disease:

1 - What is the reservoir (or reservoirs) for the VSV?

2 - How is VSV transmitted from one host to another?

3 - If a viremia of high titer and long duration has not been observed in any vertebrate hosts, how would arthropod vectors of VSV pick up the virus?

4 - Is transovarial passage of Indiana VSV in *Phlebotomus* enough to explain the survival of the virus in nature?

5 - Do NJ and Indiana VSV have different transmission mechanisms?

6 - Why have NJ VSV isolations not been made in arthropods, while they are fairly common with Indiana VSV?

7 - If lesions in cattle are produced only by inoculation of VSV at the site of mouth, foot or teat lesions, or contact of the virus with scarifications of the epithelium at these sites, how would blood-sucking vectors ordinarily produce these lesions?

8 - If the VSV or the agent producing the VS lesions is found in pastures, how do hosts such as bats, monkeys and man pick up the VS infection?

9 - How does the VSV survive between epizootics when there may be an interval of up to 10-15 years between outbreaks?

10 - Why are some VSV outbreaks in cattle characterized by teat lesions only? Is this due to a strain difference of the virus? What type of

vector would produce only teat lesions? If the lesions are caused by contact with the virus in pastures, why are mouth and foot lesions not seen also, at the same time that teat lesions occur?

11 - Is VSV transmitted mechanically during milking from one cow to the next on the hands of the milker, or on the cups of milking machines, or is the virus already present in the tissues, with the vesicular lesions being provoked by injury to the teats?

12 - Are persisting VS SN antibodies in cattle due to VS virus which persists in the host for long periods?

13 - How are cattle infected with VSV in herds where no clinical cases are seen, but where a large proportion of the herd may have antibodies for VSV?

14 - Even though vesicular stomatitis virus given subcutaneously or IM will not cause lesions, it will produce a rise in antibody titer and some protective immunity. However, the immunity produced may not protect against intralingual challenge. In view of these observations, how does VSV given IM as a vaccine provide suitable protection during VS outbreaks, as reported?

15 - Does inoculation of cattle IM with live VSV used as a vaccine produce long-lasting inapparent VS infections? Would these cattle be more prone to vesicular lesions later, during a natural epizootic? If subjected to stress, experimentally?

16 - If VS outbreaks are seen mainly during the rainy season, how can we explain some outbreaks or cases which occur during the dry season, or during a period of low rainfall?

17 - If VSV is present in pastures, why are adjacent pastures not affected more frequently? Why is the distribution of affected herds usually so spotty?

18 - Why are regional outbreaks seen, and what factors limit the appearance of the disease to these circumscribed areas?

19 - Why is there sometimes a considerable difference in the morbidity rates in affected herds in the same regional outbreak?

20 - Why are adult cattle and horses more frequently affected with clinical VS than animals under one year of age?

21 - Why is the incidence of VS highest during the rainy season?

22 - How could enough arthropod vectors of VSV be infected at a high enough level to produce a simultaneous, widespread outbreak in cattle and horses in an epizootic area?

23 - Can the distribution of VS in the Western Hemisphere, in enzootic and epizootic areas, be related with the distribution of any particular species of arthropods, or any particular set of special climatic conditions? Why is VS not found currently in the Eastern Hemisphere?

24 - If VS in swine can be spread by contact, why are clinical cases not more numerous, and why is the disease not more prevalent in swine production area?

25 - How could sentinel monkeys in cages in the tropical forest areas in Panama be infected with Indiana VSV except by flying, biting arthropods?

26 - Do cattle that develop VS lesions during a VS outbreak have antibodies for VSV before the appearance of the lesions?

PROPOSAL FOR FURTHER FIELD STUDIES

Because of the complex transmission patterns and ecological requirements that may exist for VSV, the disease is difficult to study in the field. Retrospective studies of epizootics, or sporadic surveys of animals in enzootic areas are valuable but may provide little more information than is already available.

An approach which would seem to offer considerable promise of revealing some of the missing epidemiological data about VS is a prospective, longitudinal study in an area where both NJ and Ind. VS are known to be enzootic. Such an area can be found in certain parts of Mexico, Central and South America.

The basic field study should entail a continuing ecological investigation of VS in a circumscribed enzootic area selected on the basis of serological surveys. Two or three premises could be selected where a sufficient number of cattle, horses and swine are being kept. The past clinical history of the herds could be collected, and individual

animals could be followed clinically for the duration of the study. Blood specimens could be collected at the outset of the study for CF and SN antibody tests and these could be repeated on the same animals every 2-3 months. Records could be kept of the movement of the animals, additions to the herds, type of pastures and forage available, and types of feed provided. A careful record could be maintained of a variety of local climatic phenomena (rainfall, temperature, humidity, storms, flooding, etc). Parallel serological studies could also be made on representative wild animals and on selected human residents in the area. Entomological studies could be carried out to determine the possible arthropod vectors present and arthropods collected, whenever indicated, for the isolation of virus.

Basically, the investigation would attempt to study over a number of years in a small area enzootic for VS any and all factors which might have some relation to the reservoirs and transmission of VSV in nature. The patterns of inapparent infection with VS could be followed from birth of some of the cattle under observation and hopefully, if clinical VS cases were to occur during the study period, their appearance could be related to some concomitant environmental events in the area. The occurrence of new infections, whether accompanied by clinical lesions or not, possibly could be correlated with the isolation of VSV in vectors or plants, or in other agents that are still unsuspected, or could be shown to be related to respiratory spread through close contact of the affected animals.

Since practically no field studies of VS are being carried out anywhere at the present time, it is essential that some type of prospective long-term ecological studies of this disease be started as soon as possible, to provide some understanding of the natural history of this puzzling disease. At the same time a greater effort should be made to use the many findings resulting from the wide-scale use of VSV in the laboratory, in the study of molecular biology of viruses, in the hope that they may provide a better understanding of the epidemiological findings in the field.

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