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A REVIEW OF EXPERIMENTAL STUDIES AND FIELD OBSERVATIONS ON VESICULAR EXANTHEMA OF SWINE

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1. Natural History

1. Introduction:

The announcement by Traum (1934) in 1932-33 of a Vesicular disease in swine clinically indistinguishable from foot and mouth disease in this animal, prompted the initiation of experimental studies on this disease.

2. History and Epidemiology:

In April of 1932 a disease clinically indistinguishable from foot and mouth disease in swine was encountered in garbage-fed hogs near Buena Park, Orange County, California. Spread of the disease was limited to four premises in Orange County, three just across the line in Los Angeles County and one in nearby San Bernardino County. In all probability this represents the first recorded appearance of vesicular exanthema of swine, (Traum, 1934, B.A.I. Report, 1932, Hurt, 1932, and Duckworth, 1935). No species other than swine was found to be infected. In this particular outbreak, in the limited number of tests performed, the virus failed to induce lesions in 24 guinea pigs, 2 calves, 2 heifers, 1 adult cow and 2 horses, (Traum, 1934). On the basis of these tests an initial diagnosis of foot and mouth disease was made, and the virus collected from two ranches during this outbreak was ordered destroyed.

From the Department of Bacteriology, Naval Biological Laboratory, (Madin) and the School of Veterinary Medicine (Traum) University of California. These studies were supported in part by a grant from the Bureau of Animal Industry, Research Adminst., U. S. Dept. of Ag., and by contracts between the Office of Naval Research and the University of California as well as by the Biological Division, Chemical Corps., Camp Detrick., Frederick, Maryland.

The opinions contained in this report are not to be construed as reflecting the views of the Navy Department or the Naval Service at large (Article 1252, U. S. Navy Regulations, 1948).

Grateful acknowledgement is given to Dr. William Takahashi, Department of Plant Pathology, University of California for the inoculation of the tobacco mosaic virus filtrates. Our sincere thanks to Mrs. K. B. DeOme and Mr. R. J. Vonhof for their valuable tachnical assistance.

As regards the actual diagnosis of this particular outbreak of a vesicular disease, the true etiology must always remain questionable. However, there is strong circumstantial evidence to indicate that the disease was vesicular exanthema and not foot and mouth disease. Traum (1934) in discussing this original outbreak wrote "The true classification of the virus causing the 1932 swine outbreak of foot and mouth like disease must be considered as not having been definitely determined, even though a diagnosis of foot and mouth disease had been made and eradication carried out accordingly. It is believed, if more horses had been used in the tests, that lesions would have been produced, thus making the virus of 1932 and 1933 alike in every respect."

In March of 1933, a disease in swine clinically similar to the 1932 outbreak appeared in San Diego County, California. Virus from this outbreak was collected and inoculated into a variety of animals. These animal tests resulted in infecting 15 swine, 4 of 9 horses, but none of 7 cattle and none of 37 guinea pigs. Similar results, but on a larger scale, were obtained by Mohler (1933) and Reppin and Pyl (1934). Observers of the animal tests in all cases, with experience in foot and mouth disease, saw no definite points of clinical difference between that disease in swine and the one produced by the San Diego virus. These reports were later confirmed by the British workers. (Report 1937).

Cross-immunity tests against vesicular stomatitis virus (types Indiana and New Jersey) and foot and mouth disease virus (Types A, O, C) showed the San Diego virus was immunologically different from these virus entities. Again to quote Traum (1934) "Thus, we are confronted by a vesicular disease in swine which so far has shown as much difference in experimental inoculations and immunological tests from both vesicular stomatitis and foot and mouth disease, as does foot and mouth disease from vesicular stomatitis and, although great similarity exists between the viruses of vesicular stomatitis and foot and mouth disease, we have been designating them as separate diseases. It therefore seems that with the information at hand the swine disease discussed above should be recognized as a new entity. Vesicular exanthema of swine is suggested as a name for this disease."

Following the second outbreak in 1933, the disease appeared for the third time in 1934 involving 3 counties in Northern and 2 in Southern California. In 1935 the disease appeared in only 4 counties and involved a limited number of animals. In 1936 only one county was involved, but almost four times the number of animals were attacked. The period from June of 1936 until December of 1939 was noteworthy in that no cases of Vesicular exanthema were reported.

In December of 1939 it again occurred in the San Francisco Bay Area and spread with such alarming rapidity that by 30 June of 1940 it had occurred on 123 premises involving over 222,500 hogs or approximately one-fourth of all the swine in the state of California. Table 1, taken from White (1940) shows the number of premises on which the disease occurred during the period 1932-40, and the approximate number of swine involved.

Since the 1940 outbreak which firmly established this disease in California, yearly occurrances have been the rule. Table 2 shows the number of reported outbreaks by county and by year for the period 1932-1951. These same figures are graphically illustrated in figure 1 showing total outbreaks by counties over the same 20-year period. As is shown in both table 2 and figure 1, only 15 counties have failed to report cases of vesicular exanthema over the period

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covered. It should be pointed out that the majority of these have a very small swine population, which is probably a large factor in their freedom from the disease.

In 1948 and again in 1949 the virus appeared in a number of swine being shipped to the port of Honolulu. These animals had been loaded from California ports and, it is assumed, had come in contact with the virus prior to shipment. Prompt quarantine action prevented the spread of the disease to the Hawaiian mainland, and as far as is known, no further outbreaks have ever been reported in this area.

In June of 1952 the disease suddenly appeared in Laramie, Wyoming. The source of the virus responsible for this outbreak appeared to be garbage containing pork from California. Swine from the Laramie area were then shipped to Nebraska, causing spread of the infection to hogs used by a commercial biological plant. Some of these animals were subsequently shipped to the Omaha stockyards. From these yards infected or exposed hogs were shipped to the states of Washington, New Jersey and California. By this means numerous railyards, feed and rest yards of three railroads became contaminated, resulting in the spread of the infection to at least 32 states, as shown in figure 2, Mulhern (1952).

The "escape" of the virus of vesicular exanthema from the confines of the state of California, unfortunately now poses a new problem for the swine industry of this country. What the effects of this new problem will be cannot be ascertained for the present. If the experience of the swine industry in California represents any guide, it is reasonable to assume that the effects of this disease on an almost nation-wide scale will be of considerable magnitude and in all probability will persist for many years to come.

The virus is spread from infected to well animals principally by the direct contact route. There is no question but that the vesicles which appear on the snout, mouth and feet of infected animals are rich in virus and that upon rupture the escape of this virus is inevitable. Such infected hosts in direct and extremely intimate contact with other swine constitute a direct avenue of spread. Fomites, such as feed, water, feed troughs, bedding and the contaminated physical structures housing swine probably constitute not only a reservoir for the virus, but aid mechanically in spread of the disease. In California the disease is most prevalent on those hog ranches which feed raw garbage (table 3). The inclusion in such refuse of raw pork, trimmed from carcasses of previously infected swine may contribute to the spread and establishment of this disease. Hog feeding ranches which use only grain are not immune from this disease, but the observed incidence of reported outbreaks is much lower than in the garbage-feeding establishments. It is also possible to "clean up" grain feeding ranches following an outbreak such that the disease does not recur, an accomplishment which has never been possible on garbage-feeding premises. There appears to be little doubt that if the practice of feeding raw garbage could be controlled a large reservoir of vesicular exanthema could be eliminated and the problem of controlling this disease would be significantly aided.

Other modes of spread are not known. Insect vectors, except as they may mechanically transmit the virus from animal to animal, do not appear necessary to the spread of the disease. The importance of the carrier state is completely unknown. At the present time, therefore, the only understood mode

of transmission is by direct contact and the feeding of raw garbage containing infected pork. The experiments of Crawford (1934) in this regard unfortunately did little to prove experimentally the degree of transmissability of the virus. In some cases he was able to observe practically no spread of the disease when infected and susceptible animals were placed in the same building wherein no precautions were taken to prevent spread. On the other hand animals did become infected in subsequent experiments done under similar conditions. In our own experience little difficulty has been found in controlling the spread of the virus so long as reasonable precautions have been taken in going from infected to susceptible animals. These precautions have included thorough disinfection of the personnel after leaving an infected area, prompt clean-up of contaminated pens with 2per cent lye soln., and autoclaving of all infected animal carcasses, bedding, etc. prior to the introduction of susceptible animals again in the experimental area. Inasmuch as all of our experimental work has been conducted under such working conditions we have had no opportunity to observe the spread of the disease under experimental conditions. The field evidence with this virus, however, gained over the last two decades, shows that a high degree of transmissability is to be expected.

3. Etiology:

a. Proof of a filterable virus:

The field experience with the causative agent of vesicular exanthema had strongly indicated that in all probability a filter passing virus was responsible for the disease. The pathology of the lesions produced, the complete absence of any purulent exudate, and the striking similarity of the lesions to those produced by the foot and mouth disease virus and vesicular stomatitis were all suggestive of a filtrable virus.

Filtration of field material through Seitz and Berkefeld "N" filters were found capable of producing the disease even though such filtrates were shown to be bacterial free. Crawford (1934) showed that infected horse epithelium, when passed through a Berkefeld "N" filter, was still capable of producing the disease in horses. The bacterial integrity of this filter was controlled by mixing Brucella abortus with the suspect material. Similar results were obtained by the British workers (Report 1937) who filtered infectious material through Switz E. K. filters and were able to produce the disease at will with such filtrates.

These early experiments left little doubt that the etiological agent was a filtrable virus. Conclusive proof of this was shown by us when we were able to recover infectious filtrates from gradacol membrane filters with an approximate pore diameter (A. P. D.) of 44 mu. (See section III).

b. Plurality of virus types:

The early field history of vesicular exanthema indicated the possible existence of a plurality of types. It was noted on a number of occasions that animals recovered from the disease would remain well despite intimate contact with infected swine. On the other hand, animals recently convalescent would "break" with the disease.

In 1933 and 1934 a series of virus collections were sent to the Bureau of Animal Industry Laboratories at Bethesda, Maryland. Crawford (1934) working with this collection, was able to find four immunologically

distinct types, which he named A, B, C, D. Two of the types (B and D) were found to be infectious only for swine, while the other two types (A and C) were infectious for horses as well as for swine. According to Crawford (1934) not only were there complete immunological differences among these types, but certain types produced more severe clinical symptoms than others. Thus, both the B and D types caused more severe reactions than the A and C. The D type differed from the B type in that it caused a swelling of the entire snout.

Following the severe outbreak of the disease in 1940, a series of virus collections was made in the Colma area of California in October and November of that year and March 1942. From these collections we were able to establish the identity of at least three immunologically distinct types, A, B, and C, as shown in table 4.

These results clearly indicate that at least three virus types were present when these tests were undertaken. That the response obtained must be due to a plurality of immunologically different types is proven by the fact that all animals in these experiments were inoculated with another type within 4-6 weeks after the original inoculation. This period of time is not long enough for the immunity to a given type to be impaired.

We did not observe any clear cut differences in the type or severity of the clinical symptoms produced by different types as reported by Crawford (1934).

It is unfortunate that Crawford's original types were not available to us so that an actual comparison could have been made to determine which, if any, were actual duplicates. In all probability some duplication would have been found. It is also unfortunate that the three types originally isolated by us are likewise no longer available. At the present time only two strains of this virus are available. One of these currently designated "F" strains was isolated in 1949 and the other currently designated "B" was isolated in 1951 by Dr. R. A. Bankowski.

It is suggested that since both the original strain isolates designated by Crawford and ourselves are no longer available, thus making it impossible to compare the present "F" and "B" strains of vesicular exanthema, that a new start be made toward initiating a type collection. It is proposed that the present California "F" obtained in the Fontana area be renamed Type "A" and the present so-called "B" strain retain that designation, and that we recognize these two as the only available type isolates at the present writing. As new strains are found it is recommended that they be given the next letter designation.

4. Clinical Picture:

Vesicular exanthema is an acute, febrile, infectious disease of swine, characterized by the formation of multiple vesicles on one or more parts of the body. These may include the epithelium of the snout, lips, tongue and mucosa of the oral cavity and the sole, interdigital spaces and the coronary band of the foot. Occasionally the udder and teats of nursing sows become involved.

The course of the disease is short, usually about 1 week, the mortality is low, and recovery following uncomplicated virus infection is complete. The incubation period in both natural and experimental vesicular

exanthema varies from 24 to 72 hours in the vast majority of cases, with extremes ranging from 12 hours to 12 days. All ages of swine, as well as all breeds, appear to be equally susceptible.

The introduction of virus into susceptible swine produces within the incubation period one of two reactions: (1) the appearance of so-called "primary" vesicles on the snout, lips, tongue and mucosae of the oral cavity or, (2) the appearance of so-called "secondary" vesicles on the sole, interdigital spaces, or coronary band of the foot. As a rule inoculation of the virus intradermally into the snout and mucosae of the oral cavity whether by needle or scarification, produces what has come to be taken for the classical picture, first "primary" lesions at the site of inoculation, followed by "secondary" lesions some 48 to 72 hours later. This is also true, but to a lesser extent, of subcutaneous or intramuscular inoculations. In the case of intravenous inoculation no set pattern may be expected. One may obtain only snout, mouth, tongue or only foot or a combination of such lesions in as many varieties as there are mathematical possibilities. Occasionally this unpredictable lesion pattern is also found under natural conditions, a fact which should be kept in mind during "suspect outbreaks."

In the classical case of intradermal inoculation of the virus into the snout, a condition which is believed to simulate natural infection, a diphasic sympatomatology is produced. In phase 1, lasting from 48-72 hours, there is an initial rise in temperature as shown in figure 3, followed by some degree of anorexia and listlessness, usually within the first 24 hours. These two initial signs are usually immediately followed or co-existent with the appearance of "primary" vesicles. These consist of blanched, raised areas of stratified squamous epithelium varying from 10 to 30 mm. in diameter which are filled with a watery serous fluid rich in virus. Such areas of "blanching" and "lifting" usually follow directly along the paths blazed by the inoculating needle to begin with, and subsequently either spread to immediately adjacent areas or become confluent with neighboring vesicles. Such vesicles may be raised some 10-20 mm. from the surface and to all appearances are very comparable to the blister formation accompanying burns or excessive dermal friction. The epithelial coverings usually "lift" with but the slightest pressure to reveal a raw, bleeding and very tender corium surface.

Following rupture of the vesicles, the lesions usually tend to spread so as to involve the immediately adjacent mucosa of the lips and cheeks. This spread is believed due to the virus liberated from the vesicles, as the new lesions conform to the path taken by the escaping fluid. The subcutaneous tissues of the snout are usually hyperemic and swollen and become very sensitive to external pressure. In some cases this swelling may be so noticeable as to suggest the term "bull nose". In most cases the mucosae of the lips become involved, particularly around the opposing canine teeth. These vesicles are usually quickly ruptured and the lesion there represented by a circular erosion covered with a yellowish fibrinous membrane. The tongue is often involved and is sometimes accompanied by considerable swelling productive of attacks of slobbering.

These primary lesions are almost invariably accompanied by serious temperature changes, occasionally reaching 108° F but more commonly between 106-107°F. Anorexia and lassitude are very often present at this time. It should be noted that while the above symptoms constitute the rule, there are a disturbing number of cases wherein no significant thermal reactions are

present even though typical lesions are clearly evident. These same animals do not show the anorexia and lassitude, in fact may escape detection as being vesicular exanthema cases completely even under experimental conditions, unless the most critical examination is made of the individual animal for lesions.

Healing of the primary lesions usually begins immediately upon rupture, and the raw surfaces become covered by a yellowish-fibrinous membrane. During this period the temperature gradually recedes and the animal may regain its appetite. These signs usually signify the end of phase one, and persist for 24 to 72 hours.

At the end of the 24-to-72 hour period phase two is ushered in with the appearance of the secondary vesicles. This lasts until the secondary lesions rupture, a period of from 3 to 7 days, after which recovery is rapid and complete from uncomplicated vesicular exanthema, or it may be unpredictably prolonged due to secondary invaders. The initial appearance of foot lesions is usually signified by a characteristic hesitant gait, which is best described in terms of the field veterinarians by the word "ouchy". The animal may continue to walk in this halting fashion for several days, or may simply refuse to move until the pain and swelling have decreased. This may be accompanied by edematous swellings of the legs and joints, and is particularly common in the case of the hind legs. The breaking of the vesicles is almost immediately followed by a drop in temperature and a gradual return of the animal to its normal eating habits. It is at this point in the disease that the bacterial contaminants are often encountered attacking the exposed tissues of the feet. Where these gain a foothold the prognosis is usually poor. Animals which have been severely involved with the virus phase for several days are prone to pneumonia and enteritis. This is especially true of fat hogs in feed lots or stockyards.

As previously indicated overall mortality from vesicular exanthema is very low, probably less than 5 per cent. In the case of suckling pigs, however, the infection may assume severe proportions and be responsible for a very high mortality rate. This is due to a combination of factors including the inability of the suckling pigs to feed due directly to the presence of lesions, and also due to secondary bacterial invaders. Frequently, this picture is further complicated by the presence of vesicles on the udders of nursing sows, and a drop in milk production. Hurt (1939-1940) and other field observers have called attention to the apparent inability of many sows to carry their litters to term following infection with vesicular exanthema.

The above signs and symptoms of vesicular exanthema are those most commonly experienced with this disease under field and laboratory conditions. For other discussions on the clinical picture see Traum, 1936; Hurt, 1940, Wicktor and Bradbury, 1936; White, 1940; British Report, 1937; Crawford, 1934; Anderes 1952.

5. Economic Aspects:

The economic importance of any non-lethal disease of food-producing animals is exceedingly difficult to measure in terms of dollars and cents and vesicular exanthema is an excellent example of such a disease. In baby pigs the disease is often so severe as to make subsequent marketing of surviving animals uneconomical. The high mortality

rate among suckling swine, coupled with the adverse effects of the disease on both sow and surviving piglet alike, has forced a number of hog farms to cease their own breeding operations and to purchase "feeder" pigs as a more economical way of raising market swine. Feed lot animals are usually set back about 30 days on the average as far as market "finish" is concerned. Losses in fat hogs are extremely severe inasmuch as this particular class of animal usually experiences the most crippling lesions.

Losses sustained by packing houses may reach considerable proportions due to the quarantine restrictions, extensive clean-up and disinfecting operations, loss of meat parts such as hams and feet, and death losses due to downed hogs and secondary bacterial invaders so prevalent in infected swine under transportation and stockyard conditions. For other discussions see Hurt (1939-1940) (1946-1947) (1949-1950).

II. Biological Properties of the Virus:

1. Introduction:

In 1936 a research program designed to investigate such fundamental properties of this virus as would aid in a better understanding of the disease, and ultimately lead to its control was initiated. This program has had as its most frustrating obstacle the fact that the only reliable experimental host is the pig. These animals available to us in only limited numbers have formed the basis for the experimental results herein reported for the first time.

2. Experimental Studies in Swine:

a. General techniques:

It is believed that for purposes of clarity a description of the general techniques used with this virus would be pertinent. The following methods regarding handling and preparation of virus materials, inoculation of animals, their subsequent observation and husbandry have been used consistently in this laboratory.

1- Virus techniques:

Virus material is stored in 50 per cent glycerine phosphate buffer pH 7.4 at 34-40°F., in the form of unground vesicle coverings taken either from primary or secondary lesions. For the preparation of inocula 10-20 per cent (by weight) suspensions are made by grinding the epithelium and suspending in either Sorensen's phosphate buffer pH 7.4 or 1 per cent peptone water. This suspension is allowed to sit at 34-40°F. for at least 24 hours and centrifuged at 1500 r.p.m. for 10 minutes. The clear supernatant is removed, filtered and in recent years has been mixed with penicillin (100 units/ml) and streptomycin (1mg/ml.) The sediment remaining after centrifugation is saved for future use as a virus source.

2- Animal techniques:

Test swine 25-100 pounds in weight and known to be susceptible are placed in their pens 1 week prior to inoculation with the virus. Temperatures are taken daily during this interval. Feed is supplied once daily in the form of laying mash only and running water is kept available at all times. Animals are restrained depending on their

size by either strapping in a hog board or using complete nembutal anesthesia. Intradermal inoculations of 0.2 to 1.0 ml are made into the snout, lips or oral mucosae using a 25 gauge 1 inch needle. Intravenous inoculations of similar amounts are made either into the marginal ear vein or via the anterior vena cava. Other inoculations such as intra-muscular or subcutaneous are made in the standard manner into the gluteal muscles or the subcutaneous tissues of the axillary space.

All animals are observed daily following inoculations and temperatures taken. Thermal reactions of 104° F are usually considered significant. Vesicle material is harvested prior to rupture in order to obtain the highest possible viral content. All animals are observed for a total period of 14 days following the appearance of vesicle formation. This time period has been found adequate to allow for all secondary lesions, and to date has covered the most prolonged single incubation period (12 days).

At the end of this time period animals are either destroyed and their carcasses autoclaved or they are retained for further tests. A 2 per cent sodium hydroxide solution is used for purposes of disinfection and all pens are thoroughly washed with this solution and allowed to stand idle 2 days prior to the introduction of other susceptible swine.

b. Infectivity as Determined by Dilution:

The difficulty of titrating a virus such as vesicular exanthema immediately becomes apparent when the problem of the host animal is considered. Normally a given virus is titrated on a rather large number of animals, per dilution and over a considerable range of such dilutions. Various strains of the virus are usually titrated and often where available titrations are carried out in several hosts and by usually more than one route of inoculation. With the hog as the only suitable host we have not even been able to approach any but the simplest of the above criteria.

Virus was first filtered through a 488 mu gradacol membrane and then absorbed on aluminum hydroxide-gel C. There is little doubt that considerable virus was lost through ultra-filtration and the dilutions prepared were therefore somewhat greater than actually indicated. Hundredfold dilutions were prepared from a 1/10 suspension of the virus-gel. A series of swine were then inoculated with dilutions of 1×10^{-5}, 1×10^{-6} and 1×10^{-7} The results of these inoculations are shown in Table 5. As indicated the infecting dilutions appears to be 1×10^{-6}.

A similar test carried out with the so-called "F" strain of virus gave a lower figure of 1×10^{-3} for infectivity, but was immunogenic at 1×10^{-5}. The writers are of the opinion that if virus is collected under optimal conditions and properly preserved titers similar to those reported above at 1×10^{-6} would probably be the rule rather than the exception. Much more work on all possible strains of this virus is needed to establish some figures for a minimum infecting dose.

c. Recovery of virus from blood and spleen:

The questions of where and when the virus may be recovered in the body of the host at sites other than those demonstrable by the appearance of visible lesions, is one of considerable theoretical and extreme practical important. We have initiated preliminary studies on this question,

leading in time to a complete study of the pathogenesis of the virus.

Four test swine were each inoculated with 1.0 ml. of the same virus suspension intradermally into the snout. All animals showed definite evidence of vesicular exanthema 24 hours after inoculation. One animal was sacrificed at this time and the spleen and blood removed. Similarly one animal was sacrificed at 48, 72 and 96 hours and the same tissues taken and preserved. Aliquots of these tissues were subsequently inoculated into susceptible swine with the results shown in table 6. As indicated, both the blood and spleen were positive at 24 and 48 hours after inoculation. The blood was negative at both 72 and 96 hours. The spleen unfortunately could not be tested after the 48th hour. Both positive results with blood and spleen tissue taken at 48 hours were again confirmed at least twice.

While these experiments were somewhat encouraging inasmuch as they indicated at least two other sources of virus besides vesicle material, and proved the existence of a viremia, they left unanswered a much greater number of questions. Some of these are: (1) How high a titer is reached in the blood and spleen. (2) How long after 48 hours does virus persist in the spleen. (3) Is the viremia present prior to 24 hours. (4) Does the pattern described above follow for more than one strain of the virus. (5) What other tissues may harbor the virus. These and other questions, it is hoped, will be answered by the studies on pathogenesis.

d. Immunology:

Recovery from vesicular exanthema results in the establishment of a solid immunity to a second attack by the homologous strain of virus for at least six months. This immune state is present within three weeks after the animal has been exposed to live virus. It is interesting to note such immune animals will tolerate very large doses of virus directly into the snout or intravenously without the slightest sign of even primary infection. However, absolutely no protection is afforded against heterologous strains, and this is proven in the laboratory and reflected in the field by recurrent outbreaks in the same swine population within very short periods of time.

It is believed from experiments carried out on hamsters but unfortunately not on swine, that demonstrable neutralizing antibodies are present in convalescent swine serum.

3. Attempts to extend the host range:

The inability to propagate this virus in any other than the definitive host has focused attention upon the critical need for a suitable laboratory host. A large series of common laboratory animals have been tested as well as certain of the large domestic animals. The results of these studies are presented below:

a. Large animals

1. Adult cattle and calves:

In all of the field outbreaks since the first appearance of this disease quarantine regulations have made it mandatory that at least a calf or cow be inoculated intradermally on the lips and snout with fresh swine virus material obtained from the outbreak in progress. In no single instance has a lesion indicative of this virus ever been produced, nor has there ever been significant thermal reactions related to this virus. These field experiences have been confirmed by us on numerous occasions in the laboratory as well as by Crawford (1936) and the British workers (Report 1937).

2- Sheep:

The results of all attempts to infect this species have been uniformly negative in our hands. Similar results have been experienced by Crawford (1936) and the British workers (Report 1937).

3- Goats:

No response has over been noticed with this animal.

4- Horses:

The horse has been a subject of considerable controversy since at times lesions very suggestive of vesicular exanthema have been produced while at other times completely negative results have been obtained. In the first outbreak of the disease negative results were obtained in horses. In the second outbreak approximately 50 per cent of the animals reacted with typical vesicular lesions. Crawford (1936) showed that horses were susceptible to two of the virus types (A and C) but not to B and D. The British workers (Report 1937) were unable to infect horses in 6 individual attempts. Reppin and Pyl (1934) were able to infect horses however and considered this an important diagnostic aid. In our own experience, while we have found the horse to be quite variable in its reaction, some animals do definitely respond with mild clinical vesicular exanthema. This is particularly true when intralingual inoculations are made (Traum, 1936). In all probability this animal, while not readily susceptible, will become clinically involved when virus of sufficient potency is properly inoculated. It is also highly probable, as Crawford (1936) has already pointed out, that different virus types may act differently in this animal.

b. Small Laboratory Animals:

1- Guinea Pig:

In many cases of field outbreaks the guinea pig has constituted one of the test animals and has therefore received considerable field attention. These animals have been inoculated intradermally via scarification and tunnelling of the volar surface of the plantar pads. The vast majority of these inoculations have yielded completely negative results. In a few cases, however, this animal has "reacted" with lesions highly suggestive of vesicular lesions. In 1936 one of us (J.T.) was able to produce lesions in one guinea pig and successfully

passage this to two other animals. In the second Hawaiian outbreak (1949) one of us (J.T.) obtained definite lesions and was able to make 5 serial passages. Unfortunately this virus was not properly shipped to the mainland and was dead upon arrival.

To date, however, no significant reactions have ever been obtained with this animal in the laboratory despite massive inoculations of virus by almost every conceivable route. These results have also been obtained by Mohler (1934), Crawford (1936) and the British workers (1937). In view of these results the guinea pig must be considered highly resistant to this virus.

2- Mouse:

A large number of mice have been used in several experimental series over a number of years. These have included X-irradiated and normal Namru strain mice, the Agouti, C57 black, hybrid black, and bald mice. These animals have been inoculated via a variety of routes including intradermal, intraperitoneal, intranasal, and the intracerebral with massive doses of virus. A large series of ages has been attempted including 2, 4, 7, 10, 14 day old suckling mice as well as numerous adult ages.

In all cases these animals have never shown a significant lesion which could have been thought due to the virus of vesicular exanthema. In some instances where suspect lesions have been noted, every attempt has been made to passage this material into other mice, but the results have been uniformly negative.

3- Rat:

Two species of rats, the white rat (Crawford, 1936) and the wild rat (Rattus norvegicus) British workers (Report 1937) have been shown to be refractory to this virus.

4- Rabbits:

Attempts in this laboratory to infect the common laboratory rabbit have proven completely negative.

5- Hedgehog:

All attempts to infect the hedgehog have proven negative. Crawford (1936), British workers (Report 1937), Mohler (1934). The British (Report 1931, 1937) have shown the susceptibility of this animal for the foot and mouth disease virus.

6- Chick Embryo:

Attempts to propagate this virus in the developing chick embryo have been made for a number of years. A total of some 30 virus samples taken from four different field outbreaks (1936, 2 in 1940, and 1949) have been tested. The inoculum in all cases consisted of

ground centrifuged vesicle material definitely proven to produce clinical vesicular exanthema in swine and was used in as great a concentration as possible.

Bacteria were removed from vesicle suspensions either by filtration or antibiotics. As a rule 6-10 embryos were used per dilution of virus. A large variety of embryo ages (8-14 days) have been used, the most commonly used age being 11 days. Five routes of inoculation have been investigated: (1) allantoic cavity, (2) chorio-allantoic membrane, (3) yolk sac, (4) intravenous, and (5) intra-embryo including intracerebral. The amount inoculated varied from 0.2 to 1.0 ml depending upon the route used. Incubation was carried out at both 34-35 C and at 37-38 C. for varying periods of time.

Following inoculation, all embryos were carefully examined and sub-passages made of any material derived from embryos suspected to be affected by the virus inoculum. Aliquots of embryo material from the 1-2, 5-6, and 9-10 passages were inoculated into susceptible swine.

The results to date with all embryo work, regardless of the route of inoculation or the inoculum used, have been uniformly negative. Sub-passage of potentially infected egg material into swine has also been completely negative. These swine, when challenged with known live virus two to four weeks after inoculation with the embryo material, have always responded with clinical vesicular exanthema indicating no degree of immunity had been conferred by exposure to embryo material.

7- Hamster:

In 1941 work was initiated by one of us (S.H.M.) on the possible use of the hamster (Cricetus auratus) as a laboratory host. An initial series of these animals were inoculated via two routes, intracranial and interperitoneal and all remained normal. Another series of animals inoculated on the ventral surface of the abdomen via the intradermal route showed small vesicle-like lesions surrounding the site of inoculation 24 hours later. These lesions were sharply circumscribed vesicles about 5-8 mm. in diameter and showing definite lifting. The epithelium covering these vesicles was easily removed, and revealed a raw derium surface completely devoid of any evidence of pus formation. A small amount of clear fluid was present within the vesicle.

These vesicle coverings were removed and passaged into other hamsters using the same mode of inoculation. A series of 6 passages was carried out using 2-3 animals per passage. In all cases, lesions similar to those originally described were produced through the first 4 passages, but began to be questionable in passage 5, and were completely unreliable in passage 6. Another group of hamsters inoculated with virus material of the same immunological type, but obtained from a different test pig, produced vesicles in about 50 per cent of the animals.

In the hope that another strain of the virus might produce more consistent results, a "B" strain was inoculated intradermally. Immediate "takes" were obtained and this vesicle material was subpassaged. A total of 8 passages involving approximately 50 animals was

carried out. In all cases clear cut evidence of vesicle formation resulted at the site of inoculation with occasional evidence of spread to immediately adjacent areas. Subsequent to this an additional 400 animals were inoculated for a variety of test purposes and gave regular and reproducible results.

After a number of passages the vesicles appeared regularly within 24 hours after inoculation and varied in size from 2 x 5 mm. to 10 x 15 mm. Rupture of this lesion usually occurred a few hours after its appearance and healing began almost immediately. Secondary lesions were never encountered. Clinically the animals showed little evidence of infection other than a slight anorexia and listlessness. Characteristic temperature curves were found following inoculation (Figure 4) and, in general, coincided with the appearance of the vesicles. All temperatures were taken via the cheek pouch cavity. Control temperatures taken on a series of 20 normal animals daily for a period of one week gave an average of 36.8 C. (36.5-37.0 C) with extremes of 35.9 to 37.6 C. Following inoculation the pyrexia became evident in 12-24 hours and was characterized by readings above 38 C persisting for a total of about 72 hours, after which they would gradually return to normal over an additional 12 hour period.

A third immunologically different strain was also tested and produced lesions, but without the degree of reliability such as experienced with the "B" type virus. Both the present California "F" and the strain responsible in part for the recent national outbreak of this disease, have been inoculated into both adult and suckling hamsters and have failed to produce any significant deviations from normal.

The encouraging results with some strains of this virus in the hamster have led to considerable speculation as to its value as a laboratory animal. It may be that this animal reacts very markedly to different virus strains and that such strain differences account for the alternate successes and failures. It may also be that the hamster is of a very low order of susceptibility and that only in the presence of an extremely high titer virus may positive reactions be expected. Whatever the reason, the hamster does not represent the answer to a reliable small laboratory animal for this virus. It may be that with further investigation this lack of reliability will be overcome, in the meantime, however, the lack of a suitable laboratory host still represents the largest single obstacle to research on this disease.

11. Studies on the Particle Size of the Virus:

The particle size of this virus has been determined by a series of ultrafiltration experiments, using the Elford type of "gradacol membranes". The methods of preparation, calibration and use of these membranes were those described by Bauer and Hughes (1936).

The following procedure was used throughout in the preparation of virus suspensions for filtrations tests. Vesicle coverings from experimentally infected swine were stored in 50 per cent glycerine phosphate buffer solution until ready for use. This material was finally ground and a 15-20 per cent tissue suspension made in Sorenson's phosphate buffer pH 7.4. This suspension was allowed to stand for 2 hours at 4C

and then centrifuged for 20 minutes at 3000 rpm. The supernatant was decanted, the tissue sediment resuspended in phosphate buffer and recentrifuged as above. The two supernatants were then pooled and diluted to a final tissue concentration of 10 per cent. The suspension, now ready for preliminary filtration, had a colorless moderately opalescent appearance.

For filtration all membranes were prepared by passing a capillary active agent "Hartley's broth" containing 1/10 ml of a 24 hour broth culture of B. prodigiosus, through the test filter at 40 cm. positive pressure of nitrogen gas. Preliminary filtrates through these membranes were tested for sterility. Only membranes yielding sterile filtrates were used in the size determinations.

Following passage of the capillary active agent, the virus suspension was filtered. In all cases an initial filtration was made through a 400-500 mu approximate pore diameter (A.P.D.) membrane to remove large particle sized material. Recovery of this filtrate was made at temperatures never exceeding 10-12 C. The filtrate was then passed through the membrane sizes appropriate for the initial size determination.

To serve as a check on the pore size of the filter membranes, a suspension of tobacco mosaic virus was used. An equal quantity of clarified tobacco mosaic virus suspension was added to the vesicular exanthema virus suspension, and a series of filtrations were then carried out using membranes having an average pore diameter ranging from 149 mu to 27 mu.

Filtrates obtained were divided into 2 portions, one for inoculation in swine by the intradermal and intravenous routes, and the other for inoculation in tobacco leaves. The latter inoculations were made into each of 10 leaves per filtrate, while 10 other leaves on the same tobacco plant were inoculated with known live virus to serve as controls. After 5 days the number of "spots" on each leaf was counted and reported. All leaves inoculated with known live virus showed definite evidence of the virus, while the leaves inoculated with the various filtrates gave the results listed in table 7. From these results it is apparent that the 114 mu filtrate definitely contained virus as did the 44 mu although in lesser quantity. The others, it is concluded, were negative, as the number of spots present on leaves inoculated with the 38 and 27 mu filtrates were too small to be significant.

The end point of 44 mu reached with the tobacco mosic virus is in close agreement with that reported by Thornberry (1937) of 45 mu. These results are taken to indicate that the pore size of the individual membranes used was certainly within the limits of experimental error for the figures obtained by calibration.

The results obtained with the vesicular exanthema virus are shown in table 8. These indicate that the end point of vesicular exanthema virus lies slightly above 39 mu. If one considers the actual particle diameter to be 1/3 to 1/2 that of the A.P.D., (Rivers 1948) then the particle size of this virus lies between 13-20 mu thus placing it in the range of some of the smallest virus elements.

IV. Studies on the Stability of the Virus:

1. Storage resistance:

In an effort to obtain information on the storage resistance of this virus an initial series of tests were made on virus stored at ordinary refrigeration temperatures for periods of two and three years in glycerine phosphate buffer in the form of unground vesicle coverings. Five animals were inoculated intradermally on the snout and three intravenously with a mixture of virus materials collected from two outbreaks which had been stored for 3 years. Negative results were obtained in all cases. Similar material stored for only 2 years proved infective however.

A similar test of some "F" strain material stored for 2 1/2 years gave positive results. Material stored for shorter time periods has never failed to infect swine. Unfortunately accurate titrations of virus content before and after storage have not been possible. This has precluded any estimation of a change in infectivity on a quantitative basis. These experiments have indicated however that this virus is reasonably stable under ordinary refrigerator conditions.

Very little information of an experimental nature is available for other conditions of storage. In one instance a 10 per cent suspension of ground vesicle coverings in 1 per cent peptone buffer pH 7.4 was viable after six weeks storage at room temperature, wherein the temperature rose to at least 80 F for several days. In another instance a similar suspension of virus in Sorensen's phosphate buffer survived 24 hours at 37. C but was not infective at 54 hours.

These preliminary results indicate that this virus probably shows considerable resistance to many environmental conditions, and it is suggested that until evidence to the contarary is accumulated, this virus be considered in the same resistant class as the virus of foot and mouth disease.

2. Reactivation of the Virus with Cysteine Monohydrochloride:

During the intensive work with strains "B" and "C" in the hamster (Section II) a number of virus samples which had been stored in the refrigerator were found to have lost the ability to infect this animal. This attenuation was believed due to a mechanical failure of the refrigerator in question, a fact not discovered for a number of days, during the hottest period of the year. The loss of these samples in particular would have been most unfortunate and it was decided to see if some of them at least could not be reactivated.

The work of a number of investigators had previously indicated that the attenuation of virus activity is often paralleled by oxidative changes, and that when these were prevented there was a reduction in the loss of infectivity. Gye and Purdy (1925) showed that the filtrate

from a Roux's sarcoma was inactivated in 24 hours at 37 C, but that the addition of a small amount of hydrogen cyanide to the filtrate caused a retention of activity for at least three days. Zinsser and Seastone (1930) found the virulence of herpes simplex virus stored at room temperature could be maintained for at least three weeks when cysteine was added to virus filtrates, compared to only 2 to 4 days storage when cysteine was omitted. These same authors also showed that a concentration of 0.1 per cent cysteine in the filtrate restored some of the virulence to an apparently innocuous suspension. This work on preservation and reactivation was later duplicated by Perdrau (1931).

Similar findings for the preservation of viruses by cysteine have been shown for neurovaccinia by Long and Olitsky (1930), and tomato spotted wilt virus, Best (1939), to indicate but a few.

These findings led us to attempt the reactivation of 6 lots of virus consisting of strains "B" and "C". Filtrates from each lot were prepared from the epithelial vesicle coverings. A 1 to 5 per cent suspension of tissue was made in Sorensen's phosphate buffer and filtered through a gradacol membrane (400 mu). All were tested in hamsters and all were found to be non-infective. To aliquots of these filtrates cysteine monohydrochloride was added to give a final concentration of 10 milligrams of cysteine for each gram of tissue. Treated filtrates were kept at room temperature for 24 hours and then stored in the refrigerator to await test. Aliquots of the same filtrate without cysteine were similarly held as controls.

At various time intervals thereafter, portions from these aliquots were withdrawn and inoculated into test hamsters. Each animal received a total of approximately 0.5 ml intradermally on the ventral surface of the abdomen. The results of these experiments are shown in table 9. As indicated all of the treated filtrates regained their infectivity to a point indistinguishable with that obtained prior to the refrigerator trouble. The minimum period necessary to "reactivate" was found to be 8 days, and once "reactivated" remained so over the longest period tested, 262 days.

It should be noted that an experiment such as this simply describes a qualitative phenomena, wherein the quantitative aspects are completely ignored. It is impossible to say from these experiments what percentage of infectivity was originally present, and therefore what was restored. The authors simply wish to point out the ability of cysteine to restore a measure of infectivity to previously non-infective filtrates. It is suggested that the addition of cysteine to stored samples of vesicular exanthema virus would be advantageous.

The ability to revive this virus is of considerable interest regarding its survival in nature. It has already been pointed out that a number of non-epidemic years have been experienced in California. The question of what happens to the virus during these periods is an extremely interesting one. Does the virus actually die out on the premises so that in order for a new outbreak to occur one must introduce new virus? If this is so it is very curious that from 1936 through 1939 there was not a single case reported, particularly in view of the exceedingly close watch maintained by regulatory officials and by

owners in the case of vesicular diseases of swine. If this be the answer it would be a most singular thing, in view of the raw garbage feeding habits practiced on over 300 hog ranches.

It is perhaps just as reasonable to assume that virus persists on the premises which have experienced the disease, and that the chance contact of a susceptible host with active virus is the determining factor. If this be the case, then anything which would preserve the virus, or even more important, reactivate previously inactive virus, would materially aid in the chance contact between animal and virus. It is suggested that in nature cysteine or its many related compounds might play such a role in maintaining the vesicular exanthema virus.

There appears to be no question but what ample cysteine and related substances are readily available under natural conditions. The decomposition of tissues produces quantities of cysteine and various epithelial derivatives such as hair, nails, horn and wool contain large quantities of cysteine which can readily be reduced to cysteine by common bacteria such as Proteus vulgaris and B. coli. Tarr (1933) Hosoya and Hidetake (1935). These sources could provide the virus with a supply of suitable reducing substances, and perhaps preserve it for long periods during which the opportunity would eventually present itself to infect a susceptible animal. If such an assumption is correct it could mean that the eradication of this disease, and perhaps others of the vesicular group, from heavily contaminated premises would necessitate great attention being paid to adequate disinfection procedures.

V. Vaccine Prophylaxsis and Treatment:

1. Vaccine Therapy:

At the present there is no available vaccine material. Experimentally we have studied two types of preparation on a very limited scale, one a formaline-killed $Al(OH)_3$ treated vaccine patterned after the so-called "Schmidt-Waldmann", Schmidt and Hansen, (1936) and Waldmann and Kobe, (1938) and Waldmann et al (1941) for foot and mouth disease, and the other a crystal violet blood vaccine similar to that used by McBryde and Cole (1936).

The formalin $Al(OH)_3$ killed vaccine consisted of the following:

7 per cent type "B" virus tissue suspension ...	... 50.00 ml
$Al(OH)_3$ Gel type C.....	.....250.00 ml
50 per cent glycerine in Sorensen's borate buffer pH 8.9	.....200.00 ml
5 per cent formalin.....	0.25 ml

This mixture was placed in a brown bottle, sealed under vacuum and mechanically mixed for one hour. It was allowed to stand for 48 hours at 25 C. and then stored in the refrigerator at 3 to 6 C. Sterility tests performed at the end of one week's storage were all negative. The efficacy of this product was initially tested on 2 swine. The route and amount of inoculum, as well as the results, are shown in table 10.

The results of this first series indicated complete protection against challenge, and the side effects produced by the vaccine itself were not objectionable. This experiment was then repeated on a larger group of animals, with only slight variations in the amount of vaccine used, and its route of inoculation. The results of this series are shown in table 11. These findings, while not as clear-cut as the preliminary series, still show a consistent degree of protection against secondary lesions and essentially against systemic effects. This latter point is best illustrated by table 12, showing the comparative temperatures of challenged vaccinated, and control animals.

The crystal violet blood vaccine was made as follows:
Infected blood.....150.00 ml
3 per cent disodium phosphate..... 18.75 ml
0.5 per cent crystal violet solution..... 18.75 ml

This mixture was placed in a 37 C incubator, shaken occasionally and removed 14 days later. A number of swine were inoculated with this product the protocol and results of which are shown in Table 13. Unfortunately this preparation failed to confirm the degree of protection noted for the other vaccine types. In these instances where two injections were given 14 days apart, only primary lesions were noted on challenge 30 days after the second injection of vaccine. On the other hand, where only one injection was given, no resistance was noticed on challenge. Even in those cases where secondary lesions were not present following two injections of the vaccine, severe systemic reactions were present. It is felt that not only must a vaccine protect against secondary lesions completely, but it should certainly reduce pyrexia and anorexia if it is to serve its full purpose and must take into account the plurality of types.

The type of vaccine testing described above does very little more than indicate the fact that some form of vaccine prophylaxsis is possible, and would in all probability, be profitable on a field scale. The complete lack of knowledge concerning the actual antigenic potency of the vaccine compared to the known infectivity of the challenge dose, places an experiment of this type in the class of an all or none phenomena. Under such a handicap, the fact that the results are as promising as indicated is extremely encouraging, and indicates a definite future for the field of vaccine prophylaxsis.

2. Treatment:

There is no known treatment for this disease. Certain precautions of a palliative nature may be taken, however, which will tend to reduce the losses from this infection. Weight losses can be reduced if infected animals are placed on soft foods or slops entirely, if they are taken off concrete or similar hard surfaces, and if adequate amounts of clean water are kept before them at all times. Clinically ill animals should be kept under shaded conditions, as the pyrexia coupled with the extreme reluctance to move, makes them susceptible to blistering and sunstroke.

Where infected animals must be maintained in crowded quarters such as under rail shipment, feed lots, or in slaughter houses, second-

ary bacterial complications may be markedly reduced by the judicious administration of penicillin and streptomycin.

VI. Differential Diagnosis:

The fact that the viruses of vesicular exanthema, vesicular stomatitis, and foot and mouth disease produce signs and symptoms which are clinically indistinguishable in the common susceptible host swine, places an unfortunate burden on the differential diagnosis of these diseases. The clinical likeness of these diseases has led, more by loose association and usage than anything else, to the tacit assumption that the viruses themselves are somehow related. We should like to point out that where comparable facts are known concerning these three viruses such as particle size, host range and immunological differences they are quite distinct. The practice of "borrowing" information from one virus and applying it to another without experimental proof is not recommended but has to be resorted to until definite information is available for the specific virus in question.

The field differentiation of these diseases is based primarily on the scheme proposed by Traum (1934), Crawford (1936) and Traum (1936). Essentially this involves the inoculation of a cow, pig, horse and a guinea pig by a variety of routes, the results indicating which of the three virus diseases is present. The typing of a given virus is done in all three diseases by the inoculation of the suspect virus into known type immune animals, the results being dependent on finding the homologous immune animals to the unknown virus. Virus types of vesicular stomatitis and foot and mouth disease may also be ascertained by complement fixation tests.

This system of animal inoculation is satisfactory as long as live virus is available, speed is not critical, typing of the individual virus is not required, and a new vesicular disease has not arisen. Despite the above shortcomings this system is the best available at the present time, and where properly carried out will usually allow a satisfactory diagnosis to be made. Since so much depends on the proper diagnosis being made, we feel a slightly revised system of animal inoculation techniques are in order along with detailed methods of obtaining and inoculating suspect virus material.

The majority of outbreaks of a vesicular nature which have occurred in this country over the last two decades have been in swine. Inasmuch as this is the one host highly susceptible to all three virus diseases, and in all probability, the most fertile ground for the appearance of another de novo vesicular disease, immediate quarantine of all infected and exposed animals should be imposed until adequate diagnostic tests can be performed. The following techniques are recommended:

1. Collection of Virus Material:

a. Select animals showing temperatures of at least 40.6 C or 105 F for the obtaining of virus materials.

b. Remove vesicle covering material only from unruptured

vesicles or freshly ruptured ones either on the snout or feet.

c. Collect as much material as conditions permit.

d. Place collected vesicle material in 50 per cent glycerine phosphate buffer prepared as follows:

Glycerine USP or equivalent.....100.00 ml
Sorensen's phosphate buffer pH 7.4.....100.00 ml

Virus material collected in this solution will resist unfavorable conditions for about 6 hours, but it should be placed under normal refrigeration as soon as possible.

e. Where glycerine phosphate buffer is not available, 1 per cent peptone water, 1 per cent skim milk, or in an acute emergency, plain tap water can be used to aid in preserving the virus.

2. Inoculation of Test Animals:

a. Wherever possible the test animals and routes of inoculation suggested in table 14 should be followed.

b. Select several large pieces of vesicle covering material and grind as fine as possible, adding either 1 per cent peptone water pH 7.4 or some of the original glycerine phosphate buffer as the diluent. A concentration of at least 1 part of tissue to 10 of diluent is recommended. Strain through several layers of gauze.

c. Where intravenous inoculations are to be made it is necessary to accompany such field filtrates with 300,000 units of penicillin and 500 mg of streptomycin intramuscularly.

d. Intradermal inoculations should be made directly into one side of the snout, using a minimum of 0.5 ml of filtrate, while the other side should be lightly scarified and 0.5 ml of filtrate briskly rubbed into the lines of scarification.

e. We have found it advantageous to make intradermal inoculations into the mucosae of at least one, and preferably both upper lips. This route of inoculation will often show positive reactions where snout inoculations fail. It is also possible to distinguish more easily lesions due to bacterial contamination here than on the snout.

f. Temperatures should be initiated 24 hours after inoculations and continued every 12 hours until clean cut lesions are produced. It is important to remember that lesions may occur without significant thermal reactions.

g. All virus material remaining after inoculation must be saved until released by competent authority. There is always the possibility of atypical results requiring extensive laboratory retesting of field material, as well as the possibility of new vesicular diseases arising which can only be determined by having such materials available.

3. Serological Tests:

At the present time a highly desirable adjunct to the animal inoculation scheme would be a serological test capable of differentiating these viruses. Currently an adequate complement fixation test is available for both foot and mouth disease Brooksby (1948) and for vesicular stomatitis, Camargo etal (1950) and Madin and McClain (1951). The writers have attempted complement fixation tests with the vesicular exanthema virus for a number of years, but the results while encouraging, have not yet culminated in a clear cut reliable test comparable to that used with either of the other virus entities. The great difficulty appears to be a matter of proper antigen concentration and purity and it is hoped that this problem will be overcome in the near future. To date it has not been possible to show any cross fixation between vesicular exanthema immune serum and vesicular stomatitis antigen despite repeated attempts.

One of us (SHM) has repeatedly attempted the hemagglutination technique originally described by Hirst (1941), but so far all attempts have proven futile. Michelsen (1949) using rat erythrocytes with the foot and mouth disease virus, wherein such a test was finally achieved, indicates that an exhaustive search for the proper animal species may yet yield results with vesicular exanthema virus. At present consistently negative results have been obtained using swine, chicken, rabbit, horse, guinea pig and human cells with this virus.

The problem of detecting neutralizing antibodies has been attempted in connection with the hamster as an experimental host. These results have been encouraging and indicate a possible diagnostic approach. To date, however, it has not been feasible to attempt this on swine.

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TABLE 1

OUTBREAKS OF VESICULAR EXANTHEMA OF SWINE IN THE STATE OF CALIFORNIA FOR THE PERIOD 1932-1940. (MODIFIED FROM WHITE)

YEAR	SEASON	NO. PREMISES INVOLVED	NO. SWINE INVOLVED	TOTAL SWINE IN STATE	PER CENT OF TOTAL SWINE INFECTED
1932	Apr - May	3	18,000	672,000	3
1933	Mar - Apr	4	5,500	706,000	0.7
1934	June - Dec	31	95,000	660,000	14.4
1935	Feb, May June	4	13,000	530,000	2.0
1936	Apr - June	14	19,000	610,000	3.1
1939 - 1940	Dec - June	123	222,500	885,000	28.0

TABLE 2

TOTAL VESICULAR EXANTHEMA OUTBREAKS REPORTED IN CALIFORNIA BY COUNTY FOR PERIOD 1932 - 1951

COUNTY	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951
Alameda			x*						6	4		7	8	6	7	12	,	4	11	7
Alpine		no outbreaks reported																		
Amador		no outbreaks reported																		
Butte													3			2				
Calaveras																			1	
Colusa		no outbreaks reported																		
Contra Costa			x						5	1		3	5	3	1	12	2	9	8	4
Del Norte																			1	1
Eldorado		no outbreaks reported																		
Fresno									4	6		1	3			7		1	3	2
Glenn										2										1
Humboldt										2		1		1						1
Imperial										3								1	2	1
Inyo													2			1				1
Kern									1	2			1	2		1	1	1	3	1
Kings									1										1	
Lake		no outbreaks reported																		

Lassen		no outbreaks reported																	
Los Angeles	1		x	x				30	18		21	40	13	1	12		21	41	2
Madera		no outbreaks reported																	
Marin								3	2		1	1			1		1	1	1
Mariposa																		3	
Mendocino																	1		
Merced								1	1			1			1		1	3	
Modoc		no outbreaks reported																	
Monterey								1	4		1	1			6		2	6	
Mono		no outbreaks reported																	
Napa								1	1						1		1	1	
Nevada		no outbreaks reported																	
Orange	1							10	12		7	8	1		3		9	4	
Placer												1			2				
Plumas		no outbreaks reported																	
Riverside								1	8		2	4	1				1	2	
Sacramento								4	1		2	2						5	
San Benito		no outbreaks reported																	
San Bernardino	1		x					8	12	2	9	6			2	1	1	2	1

San Diego		4			1				1	13		10	7		22	4		7	25	3
San Francisco									2			1					1			2
San Joaquin			x						14	5		14	18	6	1	4	1	1	16	3
San Luis Obispo									1	4	2	1	1			1				3
San Mateo			x	x				15	40	27	11	40	45	23	20	32	8	32	23	15
Santa Barbara										1									3	
Santa Clara									13	9	1	8	5	1	1	13	4	5	2	2
Santa Cruz									7	1		1	1			4		1	4	2
Shasta										1						1				
Sierra		no outbreaks reported																		
Siskiyou		no outbreaks reported																		
Solano									5	1	1		1	1		2				1
Sonoma									1	1		1	1					3	1	1
Stanislaus									4	1		3	1	1		3		2	4	3
Sutter												1					1			
Tehama																			1	
Trinity		no outbreaks reported																		
Tulare									1	2			1			8				
Tuolumne																1				

Ventura									1	2		3	3	1					2	
Yolo													1			4			2	
Yuba																2	1		1	

x* = Number of cases not reported for each county; 1934 - 31 cases, 27 in Northern and 4 in Southern part of state; 1936 - 14 cases, 13 in San Francisco Bay area, 1 in San Diego.

TABLE 3

OCCURRANCES OF VESICULAR EXANTHEMA OF SWINE IN GARBAGE VS GRAIN FEEDING RANCHES AND IN PUBLIC STOCKYARDS DURING THE PERIOD 1940 - 1951

YEAR	TOTAL OUTBREAKS	GARBAGE RANCHES	PUBLIC STOCKYARDS	GRAIN FEEDING RANCHES
1940	169	161	1	7
1941	110	95	1	14
1942	15	15	0	0
1943	139	126	10	3
1944	171	154	10	7
1945	60	58	2	0
1946	53	52	1	0
1947	143	129	4	10
1948	25	25	0	0
1949	105	101	4	0
1950	184	169	9	6
1951	58	53	4	1
TOTALS	1,232	1,138	46	48

TABLE 4

RESULTS OF INOCULATING INDIVIDUAL SWINE WITH DIFFERENT VESICULAR EXANTHEMA VIRUS TYPES

ANIMAL NUMBER	RESULTS OF INOCULATION WITH					
	ORIGINAL "A" VIRUS	REINOCULATION "A" VIRUS	ORIGINAL "B" VIRUS	REINOCCULATION "B" VIRUS	ORIGINAL "C" VIRUS	REINOCULATION "C" VIRUS
31	+	-	+	-	Not Done	Not Done
32++	-	Not Done	-	-	Not Done	Not Done
34	+	Not Done	+	-	Not Done	Not Done
35*	Not Done	Not Done	+	Not Done	Not Done	Not Done
36*	Not Done	Not Done	+	Not Done	Not Done	Not Done
37	+	-	+	-	Not Done	Not Done
38	+	Not Done	+	-	Not Done	Not Done
39	+	Not Done	+	-	Not Done	Not Done
40	+	-	+	-	Not Done	Not Done
41	+	Not Done	+	-	Not Done	Not Done
50	+	Not Done	+	-	+	Not Done
52	+	Not Done	+	-	+	Not Done
53	+	Not Done	+	-	+	Not Done
54	Not Done	Not Done	+	-	+	Not Done
55	Not Done	Not Done	+	-	+	Not Done

* 35 - 36 served as Potency Controls for "B" virus inoculated animals.

\+ Clinical Vesicular Exanthema

++ No explanation available for failure of this animal to "break" with Vesicular Exanthema

\- No evidence of Vesicular Exanthema

TABLE 5

RESULTS OF INOCULATING SWINE WITH VARYING DILUTIONS

OF VESICULAR EXANTHEMA VIRUS

ANIMAL NUMBER	DILUTION OF VIRUS	ROUTE OF INOCULATION	RESULTS
16	1×10^{-5}	Intradermal	Primaries & Secondaries
17	1×10^{-5}	Intravenous	Primaries & Secondaries
18	1×10^{-6}	Intradermal	Primaries & Secondaries
19	1×10^{-6}	Intravenous	Primaries & Secondaries
20	1×10^{-7}	Intradermal & Intravenous	Negative

TABLE 6

THE INFECTIVITY OF BLOOD AND SPLEEN TAKEN FROM ANIMALS AT 24, 48, 72 AND 96 HOURS AFTER INOCULATION WITH VESICULAR EXANTHEMA VIRUS

ANIMAL #	SACRIFICED ____ HOURS AFTER INOCULATION	TISSUE TESTED	NUMBER ANIMALS INOCULATED	RESULTS OBTAINED
67	24	Blood	2	Positive
		Spleen	2	Positive
68	48	Blood	2	Positive
		Spleen	2	Positive
70	72	Blood	2	Negative*
		Spleen	Not Done	
73	96	Blood	2	Negative*
		Spleen	Not Done	

*Note: These 4 animals, challenged one month later with homologous virus, were all susceptible.

TABLE 7

FILTRATION RESULTS WITH TOBACCO MOSAIC VIRUS

THROUGH GRADACOL MEMBRANES

APPROXIMATE PORE DIAMETER (A P D) OF MEMBRANES USED	FILTRATION RESULTS	
	NUMBER OF LEAF SPOTS	PRESENCE OF TOBACCO MOSAIC VIRUS
114 mu	200	#
52 mu	0	-
44 mu	18	#
38 mu	6	-
27 mu	2	-
27 mu	0	-

#Tobacco mosaic virus present

-Tobacco mosaic virus absent

TABLE 8

FILTRATION RESULTS WITH VESICULAR EXANTHEMA VIRUS

THROUGH GRADACOL MEMBRANES

APPROXIMATE PORE DIAMETER (A P D) OF MEMBRANE USED	TITRATION RESULTS	
	NUMBER OF ANIMALS INOCULATED	PRESENCE OF VIRUS
[illegible] M.	2	#
[illegible] M.	2	#
[illegible] M.	2	#
[illegible] M.	2	#
[illegible] M.	2	#
[illegible] M.	2	-
[illegible] M.	2	-
[illegible] M.	2	-
[illegible] M.	2	-

#Virus present as indicated by clinical vesicular exanthema.

-Virus absent.

TABLE 9

THE RESTORATIVE EFFECT OF CYSTEINE* ON VARIOUS LOTS OF INACTIVE VESICULAR EXANTHEMA

FILTRATE DESIGNATION	DAYS FILTRATE EXPOSED TO CYSTEINE TREATMENT	RESULTS OF HAMSTER INOCULATIONS	
		CYSTEINE TREATED FILTRATE	UNTREATED FILTRATE
31	4 days	-	-
	13 days	+	-
	19 days	+	-
	32 days	+	-
	38 days	+	-
52 - 53	6 days	-	-
	10 days	+	nd
	14 days	+	nd
	35 days	+	nd
54 - 57	10 days	+	-
	17 days	+	-
44 - 5	14 days	+	-
	23 days	+	nd
52 - C	14 days	+	-
	23 days	+	-
	35 days	+	-
4,5,6C	16 days	-	-
	19 days	-	-
	21 days	-	-
	27 days	+	-
	33	+	-

* Cysteine monohydrochloride in a final concentration of 1-100.
ND Not Done

TABLE 10

PRELIMINARY VACCINATION RESULTS USING SCHMIDT WALDMANN TYPE OF VESICULAR EXANTHEMA VACCINE

TEST SWINE NUMBER	VACCINE		CHALLENGE	
	ROUTE AND AMOUNT INOCULATED	RESULTS	ROUTE AND AMOUNT INOCULATED	RESULTS
42	6.0 ml ID into skin of flank 0.5 ml ID into snout	Induration with local inflammation for 96 hours. No system reaction.	Challenged 20 days later with 0.5 ml live virus ID into snout.	No evidence of clinical vesicular exanthema
43	20 ml subcutaneously in groin and axillary space. 0.5 ml ID into snout.	Slight swelling sites of inoculation. No systemic reaction.	Challenged 20 days later with 0.5 ml live virus ID into snout.	No evidence of clinical vesicular exanthema.
44	Control	Control	Challenged 20 days later with 0.5 ml live virus ID into snout.	Frank Vesicular Exanthema
45	Control	Control	Challenged 20 days later with 0.5 ml live virus ID into snout.	Frank Vesicular Exanthema

TABLE 11

VACCINATION RESULTS USING SCHMIDT WALDMANN TYPE OF VESICULAR EXANTHEMA VACCINE

TEST SWINE NUMBER	VACCINE		CHALLENGE - 20 DAYS LATER	
	ROUTE-AMOUNT INOCULATED	REACTIONS	ROUTE-AMOUNT INOCULATED	RESULTS
46	6.0 ml ID divided doses right and left flank. 0.5 ml ID into snout.	Slight swelling and induration at sites of inoculation. No systemic reaction	0.7 ml ID into snout and scarification.	Negative
47	4.5 ml ID divided doses right and left flank. 0.5 ml ID into snout.	Slight swelling and induration at sites of inoculation. No systemic reaction	0.7 ml ID into snout and scarification.	Primary snout lesions only
48	6.0 ml ID divided doses right and left flank.	Slight swelling and induration at sites of inoculation. No systemic reaction	0.7 ml ID into snout and scarification.	Primary snout lesions only
49	20 ml subcutaneously -10 ml each groin.	Slight swelling and induration at sites of inoculation. No systemic reaction	0.7 ml ID into snout and scarification.	Primary snout lesions only
50	10 ml subcutaneously 5 ml each groin.	Slight swelling and induration at sites of inoculation. No systemic reaction	0.7 ml ID into snout and scarification.	Primary snout lesions only
51	5 ml subcutaneously in groin. 0.15 ml given ID right snout	Slight swelling and induration at sites of inoculation. No systemic reaction	0.7 ml ID into snout and scarification.	Primary snout lesions only
52	Control	Control	0.7 ml ID into snout and scarification.	Severe Primary and secondary
53	Control	Control	0.7 ml ID into snout and scarification.	Severe Primary and secondary

TABLE 12

TEMPERATURE RESPONSE OF VACCINATED AND CONTROL SWINE FOLLOWING CHALLENGE WITH VESICULAR EXANTHEMA VIRUS

DAYS AFTER CHALLENGE	TEST PIG NUMBERS											
	VACCINATED								CONTROLS			
	42	43	46	47	48	49	50	51	44	45	52	53
-2	39.7	39.7	38.8	38.9	38.6	39.2	38.6	39.6	—	—	—	—
-1	39.7	39.1	39.2	39.1	38.9	39.5	39.2	39.6	—	—	—	—
0	39.0	38.7	39.5	39.5	39.7	39.5	39.4	39.9	39.8	39.0	39.2	39.3
1	39.8	38.8	39.9	39.8	39.5	39.9	39.7	39.7	40.3	41.3	40.8	41.0
2	39.3	39.9	40.0	40.5	39.6	40.0	38.8	39.3	41.6	41.4	40.0	40.7
3	39.0	39.0	39.8	39.3	39.3	40.2	39.5	40.0	40.7	41.2	40.5	40.6
4	39.0	38.7	39.3	39.3	39.0	39.6	38.9	39.3	40.7	40.9	40.3	40.2
5	38.8	39.0	39.6	39.7	39.3	39.7	38.8	39.5	40.6	40.3	39.6	39.7
6	38.8	39.7	39.5	39.3	39.1	39.0	38.8	39.2	40.3	39.2	38.6	39.3
7	38.5	38.7	39.3	39.6	39.0	39.8	38.7	38.6	39.8	39.7	38.6	39.9

TABLE 13

VACCINATION RESULTS USING CRYSTAL VIOLET BLOOD

VACCINE TYPE OF VESICULAR EXANTHEMA VACCINE

TEST SWINE NUMBER	VACCINE		CHALLENGE	
	ROUTE* AND VACCINE	REACTIONS	ROUTE AND VACCINE	RESULTS
82	ID 5 ml	Slight swelling at site of inoculation	30 days after vaccination 0.5 ml ID into snout	Primary lesions
83	ID 5 ml	Slight swelling at site of inoculation	30 days after vaccination 0.5 ml ID into snout	Primaries & Secondaries
84	IM 5 ml	Slight swelling at site of inoculation	30 days after vaccination 0.5 ml ID into snout	Primaries & Secondaries
85	IM 5 ml	Slight swelling at site of inoculation	30 days after vaccination 0.5 ml ID into snout	Primaries & Secondaries
87	ID 5 ml 14 days later ID 5 ml	Slight swelling at site of inoculation	30 days after 2nd vaccine dose. 0.5 ml ID into snout	Primary lesions only
88	ID 5 ml 14 days later ID 5 ml	Slight swelling at site of inoculation	30 days after 2nd vaccine dose. 0.5 ml ID into snout	Primary lesions only
89	IM 5 ml 14 days later IM 5 ml	Slight swelling at site of inoculation	30 days after 2nd vaccine dose. 0.5 ml ID into snout	Primary lesions only
90	IM 5 ml 14 days later IM 5 ml	Slight swelling at site of inoculation	30 days after 2nd vaccine dose. 0.5 ml ID into snout	Primary lesions only

* IM = Intramuscular injection
ID = Intradermal injection

TABLE 14

CLINICAL RESPONSE OF THE THREE VESICULAR VIRUSES IN THE IMPORTANT HOSTS AND BY VARIOUS ROUTES OF INOCULATION

TEST SPECIES	ROUTE OF INOCULATION	NO. ANIMALS NEEDED	RESPONSE EXPECTED IF UNKNOWN VIRUS IS:		
			VE	VS	F & M
Swine	Intradermal snout, lips, plus scarified snout.		#	#	#
	Intravenous		#	#	#
Horse	Intramuscular	1	#	#	-
	Intralingual	1	* -	#	-
Cow	Intradermal snout upper lip.	1	-	#	#
	Intramuscular	1	-		#
Guinea Pig	Intradermal volar surface of the plantar pads	2	-	#	#
Mouse	Intranasal in 2-3 week old animals	4	-	Fatal encephalitis 5-7 days	-

Produces typical disease process - No clinical reaction ⁋ Disease process rarely occurs
* Usually very mild evidence of vesicular disease approximately 50 per cent of the time.

FIGURE 1

Outbreaks of vesicular exanthema in California
(by Counties)

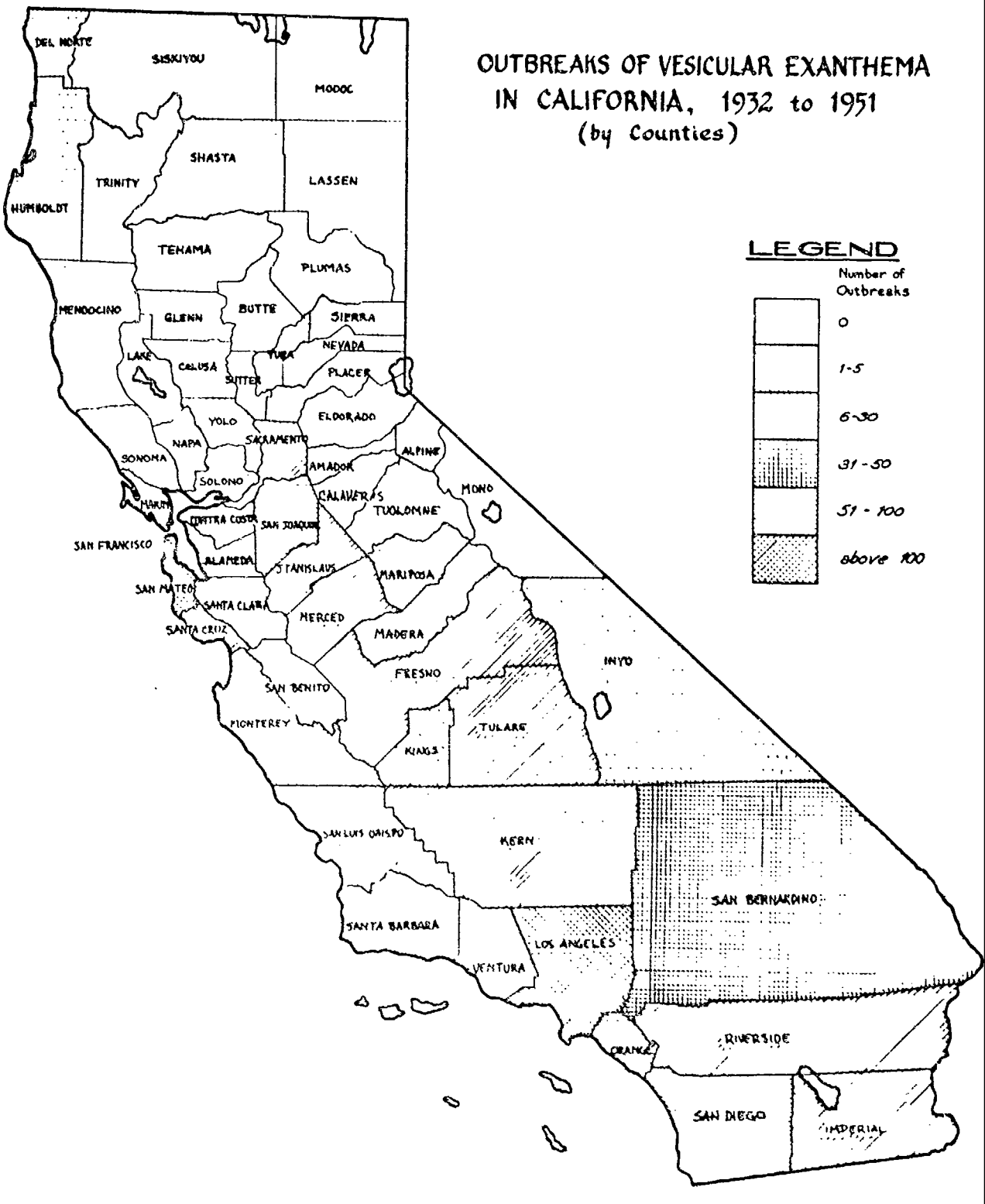
OUTBREAKS OF VESICULAR EXANTHEMA
IN CALIFORNIA, 1932 to 1951
(by Counties)
LEGEND
Number of Outbreaks
0
1-5
6-30
31-50
51-100
above 100
DEL NORTE
SISKIYOU
MODOC
TRINITY
SHASTA
LASSEN
HUMBOLDT
TEHAMA
PLUMAS
MENDOCINO
GLENN
BUTTE
SIERRA
NEVADA
LAKE
COLUSA
YUBA
SUTTER
PLACER
YOLO
EL DORADO
NAPA
SACRAMENTO
ALPINE
SONOMA
AMADOR
SOLANO
CALAVERAS
MONO
MARIN
CONTRA COSTA
SAN JOAQUIN
TUOLOMNE
SAN FRANCISCO
ALAMEDA
STANISLAUS
MARIPOSA
SAN MATEO
SANTA CLARA
MERCED
SANTA CRUZ
MADERA
INYO
FRESNO
SAN BENITO
MONTEREY
TULARE
KINGS
SAN LUIS OBISPO
KERN
SAN BERNARDINO
SANTA BARBARA
LOS ANGELES
VENTURA
RIVERSIDE
ORANGE
SAN DIEGO
IMPERIAL

FIGURE 2

Extent of spread of vesicular exanthema
as of December 24, 1952

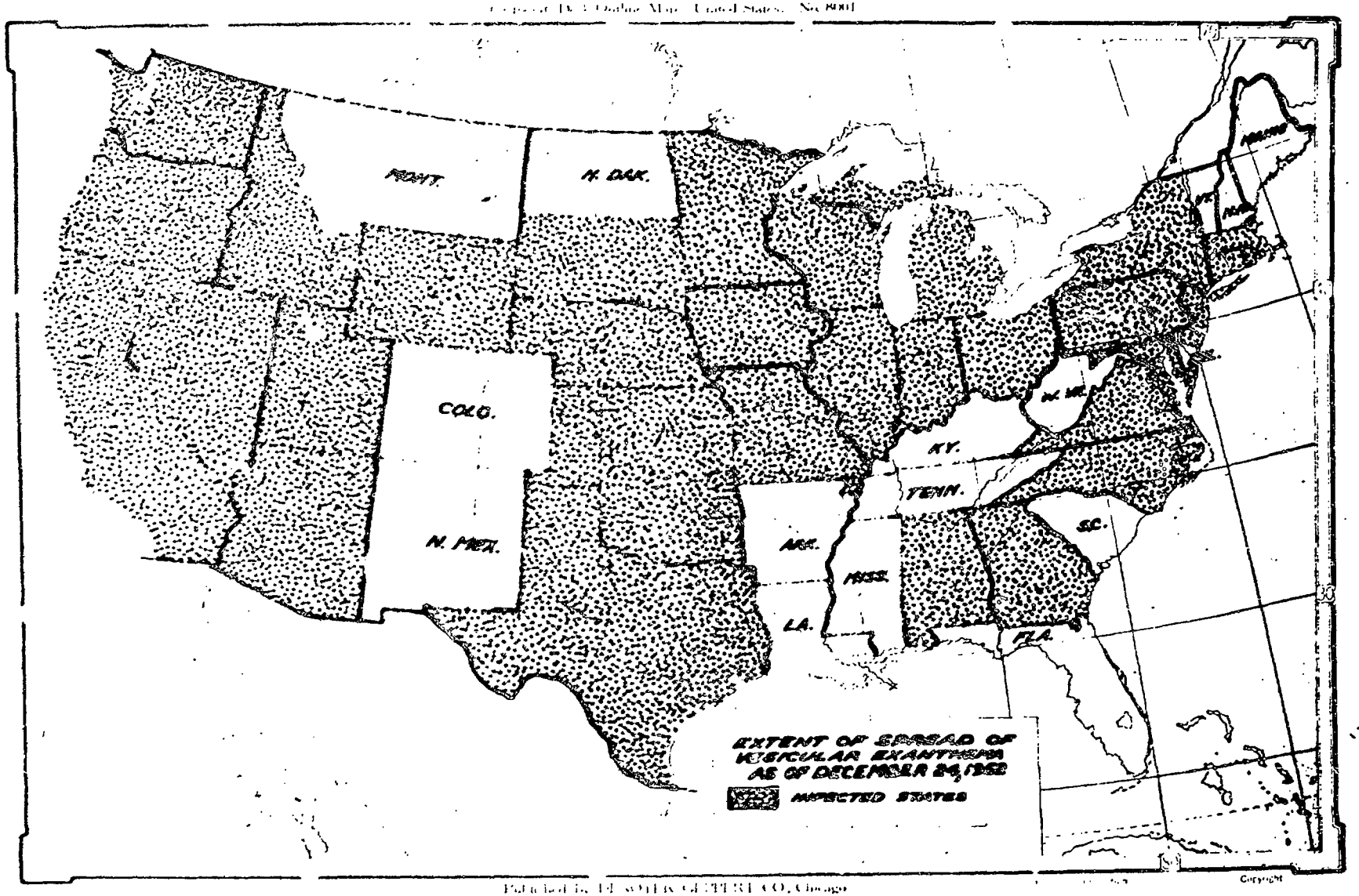
MONT.
N. DAK.
COLO.
N. MEX.
MICH.
MISS.
LA.
N. HAMP.
VT.
MAINE
N. YORK
KY.
TENN.
S.C.
FLA.
EXTENT OF SPREAD OF
VESICULAR EXANTHEMA
AS OF DECEMBER 24, 1932
INFECTED STATES

FIGURE 3

CHARACTERISTIC TEMPERATURE CURVE FOLLOWING INTRADERMAL INOCULATION OF SWINE WITH VESICULAR EXANTHEMA VIRUS

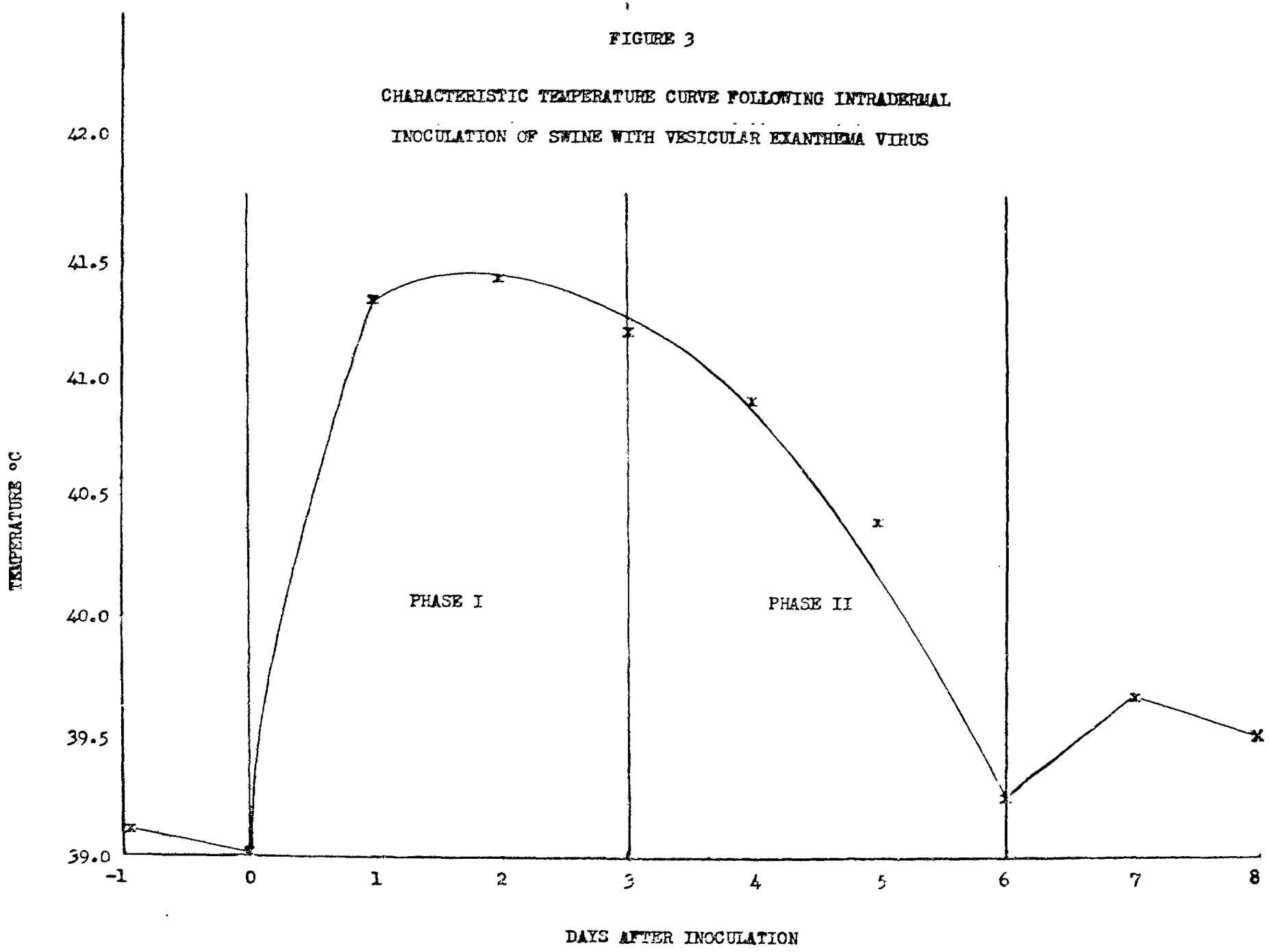

FIGURE 4

TEMPERATURE CURVES OF THREE HAMSTERS FOLLOWING INTRADERMAL INOCULATION OF ONE STRAIN OF VESICULAR EXANTHEMA VIRUS COMPARED TO NORMAL ANIMAL