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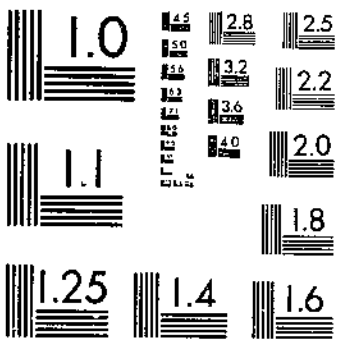
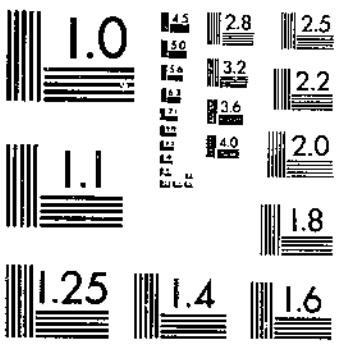


MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

TB 76 (1928)

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UNITED STATES DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

REPORT OF FOOT-AND-MOUTH-DISEASE COMMISSION OF THE UNITED STATES DEPARTMENT OF AGRICULTURE

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With an introduction by JOHN R. MOHLER, *Chief, Bureau of Animal Industry*

INTRODUCTION

Public sentiment in the United States has recognized for many years the necessity for aggressive measures in dealing with foot-and-mouth disease, which is one of the most contagious animal diseases known. Outbreaks of this malady occurred in the United States in 1870, 1880, 1884, 1902, 1908, 1914, and 1924, but with the exception of that of 1914 they were of relatively short duration and were limited to small areas. Slaughtering infected and exposed animals promptly, and burying or burning their carcasses, have been the chief means of eradication. This drastic method, together with the exercise of vigilance to prevent the introduction of the disease into the country, has entailed considerable hardship and expense to stock owners and to the State and Federal Governments. Nevertheless, it has been the necessary price paid for the protection of our domestic animals, and while large in the aggregate, it is very small compared to the losses that would inevitably occur should the scourge at any time be allowed to get beyond control.

Foot-and-mouth disease has been recognized for centuries, but until comparatively recent years exact knowledge concerning the virus and certain aspects of the disease has been wanting. Extensive studies have been conducted in countries where foot-and-mouth disease is enzootic and the United States is deeply indebted to the scientists of those countries for their valuable contributions to our knowledge concerning this malady. On account of the danger of the virus escaping and starting new outbreaks, the scientists of the United States have not engaged regularly in its investigation. However, some observations and researches have been made in connection with outbreaks in the United States which have been outstanding. For instance, the 1908 outbreak was traced to contaminated smallpox vaccine imported from a foreign country several years preceding its use. This investigation proved that symbiosis existed with the infections of vaccinia and foot-and-mouth disease. Animals vaccinated with the mixed virus, as a rule, showed only lesions of one of

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ses, namely, vaccinia; nevertheless the infectious principle of [illegible] mouth disease remained in the vaccinal eruption.

In another instance the disease was carried from a serum-producing plant to five States through contaminated anti-hog-cholera serum collected from hogs which showed no symptoms or lesions of the disease at the time they were slaughtered and their blood collected. Proof of this contamination was not obtained until the sixty-second animal was injected in the fifth series of tests. In the 1914 outbreak an outstanding investigation was conducted on a group of 740 recovered cases of foot-and-mouth disease in order to determine the presence or absence of any disseminators of the virus (virus carriers) among the survivors. Such carriers have been known to persist in certain recovered cases of typhoid fever and diphtheria of man. However, in this particular group of recovered foot-and-mouth disease cases no virus carriers were found after numerous prolonged and carefully conducted tests had been made.

The initial outbreak in California in 1924 was traced to garbage containing contaminated meat scraps from carcasses which had been purchased in a foreign country where the disease existed. In conducting tests of premises with live animals preparatory to restocking it was established that the virus of foot-and-mouth disease, under favorable conditions, will remain virulent for a long time. In one instance, as clear-cut as if conducted and controlled under laboratory conditions, test animals became infected when they were placed on quarantined premises, although 345 days had elapsed following the slaughter of the originally infected herd and the disinfection of the barn and other buildings. The eradication of the disease among the deer in the Stanislaus National Forest, Calif., requiring the destruction of thousands of these creatures, involved considerable investigation before definite plans could be developed, since never before in any country had an attempt been made to eradicate this disease among deer or other wild animals.

While its scientists have not been regularly engaged in research work of foot-and-mouth disease, the Bureau of Animal Industry has kept in close touch with what was being done in various parts of the world.

In order to obtain further scientific knowledge concerning this highly infectious disease—and to do so without danger to the country's extensive livestock industry—the United States Department of Agriculture proposed to Congress the appointment of a commission for studying foot-and-mouth disease in European countries where it is constantly present and where suitable experiments could be conducted. By a special act Congress authorized the appointment of such a commission and empowered it to conduct studies of foot-and-mouth disease abroad with the hope of obtaining information that might be used in suppressing most effectively any outbreaks of the malady that may later occur in the United States.

The commission appointed consisted of Peter K. Olitsky, of the Rockefeller Institute for Medical Research; Jacob Traum, of the University of California; and Harry W. Schoening, of the Bureau of Animal Industry, United States Department of Agriculture. These trained investigators went to Europe in May, 1925, visiting 11 countries, namely, France, Germany, England, Denmark, Sweden, The Netherlands, Belgium, Switzerland, Austria, Hungary, and Italy.

Officials in the countries visited welcomed the American commission and gave valuable assistance, for which acknowledgment is made.

The American investigators performed most of their work at Strasbourg, France, where Louis Boëz, of the Institut d'Hygiene, acted as collaborator. Here also Prof. A. Borrel, director of the institute, extended to the commission every courtesy and facility. Other studies were made at Alfort, France, with the valuable assistance of Professor Vallée and his staff, notably Dr. P. Rinjard, and Prof. M. Burgi, and Dr. G. Flückiger, of Switzerland, aided in obtaining experimental animals and in other ways assisted the commission. In addition, the American investigators made special studies of the extent of the disease and the methods of control in the European countries, where the officials were very generous with their time and suggestions.

The following pages contain the results of the commission's work, which was of approximately a year's duration. The findings of the commission show, as the reader will observe, that the virus of foot-and-mouth disease is extremely active and dangerous, a conclusion that must be constantly kept in mind when dealing with possible future outbreaks of foot-and-mouth disease in the United States. The ultramicroscopic nature of the active agent of foot-and-mouth-disease virus, its physical and chemical properties, and its ability to escape identification are also outstanding results of the year's study. In addition, considerable time was spent in investigating the validity of Frosch and Dahmen's experiments on cultivation of the incitant, on the carrier problem, in serological and immunological studies, in comparing the viruses of foot-and-mouth disease and of vesicular stomatitis, and on other related work.

While the length of the commission's stay in Europe limited the quantity of research work done, valuable information concerning various aspects of the disease was obtained and some facts were brought out that are additions to the knowledge concerning the disease. The full report of the commission follows.

JOHN R. MOHLER.

EPIZOOLOGY AND CONTROL OF EPIZOOTICS

The description to be presented of the epizoology of foot-and-mouth disease and the control measures used during epizootics is based on personal interviews with authorities in Europe in charge of such work and on results of the writers' observations and experiments.

Before discussing the underlying principles of the various phases of the foot-and-mouth-disease problem, it is desirable to consider first the characteristics of the disease and the incitant, the presence of the latter within and without the body, its resistance to chemical and physical agents, and the immunity that it is capable of inducing. This information in outline form will be based on findings already reported by others as well as on the results of the commission's experiments.

CHARACTERISTICS OF FOOT-AND-MOUTH DISEASE

Practically all cloven-footed animals, especially cattle, hogs, sheep, and goats, are susceptible to this malady. Under natural conditions the disease manifests itself, after an incubation period of from about 18 hours to 3 weeks, in a general systemic disturbance. The first

symptom usually observed is fever, followed by the appearance of vesicles, most frequently on the mucous membrane of the mouth and on the skin between and above the claws of the feet, and at times also on the teats and udder. In hogs and sheep the feet are the most frequent seat of the vesicle formation. These vesicles rupture as a rule within 24 hours, leaving a raw, eroded, red surface and, if the lesions are in the mouth, there is excessive salivation and drooling.

The affected areas then heal more or less rapidly, but they may sometimes, especially in the feet, encourage the invasion of secondary infections with subsequent inflammatory changes. During the attack the animal loses considerable weight and, in milking cows, there is a reduction in the milk supply. The financial loss resulting from the deterioration of animals is probably greater than that caused by mortality, which is estimated at about 3 per cent in ordinary, mild epizootics. In malignant forms of the disease, however, as high as 50 per cent of the animals in a herd may succumb or be so seriously affected that they are slaughtered. McFadyean (*50*) [1] states that 5 per cent is a fair estimate of deaths among animals affected during the last 40 years in Europe.

THE INCITANT OR CAUSATIVE AGENT

There is up to the present no confirmatory evidence that the various kinds of bacteria of the ordinary species which have been advanced as the inciting agents of the disease actually play this part. It is now universally accepted that the incitant is a filter passer. It has eluded artificial cultivation by others and by the writers. The most promising efforts in the direction of culture of this filter passer were those of Frosch and Dahmen (*21, 22, 23, 24, 25*) (described later), but the results of a German commission, a British committee, this American commission, and others did not confirm their findings. Hence, at this time the causative agent may be regarded as noncultivable by means now available.

CHARACTERISTICS OF THE INCITANT

Many characteristics of the etiological agent are directly concerned in the study of animal diseases and of control measures. The studies undertaken by others and by the authors indicate that the virus behaves differently in some respects from ordinary bacteria, although it has a character similar in many ways to that of other filter-passing viruses.

The writers have found that the active agent can induce the experimental disease in dilutions of 1 to 10,000,000 and that, generally, the period of incubation and the severity of the experimentally induced disease are proportional to the concentration of the incitant; that is, the greater the concentration the shorter the period of incubation and the greater the severity of the symptoms. The marked activity of the virus, as manifested by pathogenicity in very high dilutions, has therefore an obvious connection with the difficulty of controlling outbreaks.

The virus is contained in the blood during the initial febrile stage of the disease. It is also present in the fluid (the so-called lymph) and in the coverings of the vesicles. The commission's limited experi-

[1] Italic numbers in parentheses refer to "Literature Cited," p. 168.

ments with cattle on this phase of the problem reveal that the blood was active when injected into guinea pigs in two of five tests. The fluid and the coverings of vesicles of cattle and hogs were infectious for guinea pigs for not longer than six days after the first appearance of symptoms. In most instances, however, such materials were inactive the fourth day after the beginning of the disease.

Saliva, urine, milk, and probably other secretions and excretions also contain the active agent. The writers were unsuccessful, however, in demonstrating the presence of virus in cow's urine. Only two attempts were made, and in one the blood removed five hours previously proved to be active.

Bielang (*8*) examined the feces of three cattle and five hogs at varying intervals, from 24 to 120 hours, after artificial infection of the animals; in no instance was the experimental disease induced with these materials in guinea pigs. The number of tests with these excretions is too few to permit general conclusions. No tests were made by the writers with milk or other secretions. Whatever the situation is, in view of the authors' results, the repeated findings of other investigators indicate that the virus may be present at one time or another in practically all the secretions and excretions. Whether this is due to admixture of these materials with vesicular contents or coverings is immaterial; the practical bearing on control measures is that the secretions and excretions should be considered as harboring the virus.

It is noteworthy, moreover, that the oral cavities of two cattle with the experimental disease were swabbed, and the washings of the swabs were injected into guinea pigs. The latter showed as a result the typical lesions. In the case of both cattle no observable changes in the tissues were noted. Hence it appears that the virus is present in animals before the distinctive lesions of the disease appear. This conclusion is in accord with those of Bartolucci (*5*), of Vallée and Carré (*71*), and of Lebailly (*44*) and its practical signficance will be discussed in the section on control measures.

With respect to the viability of the virus outside of the animal body, the conditions under which it is kept determine in a large measure its resistance to destruction. In the laboratory, at 37° C., the active agent dies in from 24 to 48 hours, which has given rise to an impression that the virus is very fragile. But this is merely an extraordinary phenomenon, still inexplicable, and does not indicate the true character—the resistance—of the virus. For at room temperature (18° to 20° C.) the writers have kept the virus alive in artificial media more than 69 but fewer than 100 days. In the cold, in 50 per cent glycerol, it may be preserved for months. This preservation in the cold has also been demonstrated by Gins, Vallée and Carré, and the workers of the British committee. In addition, the virus resists desiccation. Roux and his coworkers found that if the incitant is dried very rapidly it can then retain its activity for 105 days. Under field conditions, on the other hand, authorities differ on the question of the length of life of the active agent. Lebailly (*44*) and Vallée and Carré (*71*) maintain that it is killed within a few days after leaving the animal. They base their conclusion on cattle experiments simulating field conditions.

In one experiment the American commission placed pieces (10 by 7 by 3 millimeters) of coverings from unruptured tongue vesicles

of cows in a sack containing hay. After 30 days these tissues were still capable of inducing experimental foot-and-mouth disease in guinea pigs. After 50 days, however, the experiment failed. In soil, in an experiment to be described later, the incitant was active for at least 25 days—the longest period tested. That the virus does not always die rapidly after leaving the animal is suggested by the fact that foot-and-mouth disease has appeared on premises where restocking has taken place from 30 to 60 days after the slaughter of infected herds. In one American instance definite evidence is available to show that the incitant persisted in the field for 345 days.[2]

It is of utmost significance therefore, in the control of outbreaks, to regard the virus as resistant and not fragile. Furthermore, a situation may arise in which a portion of the coverings of a freshly ruptured vesicle may become detached, and if this finds suitable conditions outside the animal the virus in the fragment may be active for a considerable period of time.

PHYSICAL PROPERTIES

The writers have delimited the relative size of the active agent as between 20 and 100 mμ in diameter, and its charge as electropositive—the isoelectric point being at P_H = about 8. The minuteness of size and the charge indicate the possibility of its entering into firm combination with proteins, ordinarily electronegative, or its capability of hiding in or being protected by larger colloidal agglomerations. Indeed, the writers have shown that the colloidal protective action is very marked and explains the abnormal resistance which the virus displays toward such antiseptics as alcohol, acetone, bichloride of mercury, and cresol compounds.

CHEMICAL PROPERTIES

Loeffler and Frosch (*49*) reported that the virus of foot-and-mouth disease resisted the action of 1 per cent phenol for at least five months. This has been repeatedly confirmed but since the time of those workers others have brought forward additional evidence of the remarkable resistance of the virus. For example, the investigators (*63*) of the British committee have shown that 25 to 50 per cent alcohol does not destroy the incitant in less than 3 days, but that it can withstand chloroform for 27 days and ether for 10 days. Abe (*2*) states that from 2 to 3 days are required for 70 to 75 per cent alcohol to kill the virus. The writers have confirmed the fact of this general resistance, using in their experiments as examples of the fat solvents, alcohol 20 to 60 per cent and acetone, and as examples of the general disinfectants, bichloride of mercury, cresol, or cresol compounds, and chloronal. They have explained the resistance of the virus to these materials, which are so rapidly destructive to ordinary bacteria (60 per cent alcohol kills staphylococci within one minute), on the basis of protection by coagula. In the laboratory tests, these antiseptics are added to pure cultures of ordinary bacteria. The action is then direct. In the case of the virus, however, which is noncultivable and

[2] As this report goes to press a publication of Trautwein (*68*) has appeared in which this investigator reports experiments on the survival of the virus under conditions simulating those of nature. He found that lymph admixed with sand and with stable litter, or lymph placed on watch glasses in stables, remained infective from 5 to 11 days. Vesicle coverings about the size of a bean and 1 millimeter thick were active in water 41 days, in dried stable litter 43 days, and suspended in air 67 days.

therefore admixed with tissue or with exudate, the chemicals cause a more or less heavy coagulation of proteins and these coagula protect the virus from the effects of the chemical agents. If the coagulation is prevented, as was the case in the test with alcohol made by the writers, then the virus is, on the contrary, more sensitive to the reagent than are staphylococci. As a corollary, such chemicals as sodium hydrate and antiformin that do not coagulate the proteins are highly virucidal. Sodium hydrate in from 1 to 2 per cent solutions or antiformin in 1 per cent solution can kill the virus within one minute.

CHEMICAL DISINFECTANTS IN PRACTICE

In the control of outbreaks of foot-and-mouth disease, one or another of the usual chemical disinfectants is recommended by the governmental authorities of the countries involved. These in general are similar to those used in bacterial diseases of man and animals. Among them may be mentioned compound cresol solution, bichloride of mercury, formaldehyde, phenol and chloride of lime or air-slaked lime in dry or liquid form. Thus, in Switzerland, in accordance with an executive order to the 1920 federal regulations governing the control of infectious animal diseases the following chemicals are advised: Milk of lime, chloride of lime solution, saponified cresol solution, phenol, bichloride of mercury, and formaldehyde. In Germany the chemicals designated by the Deutscher Veterinär-Kalender 1926 and 1927 (German veterinary calendar) (*62*) are: Lime, chlorinated lime (dry or in solution), saponified cresol solution, bichloride of mercury, and formaldehyde.

A discussion of the practical applications of disinfectants is given in the section on control measures.

MODE OF SPREAD

On the mode of the spread of foot-and-mouth disease, several points of interest may be gathered from the writers' work and from recent observations of others.

DISEASED ANIMALS AND CARRIERS

The most common cause of the spread of the disease is, of course, the infected animal itself. As already stated, the virus is contained in the fluid and the coverings of the vesicles and also in the blood during the febrile stage of the disease. At this time the saliva, urine, milk, and probably other secretions and excretions are also infectious.

The active virus leaving the infected animal contaminates its surroundings and can be carried in a mechanical way by animate beings (man, horses, dogs, birds, etc.) and inanimate objects (litter, feed, stable utensils, etc.). When such contaminated material comes in contact with susceptible animals, the latter can readily become infected. Under conditions favoring the persistence of the virus outside of the body, the danger of spreading infection thereby is considerable. In view of experimental evidence it is probable that the period in which infected animals spread the virus most actively is in the early stages of the disease, even before any lesions may be observable. At this time the blood and certain secretions and excretions contain the incitant and undoubtedly large quantities of the active agent are escaping from the body.

On the other hand, the fact that the virus loses its activity in a relatively short time within the body suggests that animals in the later stages of the disease play little part in its spread. In spite of this, however, it is believed by investigators and sanitarians, with only a few exceptions, that virus carriers exist, and that such animals may harbor the virus for a long time after recovery. The percentage, however, is believed to be small. The field evidence during a period of years presented to support this view is very strong; and numerous instances are reported in which the disease occurred in clean herds shortly after the addition to the herds of animals which previously had foot-and-mouth disease, other sources of infection having been eliminated. Animals even eight months after recovery, and indeed in several instances after more than a year, have been held responsible for causing outbreaks of the disease.

An extensive experiment made by the writers on the problem of the carrier, reported in detail elsewhere in this report, failed to demonstrate the presence of a carrier of the virus in 20 specially selected animals recovered from foot-and-mouth disease. However, in another experiment active virus was found in the scrapings of the feet of a cow, examined post-mortem, 34 days after inoculation with foot-and-mouth-disease virus.

CATTLE PRODUCTS

Milk, meat, and the raw by-products of slaughter of infected animals may also be implicated in distributing the virus. Milk from animals in the early stage of the disease, or contaminated by udder lesions, may contain the virus and when fed to susceptible animals may result in infection with foot-and-mouth disease. While evidence has been presented to show that the infectiousness of milk is eliminated by souring and also by fermentation in the production of cheese (*40*), data on the question of survival of the virus in milk under various conditions are limited in the main to work reported many years ago. It is a subject that should be investigated again experimentally, in the light of present-day methods.

The meat of animals in the febrile stage of the disease or any part of a carcass of those slaughtered in this stage may be infectious. The feeding to susceptible animals of such uncooked meat or product obviously can result in infection. It has been stated, however, that the formation of lactic acid in the meat of animals shortly after slaughter is sufficient to destroy the virus of foot-and-mouth disease. But this can not be accepted as final evidence that carcasses of such animals do not harbor active virus. More confirmatory evidence must be brought forward and at present the carcasses of animals slaughtered in the febrile stage of the disease should be considered as dangerous.[3]

[3] In a recent publication (*37*) there appears a report of an extensive investigation of this subject made by the British foot-and-mouth-disease research committee. It found that in guinea pigs that were bled out and kept at a temperature of from 2° to 7° C., the blood from tissues around the throat was virulent after 21 days, while bone marrow of such animals contained active virus for periods of from 21 to 87 days. Muscular tissue in one case remained infectious for 7 days and kidney tissue in one case for 54 days. In unbled guinea-pig carcasses kept at cold-room temperature, the blood was infectious from 35 to 46 days, while bone marrow was found to contain active virus at intervals up to 90 days. In experiments with large animals that were slaughtered during the febrile stage of the disease and the carcasses kept at chilling temperature, active virus was found in the bone marrow of bacon carcasses after 42 days, and also in the bone marrow of such carcasses after 42 days when treated by dry or wet salt processes. In beef and bacon carcasses kept at freezing temperature active virus was found in the bone marrow for 76 days. No evidence was found by feeding or inoculation experiments to show that muscular tissue itself contained virus. These results clearly indicate the dangerous part that carcasses or parts of carcasses of animals, slaughtered in the period of blood infectivity, may play in the spreading of infection.

Cognizance of this fact is taken in sanitary police regulations of various countries in their efforts to control foot-and-mouth disease. For example, England has recently prohibited the importation of fresh meat from continental European countries in which foot-and-mouth disease is enzootic and is protected by regulations which prohibit the importation of cloths, sacks, etc., used in wrapping meats to be brought in contact with animals, unless previously sterilized. Boxes and all other meat containers are kept from contact with animals. Similar restrictions were placed on the importation of meats from certain sections of Sweden by Norway.

BIOLOGICAL PRODUCTS

That biological products, in the manufacture of which animals susceptible to foot-and-mouth disease are used, can be the cause of the dissemination of the disease, has been proved in two instances in the United States. In 1908 smallpox vaccine from Japan, which was later proved by Mohler and Rosenau (*56*) to be contaminated with foot-and-mouth-disease virus, was found to be responsible for an outbreak. In the outbreak of 1914, hog-cholera virus and serum (*54*) prepared from hogs infected with foot-and-mouth disease were responsible for an extension of the epizootic.

SPREAD BY MAN

Man is next in importance to infected animals themselves as a factor responsible for the spread of the disease. Close contact of man with animals, intercourse among people, travel, and the nature of the virus make this point easily comprehensible. It is generally held that man conveys the virus mechanically; that is, he carries it on his clothing or person.

Recently in Sweden, however, two physicians, Kling and Hojer, have advanced the theory that man can actually harbor the virus on the mucous membranes of the mouth, nose, and throat, thus acting as a true carrier of the incitant. Their viewpoint is based on a study of the disease in Sweden, and sufficient interest has been aroused thereby to make available funds for further research. Others in Sweden well versed in this subject are not in accord with this theory, maintaining that man plays the part only of a mechanical carrier. Experts in other countries also hold this view. As no satisfactory experimental evidence has been produced by Kling and Hojer to substantiate their theory, the present conception is that man acts merely as a mechanical carrier. The susceptibility of man to infection with foot-and-mouth disease has been seriously questioned and denied [4] as a result of actual attempts at direct inoculation with negative results. However, numerous cases are recorded in the literature of persons having contracted foot-and-mouth disease. With a few exceptions none of these cases have been proved to be foot-and-mouth disease by the inoculation of susceptible animals, so that in many of these reported cases the validity of the diagnosis can be reasonably questioned.

Field observations in the United States during outbreaks of the disease also have borne out the resistance of man. It may be stated, therefore, that man is rather resistant and rarely contracts the disease.

[4] MAGNUSSON, H. A SUMMARY OF SOME OBSERVATIONS MADE IN SWEDEN ON FOOT-AND-MOUTH DISEASE. Meeting of Scandinavian pathologists, July 6. Copenhagen, 1926. [Unpublished data.]

TRANSMISSION BY INTERMEDIATE HOSTS

That the virus may be carried and kept alive by an intermediary host has also been considered. For example, the British foot-and-mouth research committee in its first progress report, 1925 (*35*), states that negative experimental results were obtained in attempts at transference of virus from infected to healthy guinea pigs by means of the bedbug, *Cimex lectularius*. Likewise Lebailly (*45*) from his work concludes that the disease can not be transmitted naturally by flies—the house fly and a species of stable fly being used.

In an investigation into the cause of the recurrence of foot-and-mouth disease in Texas in 1925, Mohler [5] (*55*) found that cattle ticks (*Margaropus annulatus*) taken from animals in the febrile stage of foot-and-mouth disease harbored the virus. He pointed out the possibility that the virus may be carried through the eggs to the seed ticks and after several months transmitted to healthy cattle. However, later tests made with seed ticks or progeny of infected ticks failed to produce the disease. The important discovery that ticks feeding on infected cattle may contain the virus is sufficient in itself to give this insect consideration in the control of the disease in the tick area of the United States. The length of time that the virus can live in the arachnid is not known, but until more information is obtained, ticks, after engorgement on infected cattle, should be considered as possible sources of danger as disseminators of virus. The commission's limited time prevented an extensive investigation of the cattle tick as a carrier. Attempts were made to study this problem, but difficulty was encountered in getting a supply of *Margaropus annulatus*, although a few castor-bean ticks were obtained and placed on infected cattle. However, the latter species of ticks either died or failed to attach themselves to the cattle.

An experiment made by the writers to determine whether the earthworm could harbor the virus gave negative results.

The spread of foot-and-mouth disease by means of animals usually not considered readily susceptible has received attention in recent years. The disease has been produced experimentally in rats and rabbits, but not with regularity. For example, the British foot-and-mouth-disease research committee (*35*) was able to infect artificially a small percentage of white rats and wild rats. White mice, on the other hand, were resistant, but 6 out of 10 inoculated house mice showed active virus in their blood. Wood mice (*Apodemus sylvaticus*, long-tailed field mouse) invariably showed the virus in their blood after inoculation, but the disease was not transmissible by contact. The writers failed to infect 6 white rats by artificial inoculation. The writers also inoculated 4 rabbits with negative results, but Gins (*29*) and others report the transference of the disease to this species. The irregularity with which rats and rabbits respond to artificial infection, the failure of the disease to spread naturally from one animal to another, and the fact that in guinea pigs, a species highly susceptible to laboratory infection, the disease can not be transmitted naturally, indicate that rats and rabbits are probably not directly involved in the spread of the disease other than by mechanical means.

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[5] UNITED STATES DEPARTMENT OF AGRICULTURE, OFFICE OF THE SECRETARY. TEXAS FOOT-AND-MOUTH OUTBREAK IN CHARGE OF FEDERAL FORCES. U. S. Dept. Agr., Press Service Release Sept. 25, 1925. 1 p. [Mimeographed.]

The writers were unable to infect horses artificially with the two types of foot-and-mouth-disease virus. There is no report in the literature on such experimental transference of the disease, although it has been stated that horses have contracted foot-and-mouth disease after exposure to large amounts of virus by contact with infected cattle. In the light of the writers' failures to produce the disease by severe artificial exposure, and field observations in this and other countries, it is believed that the horse can play little part in the spread of the virus except by mechanical means.

DIFFERENTIAL DIAGNOSIS

Since the virus of foot-and-mouth disease has thus far eluded cultivation in vitro and since there are no serologic or allergic tests available for detecting the disease, animal inoculations, to a great extent, must be depended on for diagnosing and differentiating this disease.

All abnormal conditions of cattle, hogs, sheep, and other susceptible animals which produce inflammatory changes on the mucous membrane of the mouth or on the skin of the feet must be taken into consideration in establishing a diagnosis. None of these, except vesicular stomatitis, will give the experienced observer any great difficulty when the characterization of foot-and-mouth disease is borne in mind.

The principal feature of vesicular stomatitis is the formation of vesicles on the mucous membrane of the mouth. It affects both equines and cattle, but does not, as a rule, induce as extensive lesions in cattle as foot-and-mouth disease, nor does it spread so rapidly or so readily as the latter disease. It rarely produces foot lesions, and teat lesions have been infrequently noted.

Although, experimentally, hogs can readily be infected with vesicular stomatitis, natural outbreaks of this disease in these animals have not been reported.

The guinea pig is susceptible to both viruses, but unless it is immune to either the various types of foot-and-mouth disease or vesicular stomatitis, it is of no aid in differentiating between the two.

In doubtful cases and especially in the beginning of an outbreak when a mistake in diagnosis in either direction has such a far-reaching effect, animal inoculations should be resorted to. It has been found that the viruses of foot-and-mouth disease and vesicular stomatitis die rather quickly in the affected animals, so that to insure the virulence of the material to be tested, only lymph or the coverings of fresh vesicles should be used. This can be ground up in a sterile mortar with a small quantity of physiological saline solution. One or more susceptible cattle should be inoculated, intradermically, on the gum by syringe or by the application of the suspected material to a scarified area. One or more cattle should also be injected intramuscularly. By this latter method vesicular-stomatitis virus has not, in the writers' experience, produced manifest lesions in cattle, while active foot-and-mouth-disease virus has done so regularly. One or more horses should be exposed by applying the virus to a scarified area on the dorsal surface of the tongue. Equines are very susceptible to vesicular stomatitis, but are very resistant to foot-and-mouth disease. The development or failure of development of the disease

in the horse is the basis for differential diagnosis between foot-and-mouth disease and vesicular stomatitis. It is highly important that the animals used for test purposes should be susceptible to both diseases.

For more detailed comparison of the effects of the two viruses the section on "Comparative studies of vesicular stomatitis and foot-and-mouth disease" should be consulted.

RELATION OF IMMUNITY TO EPIZOOLOGY

That immunity follows recovery from foot-and-mouth disease is a long-established and generally accepted fact.

DURATION

It is usually stated that the duration of immunity varies from several weeks to several years. In estimating the duration of immunity, consideration must be given to two observations reported within recent years, namely, the plurality of virus and the difference between local and general immunity.

PLURALITY OF VIRUS[6]

The work of Vallée and Carré and of this commission, recorded elsewhere in this report, showed that at least two types of foot-and-mouth-disease virus exist, each of which is capable of inducing immunity against the homologous type, but not against each other. Hence in reports stating that animals which recovered from an attack of the disease have become reinfected within a short period of time, the question arises whether the same type of virus caused the reinfection. Early observations antedating Vallée and Carré's announcement can not be freed entirely from the possibility that a different type of virus may have been responsible for the second attack.

LOCAL AND HUMORAL IMMUNITY

Terni (*64*), Waldmann and Pape (*78*), and this commission have demonstrated that immunity in animals which have recovered from foot-and-mouth disease may be histogenetic or local which manifests itself by the prevention of the formation of foot-and-mouth vesicles at the point of inoculation, or it may be humoral and prevent the generalization of the disease as indicated by the failure of the development of lesions in sites other than that of inoculation. The writers found both types of immunity present soon after recovery. Immunity appears early in foot-and-mouth disease. Thus Waldmann and Trautwein (*79*) have found local immunity present in hogs at 48 hours and humoral immunity in hogs and cattle between three and four days after experimental infection. The local immunity is usually first to disappear. It can be readily appreciated that reports, especially of field observations upon the duration of immunity, may include cases in which the local immunity may have been lost, and local lesions, even though present, may have escaped observation.

[6] As this report goes to press an article by Waldmann and Trautwein (*81*) has appeared in which these authors state that there are three types of foot-and-mouth-disease virus, called by them types A, B, and C. This, in the light of greater experience, reverses the original opinion of Waldmann and Mayr (*76*) who denied the existence of the plurality of the virus. In addition to cross-immunity tests, the serum of recovered guinea pigs was used in differentiating the viruses, a method which the American commission also used.

The writers' experimental work was done within a period covering less than one year. It was not possible, therefore, to plan experiments on the duration of immunity. In spite of this the commission did not find any recovered cattle susceptible to reinfection with the same type of virus. The longest period of observation, however, was only 137 days. Most of the observations on this phase covered a three-month period.

Waldmann and Trautwein (*79*), present data which may be used in estimating the duration of immunity. They found that 4 of 5 cattle, inoculated from 7 to 8 months after recovery, exhibited local inoculation vesicles but no generalization of the disease. The fifth was solidly and wholly immune. Of 30 animals about 18 months after recovery, injected locally, 8 showed no resistance and developed both primary lesions and generalization on reinoculation, while 22 showed only local inoculation vesicles.

Although the number of cases and the periods of retest included in the work of Waldmann and Trautwein and of the commission are not numerous, nevertheless one may conclude that animals are, as a rule, wholly and solidly immune for a period of at least 3 months; that most of the animals lose their local resistance after 7 months, but possess humoral immunity; and that 18 months after infection practically all the animals have no local resistance, and a small number have no general immunity.

ACTIVE IMMUNITY

Practically all methods of inducing artificial immunity used in other diseases have been attempted in foot-and-mouth disease, but without lasting success. Loeffler and his coworkers experimented with attenuation of the virus by heating it at different degrees or by exposing it to low temperatures. The result was that the virus was either so much attenuated that it produced no immunity or was not sufficiently weakened and produced manifest lesions of the disease.

A mixture of serum and virus and progressively increasing doses of virus were tried. Some of these methods yielded encouraging results, but, upon further tests, none were found to be of value in practice. Bellfonte suggested the use of defibrinated, washed, red blood cells as a means of producing immunity without at the same time producing the disease. On further trial, this was found wanting. Cosco and Aguzzi (*13*) have recommended three or four intravenous inoculations of virulent blood, claiming that by this method the manifest lesions of foot-and-mouth disease were not produced, but that lasting immunity was established. However, a negative phase of several weeks follows this method during which time the animals are highly susceptible. Moussu (*57*) found that this method was an improvement over the old one of inoculating locally infective saliva or vesicular contents, but he did not entirely substantiate the work of Cosco and Aguzzi, since with this method he frequently produced the disease.

Waldmann and Trautwein (*80*) had some degree of success by the use of virulent guinea-pig blood upon cattle, but, even in their own hands, it later failed. Vallée, Carré, and Rinjard (*72*) have recently reported on the use of a vaccine prepared by killing the virus with formalin. In a limited number of experiments bovine animals were protected by this vaccine against artificial infection with types A

and O foot-and-mouth-disease virus. These results have not yet been confirmed and therefore judgment must be suspended.[7]

It may be stated, therefore, that at present there is no proved method of actively immunizing animals without first producing the disease.

PASSIVE IMMUNITY

The presence of immune substances in the blood of recovered animals has been demonstrated by Loeffler and his coworkers, and by others, as shown by tests upon cattle. Waldmann and Trautwein, this commission, and others have successfully used guinea pigs for the same purpose.

At present, Loeffler's hyperimmune serum and convalescent serum are being used in Europe and other parts of the world in combating foot-and-mouth disease. The hyperimmune serum is produced on the island of Riems under the direction of the Prussian ministry of agriculture. In brief, the process consists of giving large Simmenthal cattle several injections of virus in the form of vesicle material of affected hogs. This is essentially the same process as formerly used by Loeffler and his coworkers.

Convalescent serum has been suggested and used by Kitt, Nocard, Vallée and Carré, and others, but it has recently received prominence as a measure in the control of foot-and-mouth disease through the efforts of Ernst (*17*, *18*), Drescher, Zink, and others, in the Bavarian veterinary police service. They employed it on a large scale in the 1919 and 1920 outbreak of the malignant form of the disease. That outbreak was causing great losses. Loeffler's hyperimmune serum was not always available in the required quantities and was too expensive. The Bavarian officials resorted to the use of serum from recovered cases, and, as a rule, the blood was drawn from one to three weeks after recovery. In Denmark, Sweden, and parts of Germany special stations are maintained for handling and preparing this material.

As in the case of passive immunity in other diseases, the immunity conferred by the foot-and-mouth disease immune serum is only of short duration, the limits being between 8 and 14 days.

Hyperimmune serum and convalescent serum, according to the limited tests of the writers, have practically the same value, although it is stated by Loeffler, Waldmann, Trautwein, and others that the hyperimmune serum shows by test a much greater content of protective substances than the convalescent. There is no doubt that some lots of hyperimmune serum are more potent than many lots of convalescent serum, but the writers' limited comparative tests with two samples of Loeffler's hyperimmune serum and several specimens of serum from recovered cases do not indicate that difference.

There are three ways in which serum may be used (*75*): (1) Prophylactically; (2) therapeutically; and (3) simultaneously with virus.

As a prophylactic agent it has been employed mainly in Germany and to a lesser extent in other countries, to protect animals during transport, at fairs, at markets, etc. For this purpose the animals are treated with serum before they leave for their destination.

[7] In the Second Progress Report of the foot-and-mouth-disease research committee, Ministry of Agriculture and Fisheries, London, 1927 (*57*) encouraging experimental results in guinea pigs with formalin vaccine are recorded.

Under certain conditions, it is recommended that the treatment be repeated. The doses advised are usually from 15 to 20 cubic centimeters for each 100 pounds of body weight.

Therapeutically, serum is used in animals in the early stages of the disease, and especially in those showing no other symptom than the rise of temperature. They are given serum alone in doses of 10 to 15 cubic centimeters per 100 pounds of weight.

Simultaneously with virus, serum is used in infected premises and also in infected districts. Animals which show no evidence of disease are given the amount of serum mentioned and, at the same time, are inoculated locally in the mouth with material from fresh vesicles. The disease makes its appearance in these animals, but in a moderate form, with a consequent induction of immunity.

The literature covering the period of the severe European outbreak of 1919–20 contains many reports of excellent results with both hyperimmune serum and immune serum in reducing the mortality and in moderating the severity of the disease, and there is no doubt that in countries where slaughter is not practiced or is not desirable, the use of serum appears to be indicated.

The writers' own work with both convalescent and hyperimmune serums on guinea pigs is given in another section of this report. In a test made with Loeffler's hyperimmune serum on 12 cattle, given in detail elsewhere, the writers found that immune serum will protect cattle against infection by contact with diseased animals for a period of from 5 to 8 days. From 10 to 12 days after serum treatment, however, cattle exposed to similar infection contract the disease. The serum, on the other hand, does not protect, even for a day, against primary lesions as a result of local inoculation of the mucous membrane of the gum and pad.

CONTROL MEASURES

Since there is no specific agent of practical importance which can be used for controlling outbreaks of foot-and-mouth disease, reliance is placed on two methods, namely, (1) the "stamping out" or slaughter method, adopted in the United States and in England, and (2) the quarantine procedure used in most European countries. The adoption of either of these methods, however, as a means of controlling foot-and-mouth disease, depends on prevailing conditions.

In a country free from foot-and-mouth disease and protected from its introduction by geographical situation and quarantine regulations, as for example the United States, the slaughter method is the logical one to use. It has been shown in the United States that the disease can be eradicated by slaughter at a much less cost than if it were allowed to become enzootic. That this method is the most practical one for controlling epizootics under certain conditions is recognized by all authorities on foot-and-mouth disease, and many countries of Europe in their control measures against foot-and-mouth disease have regulations providing for the slaughter of infected and exposed animals. But in those countries limitations are set on its use, as is discussed later.

European authorities with whom the American commission discussed the slaughter method for combating outbreaks of foot-and-mouth disease in the United States, were practically unanimous in

their opinion that, situated as this country is, ordinarily free from the disease, the slaughter method is by far the best.

In continental Europe, however, where the disease has gained a strong foothold as a result of the long period of activity of the virus, the various countries, because of the extent of the disease, their geographical location, and their inability to prevent the reintroduction of the disease, are compelled to adopt the next best means, namely, quarantine measures. The regulations of the different European countries concerning control measures are all nearly alike, since those responsible for the promulgation of these regulations are well acquainted with what is known regarding the disease. The application of these rules, however, as well as the vigor with which the regulations are enforced, differs in various countries, depending on the extent of the disease, the geographical situation, and the economic conditions of the country. As a result of the demoralizing effect of the World War and its influence on sanitary police measures, there was an increase in the spread of infectious animal diseases. This was particularly noticeable in the case of foot-and-mouth disease. A malignant type of the disease began in Italy in 1918 and swept over a large portion of Europe within the next three years. It caused enormous losses, in some herds the mortality reaching 50 per cent of the adult animals.

In Switzerland, for example, after an extensive survey, the losses were placed at 350 million francs (about $70,000,000) (*19*). This is especially significant in view of the fact that the number of susceptible animals in Switzerland at that time was less than one-fiftieth of the number in the United States and that the area of the country is only about one-tenth of that of the State of California.

CONTROL MEASURES IN EUROPE

As a part of the writers' study of the epizoology of foot-and-mouth disease in Europe the following countries were visited in the order named: France, Germany, England, Denmark, Sweden, The Netherlands, Belgium, Switzerland, Austria, Hungary, and Italy. A general discussion of the disease found in these countries, also the research work and control measures follow. (The detailed regulations in force in the various countries are given in the chapter on regulations for the control of foot-and-mouth disease in certain European countries.)

FRANCE

It is mandatory on the part of the owner, veterinarian, or others having knowledge of the existence of foot-and-mouth disease to report it to the authorities. Notices are posted on the boundaries of the villages and also on the infected premises. Entrance to infected farms is forbidden. Animals on affected premises are not allowed to be moved except for immediate slaughter. Various regulations covering the aspects concerned in the spread of the disease are on the books. What regulations shall be enforced is left in many instances to the town or authorities of the Department in which the disease occurs, and the conditions peculiar to the section of the country determine the strictness of the quarantine. But the disease is present continually even though the quarantine regulations are more or less enforced. The Government pays no part of the burden of the

disease; the individual bears it all. Animals severely affected are slaughtered under veterinary supervision and the meat used for food. The slaughtering is usually done on the farm.

During the course of the disease the animals are treated by various means. The use of convalescent serum has been successful in lessening the severity of the disease and reducing the mortality. Fourteen days after disinfection, which is made after recovery of the last animal affected, the quarantine is raised.

From France has come much of our knowledge concerning foot-and-mouth disease, as active research work on this disease has been done in the last 25 years by a notable group of workers. At Alfort is the national research laboratory, which possesses modern laboratory buildings, including stables especially constructed for research work on foot-and-mouth disease with large animals. These stables consist of 24 large box stalls constructed of brick, concrete, and iron, and ideally arranged in groups of six with a large yard for each stall. Concrete walls establish complete separation. The animals are so fed and watered that the attendant does not come into contact with them. By means of a system of tracks and switches, feed and manure can be easily handled. It is here that the principal research work on foot-and-mouth disease has been conducted. At Caen, in the north of France, considerable work has also been done, while some laboratory investigations are being carried on at the Pasteur Institute, Paris. Experimental studies in progress at these laboratories are hampered, however, by the lack of funds.

GERMANY

Foot-and-mouth disease is a reportable disease. When it appears sporadically in a district otherwise free from infection, authorization may be given for the slaughter of the infected and exposed animals, provided it is assumed that the contagion will thereby be exterminated. Compensation for such slaughtered animals is based on market value and paid with public funds. In other cases infected premises and certain contiguous areas are placed under quarantine. The movements of animals, feed, and products, and persons are restricted as proscribed from time to time by the police. Quarantine is removed on recommendation of the veterinarian after the slaughter or recovery of affected animals and disinfection of the premises. In spite of the quarantine regulations the disease is very prevalent in Germany and the losses are heavy.

Figure 1 shows the course of foot-and-mouth disease in Germany from 1886 to 1924, inclusive. The number of infected farms during each year is given in quarterly reports. The epizootic of 1920 was the most severe in the history of Germany; 746,571 premises were reported infected for that year. In certain sections of the country the malignant type of the disease predominated. The outbreak was combated by the extensive use of convalescent and hyperimmune serums, whereby it is stated that the losses from the disease were reduced.

Research work in Germany is in more or less active progress in different institutions, among which may be mentioned the Staatliche Forschungsanstalt (Government experiment station), Insel Riems;

Institut Robert Koch, Berlin; the Veterinärpolizeiliche Anstalt (the veterinary police experiment station), Schleissheim, and at many of the laboratories of the veterinary high schools.

A visit was made to the Staatliche Forschungsanstalt, situated on the island of Riems, in the Baltic Sea, where hyperimmune foot-and-mouth-disease serum is prepared. The serum is prepared by hyperimmunizing oxen, after recovery from the experimental disease, by repeated injections of virus obtained from the vesicles of swine. After hyperimmunization the cattle are bled to death; the blood is defibrinated, centrifuged, the serum preserved with 0.5 per cent phenol and passed through Seitz filters. The carcasses of the animals are utilized for food purposes.

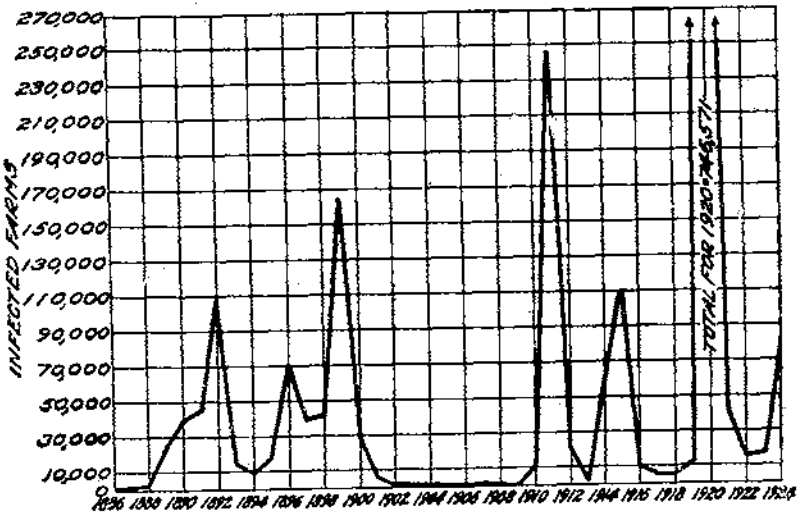

FIG. 1.—Trend of foot-and-mouth disease in Germany, 1886 to 1924

UNITED KINGDOM

In the United Kingdom the stamping out or slaughter method is used. In recent years, particularly from 1922 to 1924, there have been several severe outbreaks. Table 1 gives the number of outbreaks of foot-and-mouth disease from 1921 to 1925, the number of animals slaughtered, and the amount paid in compensation for slaughtered animals (*50*).

TABLE 1.—*Number of outbreaks of foot-and-mouth disease, number of animals slaughtered, and compensation paid for slaughtered animals in the United Kingdom, 1921–1925*

Year	Number of outbreaks	Number of animals slaughtered	Compensation paid for slaughter (£ [1])
1921	44	3,085	
1922	1,140	55,599	503,520
1923	1,854	125,098	2,209,781
1924	1,449	88,726	1,389,696
1925	260	19,963	270,000

[1] The exchange equivalent of the British pound is $4.87.

Infected and exposed animals are slaughtered. Restrictive quarantine measures, somewhat similar to those employed in epizootics in the United States, are also in use. The owners are reimbursed for slaughtered animals, and the cost of cleaning and disinfection is borne by the Government.

While the British Isles occupy a somewhat isolated position, yet their proximity to the Continent predisposes them to introductions of the disease. In spite of the severe outbreaks of 1922, 1923, and 1924, special committees (*32, 34*) appointed to investigate the methods of handling the outbreaks were convinced after a searching inquiry that the slaughter policy was the proper one to pursue, and that while the expense was considerable it was much more economical in the end than permitting the disease to become enzootic.

While the general policy in Great Britain is to slaughter all affected and exposed animals, in recent years, under exceptional conditions, isolation and quarantine have been adopted. When the involved animals were pedigreed stock of the best blood lines and where conditions favorable to a strict quarantine were available, isolation and quarantine were carried out at the owner's expense. Thus in the 1922 outbreak 67 herds were under quarantine, while in 1923–24 the number of such herds was 37. The mortality in these herds ranged from 0 to 10 per cent of the total number of animals in the herd; the average for 1922 was 2.4 per cent, and in 1923–24 it reached 3.1 per cent. It should be noted that under the slaughter method the expense of the eradication of the disease is borne by the Government, while in that of isolation and quarantine, the cost is borne by the owner. The departmental committee of 1923–24 examined as witnesses a number of owners of isolated and quarantined herds. Its report on the results of isolation in two herds is as follows (*34*):

> In one herd consisting of 141 cattle the owner placed his loss at about £2,000, which included losses from deaths of 4 cattle and 1 calf, depreciation of animals as result of the disease, as bad udders, lameness, etc.; loss in milk; labor in caring for sick animals; veterinary attendance; medicines, etc.; and costs of cleaning and disinfection. The owner stated that he wished the stock had been slaughtered in the first place.
>
> In another herd of 149 cattle the owner placed the loss at about £500, and in this case the owner stated he was satisfied with the result.

The danger of recovered animals acting as carriers of the virus is illustrated in two outbreaks of foot-and-mouth disease, the details of which are given in the chapter on carriers.

Because of the continued outbreaks of the disease, research was initiated in 1924 to throw more light on some of the obscure phases of the problem and to attempt to introduce or perfect an immunizing agent of practical value for control. When the American commission visited England active work was being done at the Lister Institute, the ministry of agriculture's laboratories at Weybridge, and at Pirbright. At Liverpool some work was in progress with rats. At the places visited there were excellent facilities for energetic research work. The large animal station at Pirbright is ideally situated and equipped, and its size (capacity about 100 box stalls) is such as to afford ample opportunity for extensive work.

DENMARK

As a result of its somewhat isolated position from the rest of the European Continent, Denmark until recently has had no severe

epizootics. Table 2 gives the number of outbreaks of the disease from 1920 to June 30, 1926.

TABLE 2.—*Outbreaks of foot-and-mouth disease in Denmark, 1920–1926*

Year	Number of herds affected	Year	Number of herds affected
1920	5,497	1924	7,941
1921	2,079	1925	53,617
1922	223	1926 [1]	45,716
1923	64		

[1] The period covered in 1926 is from Jan. 1 to June 30, inclusive.

Owing to its export trade in live animals Denmark has been subject to introductions of the epizootic by cattle dealers and traders from Germany, but by means of the slaughter, isolation, and strict quarantine methods the disease has been kept partially in check. In the fall of 1924 an outbreak, considered to be of German origin, appeared and spread with such rapidity that the slaughter method had to be abandoned entirely for economic reasons. Strict measures governing isolation and quarantine were adopted at the request of the farmers, but when about 6 per cent of all the herds had become affected, much dissatisfaction was expressed by the farmers because of the stringency of these measures. The regulations were then modified considerably in spite of the strong protests of the chief veterinary adviser to the Government, who refused to assume responsibility for the spread of the disease if the quarantine was modified. As a result of this modification the disease became so widely disseminated that almost 50 per cent of the herds were affected. This outbreak reached its maximum in January 1925, when 8,050 new outbreaks were reported for that month. Fortunately the disease appeared to be of a mild type and by the extensive use of convalescent serum for treatment of the animals, losses from the disease were reduced. However, as the outbreak was so widespread, sufficient serum could not be produced to meet the needs, and its use at times had to be limited to young stock, pregnant cows, and bulls, especially heavy animals which may suffer seriously from foot lesions.

A gradual diminution in the number of cases occurred until the early part of 1926, when the infection began anew. It appeared in the same localities that were affected the previous year and many of the animals became reinfected. The fresh outbreak began in the Amt (department) of Copenhagen and spread westward into Jutland, where it spread with great rapidity. For the month of May, 1926, more than 13,000 new cases were reported and officials believed at that time that the peak of the outbreak had not been reached.

The animals which were reinfected suffered much more than those with an initial attack. The first outbreak was of a rather mild type, principal vesicle formation being on the mouth and feet; in the recurrence, while the usual mouth lesions were noted, they were not so regularly found and numerous cases were observed in which vesicle formation occurred in the nostrils and muzzle and on the udder. In the latter cases large areas of skin sloughed off.

When the commission visited Denmark (June, 1926) the officials were in the midst of dealing with the epizootic and had no definite statistics, but they were of the opinion that about 50 per cent of the

animals that had the disease the previous year were again attacked with the malady. The convalescent serum from the 1924–25 outbreak had little influence on the course of the disease, but good results were obtained with convalescent serum from the 1926 outbreak.

That the 1926 epizootic in Denmark was the result of the operation of a different type of virus from that which was responsible for the outbreak of 1924–25 was suggested by field observations and confirmed by laboratory investigations made at Strasbourg, France, by the American commission on receipt of samples of virus from Denmark. (See chapter on typing viruses.)

The failure of the farmers to live up to the regulations, even those which were later modified, was held responsible by the officials for the extensive spread of the disease. The owners of uninfected herds made strenuous efforts to prevent the introduction of the infection, but once the disease appeared in their herds, many lost interest in preventing the spread. These conditions resulted in a further disturbance of trade caused by the British embargo prohibiting the importation of fresh meat from Denmark.

The Government veterinary laboratory at Copenhagen has a well-trained force and a well-equipped laboratory for the preparation of convalescent serum. Visits are made to farms where the animals have had the disease six weeks to two months previously, and about 8 liters of blood (citrated) are drawn from each adult animal. The owners of the animals are paid a nominal sum for each animal bled. The blood is transported by truck to the laboratory, where the serum is separated by centrifugation and chinosol added as a preservative. It is shipped out in 1-liter bottles and sold to veterinarians only. The dose for adult animals is from 200 to 300 cubic centimeters and for young stock 50 to 100 cubic centimeters are given. Its use is mainly for therapeutic purposes; animals already infected are injected with the serum and those exposed are inoculated artificially with the virus and, at the same time, with serum.

The wearing apparel of a Government inspector in visiting infected farms consists of overalls, a white linen coat and hat, and a pair of short rubber boots that fit over the shoes. Rubber gloves also are worn. After the inspector leaves the infected stable his boots and gloves are washed in cresol solution. The other clothing mentioned is left in care of the farmer, who sees to it that it is boiled and then washed. The inspector on his next trip to the farm collects the clothing.

Preparations are in progress for research work on foot-and-mouth disease and the Government has rented an island for the establishment of a laboratory.

SWEDEN

Because of its proximity to Denmark, conditions existing in that country with respect to the prevalence of foot-and-mouth disease have been reflected, in recent years, to a certain extent in Sweden. The disease has appeared sporadically in Sweden in earlier years as a result of importation by animals from other countries. Since 1898 seven outbreaks have been quickly eradicated by the slaughter of infected and exposed animals, together with disinfection and quarantine. These measures were vigorously enforced; armed guards were on duty continually to see that the quarantine regulations were carried out.

In 1924 the disease appeared in the läns (counties) of Malmöhus and Kristianstad, the points of the Swedish peninsula closest to Denmark, the latter country having an epizootic at that time.

The slaughter or stamping-out method was at first applied in these läns. The disease spread so rapidly, however, and so many valuable herds were involved that the slaughter method was abandoned for economic reasons and the isolation and quarantine method substituted. The disease was confined to the southern section of the country, a natural barrier of forests and mountains preventing its spread into the north of Sweden.

When the slaughter method was discontinued and replaced by isolation and quarantine, strict regulations were put into force. Infected premises were placed under a rigid quarantine, and no one was allowed to leave except by special permission. During the course of the epizootic the animals were kept in the stables and precautions taken to prevent the spread of infection by birds, dogs, and other means. After the last animal in the herd had recovered the animals were removed from the stables. During this procedure each animal was thoroughly scrubbed with a disinfectant solution, placed in stocks, and all loose horn cut away from the hoofs. The hoofs were then painted with an alcohol-tar preparation. The animals were then placed in an isolated field and the stables thoroughly cleaned and disinfected. After disinfection the cattle were returned to the stables and the quarantine raised if the veterinary inspector approved. During the quarantine period no milk was allowed to be taken from the premises. The Government paid all the costs of disinfection and reimbursed farmers for milk losses not covered by insurance.

The regulations covering the movements of the people were strictly enforced and in some districts public gatherings were forbidden. Many claims for compensation were presented to the Government for losses incurred during 1925 as a result of the restrictive quarantine measures. These ranged from individuals restricted from plying their trade to owners of motion-picture theaters.

The disease was treated by the use of convalescent serum, with beneficial results. It was used only therapeutically, and as a result the severity of the disease was lessened and the mortality reduced.

Table 3 gives the number of outbreaks of the disease, by months, from November, 1924, to August, 1926. It is to be noted that the epizootic in 1925 reached its maximum in March, and that in April and May, 1926, the disease flared up again.

TABLE 3.—*Number of infected herds in Sweden monthly, from November, 1924, to August, 1926*

Year and month	Number of herds infected	Year and month	Number of herds infected	Year and month	Number of herds infected
1924		1925		1926	
November	33	May	689	January	8
December	152	June	270	February	44
1925		July	72	March	305
		August	40	April	905
January	628	September	9	May	1,077
February	848	October	37	June	923
March	1,118	November	30	July	743
April	890	December	18	August	651

Conditions similar to those in Denmark in the 1926 outbreak were found to be present also in Sweden. Districts in which the disease had been prevalent the previous year again became infected and many animals that had passed through the disease in 1925 became reinfected in 1926. About 40 per cent of the animals affected in the second outbreak had had the disease the previous year. Convalescent serum obtained in 1925 failed to influence the course of the affection in 1926, while serum from the animals infected in 1926 yielded good results. As in the case of Denmark, the American commission at Strasbourg was able to confirm the field evidence that the 1926 outbreak in many sections was caused by a type of virus different from that causing the 1925 outbreak. A special laboratory for the preparation of serum was established at Malmö and the procedure of obtaining the serum has been similar to that employed in Denmark.

In 1924 a commission was appointed in Sweden to investigate the subject of foot-and-mouth disease. The commission visited various European countries where conditions relative to foot-and-mouth disease were studied.

The suggestion has been made that a special research station be built on an island where experimental work on foot-and-mouth disease could be done without danger of its spread.

The work of Lebailly relative to the rapid death of the virus of foot-and-mouth disease outside of the body has received considerable attention in Sweden. In the latter part of 1925 and early in 1926, experiments were made to test the viability of the virus under natural conditions. Infected animals, from 7 to 24 days after the first symptoms of foot-and-mouth disease, were removed from the stables and replaced by normal cattle or swine. No cleaning or disinfection was done. The accumulation of litter and manure from the infected animals was heaped in the stable and when the normal animals were brought in, this material was spread out so as to allow greater contact. Nine separate experiments of this type were made. In not a single instance did any of the normal animals contract the disease after being in the stable from 3 to 4 weeks. The normal animals in five cases consisted of cattle and calves and in four cases of cattle, calves, and swine. They were then inoculated artificially. A successful result proved their susceptibility to foot-and-mouth disease. A positive result was obtained in one experiment in which the walls of a fresh vesicle were cut away by scissors and the material allowed to fall in the litter. Thirteen days later the diseased animals were removed and one cow and one calf placed in the infected stalls. Six days later the disease appeared in these animals. As a result of these experiments, which were to be continued, a modification of the methods of cleaning and disinfection was being considered.

Investigations covering the part that man plays as a carrier of the virus are also being undertaken. Kling and Hojer have been widely quoted in the press as stating that as a result of a study of the epizoology of the disease in Sweden, man acts as a true carrier of the disease by harboring the virus within his body, probably on the mucous membranes. Nothing has yet been published on the results of their experimental work.

SWITZERLAND

Bordered by Germany, Italy, and France, countries in which foot-and-mouth disease is enzootic, the position of Switzerland is always precarious. Statistics of foot and-mouth disease in Switzerland, contained in Table 4, show that from 1886 to 1925 the country was not free of the disease for an entire year.

TABLE 4.—*Number of animals infected with foot-and-mouth disease in Switzerland, 1886–1925*

Year	Number of infected animals	Year	Number of infected animals
1886	2,964	1906	1,381
1887	2,710	1907	7,325
1888	5,642	1908	14,555
1889	21,833	1909	19,025
1890	13,492	1910	2,441
1891	23,430	1911	48,488
1892	15,941	1912	25,186
1893	24,384	1913	69,091
1894	13,878	1914	24,703
1895	4,498	1915	11,678
1896	2,824	1916	5,148
1897	10,342	1917	933
1898	106,884	1918	9,728
1899	38,304	1919	31,434
1900	12,456	1920	569,051
1901	8,110	1921	62,365
1902	16,552	1922	14,392
1903	635	1923	7,459
1904	1,494	1924	16,195
1905	4,685	1925	21,689

Vigorous methods are pursued in Switzerland in combating foot-and-mouth disease. Favored by efficient Federal and cantonal veterinary organizations, the outbreaks, except for a few years when severe epizootics prevailed over all Europe, have been kept to a minimum.

The topography of Switzerland differs considerably from that of other European countries, except possibly parts of the bordering countries. The fact that the villages and homes are situated on the mountains and that cattle are pastured on mountain sides simplifies the enforcement of extremely stringent quarantine regulations.

Measures for controlling foot-and-mouth disease include the slaughter or the stamping-out method, isolation, and quarantine. The Federal and cantonal governments bear the expense of compensation for slaughter and disinfection. Animals slaughtered are appraised at their full value, but the owner receives only 80 per cent of this sum.

Formerly slaughter was performed on the farm and the meat, after veterinary inspection, was sold for food. At the present time, however, specially constructed trucks are used to transport the infected and exposed animals from the farm direct to the slaughterhouse, where, after veterinary inspection, the carcasses or parts of carcasses considered fit for human food are sold. The trucks are constructed in a manner so that no infection can escape and so that they can be thoroughly cleaned and disinfected. Six of these trucks are placed in strategical parts of the country; in this way outbreaks

in any section can be quickly taken care of. The slaughter method is used to a large extent, especially in early outbreaks when it is thought that the disease can be eradicated by these means and also when the animals are in an accessible position. The slaughter method is also adopted when the disease appears in the locality favorable for its wide dissemination.

At the principal abattoirs in Switzerland a special section completely isolated from the main abattoir is used for the slaughter of animals affected with foot-and-mouth disease. After the slaughter the entire premises, implements, clothing of butchers, etc., are thoroughly disinfected. No one is allowed to leave this building without a complete change of clothing and until all infected animals have been slaughtered.

After removal of the animals from an infected place, the premises are thoroughly cleaned and disinfected. Manure is buried or burned or covered with lime if the quantity is large. No restocking is allowed until 30 days after disinfection.

When the slaughter method can not be adopted the animals are quarantined, for in many instances the animals are on pasture in the Alps and so far removed from the roads as to be inaccessible by truck. Quarantine extends to the premises themselves, and a surrounding zone. Roads and passes leading to infected areas are plainly placarded and entrances are guarded by police, in many cases in uniform. Movements of people and stock are restricted in the quarantined area. Warning zones, areas about 6 miles around infected premises, give notice that one is approaching the proscribed zone. The people are not allowed to leave the infected premises for at least six weeks and the children are not permitted to go to school until after that period has elapsed unless arrangements can be made for them to live at some other place. Food, mail, and other necessaries are carried by guards into the mountains and are left at neutral zones to be called for by the quarantined persons. In the quarantined area tourist traffic is practically brought to a standstill. Hotels situated there suffer a heavy loss as a result of these restrictions.

Milk from infected cattle is used on the premises and all cheese factories in infected areas are closed. During the quarantine period the infected premises are cleaned and disinfected several times. The lesions of the affected animals are treated daily with antiseptic solutions and twice during the quarantine period the hoofs are trimmed, in an effort to remove any virus protected by loose horn.

New cattle are not permitted to be brought on the premises until the eighth month after recovery of the affected animals; and animals that have passed through the disease are not allowed to be removed before that time except for immediate slaughter. Such cattle as are removed for slaughter must be sent to the special foot-and-mouth disease department of the abattoir. Cattle are imported into Switzerland for slaughter only, are considered as foot-and-mouth disease suspects, and are slaughtered only in the special department of the abattoir. In many instances in which the disease has broken out in the Alps and it has been impossible to kill the animals because of their inaccessibility, a large number have been slaughtered eventually after recovery in the fall. Many show the effects of the disease and

are known as "unvollständiggeheilt or Kummerer," (incompletely recovered) and are considered by the Swiss officials as potential carriers of the virus.

The fact that animals can be carriers of the virus after recovery has been observed many times in the field, in cases where recovered animals have been held responsible for outbreaks after they have been brought into contact with susceptible animals. It has been observed that recovered animals can act as carriers of the virus for as long a period as two years; but recurrence of an outbreak is caused most often by animals within eight months after recovery. For this reason recovered animals are kept separated from normals for eight months. All animals which are isolated and quarantined as a result of the disease are identified by a tattoo mark in the ear giving the date of the infection. This mark is permanent, and such animals can be identified easily at any time.

As a result of the vigor with which the strict quarantine regulations are enforced, the thorough cleaning and disinfection of infected premises and the permanent identification of the animals, the field evidence accumulated by the Swiss officials on the infections caused by recovered animals gives strong indication of the existence of carriers.

The disease is often treated by the use of convalescent or hyperimmune serum, and in general, good results are obtained thereby in lessening the severity of the disease and reducing its mortality.

While no permanent laboratory work is done in Switzerland on foot-and-mouth disease, research of various phases of the disease during outbreaks has been made and has resulted in many valuable contributions to the knowledge of various phases of the disease.

ITALY

Foot-and-mouth disease has been enzootic in Italy for many years and particularly severe epizootics have occurred at intervals. The disease is combated by means of isolation and quarantine, but its spread is in certain sections merely limited; in others, particularly in the parts of the country much traveled, its dissemination is very rapid until practically all susceptible animals in a community are attacked.

The losses from foot-and-mouth disease are heavy, not only as a result of quarantine measures and depreciation of recovered animals, but also of mortality.

Table 5 shows the number of infected animals and the number of deaths from 1909 to 1920. The column "Animals dead or slaughtered" includes animals so severely affected that they were slaughtered. As can be seen, the mortality from the disease in certain years is high.

The malignant type of the disease that spread widely over Europe from 1918 to 1921 had its origin in Italy. During the war much of Italy's livestock was slaughtered; new stock was then introduced and a malignant type of the disease appeared.

Italian epizoologists have noted that an epizootic of foot-and-mouth disease may be divided usually into three stages:

TABLE 5.—*Number of animals infected and number of deaths from foot-and-mouth disease in Italy, 1909–1920* [1]

Year	Animals infected	Animals dead or slaughtered		Year	Animals infected	Animals dead or slaughtered	
		Number	Per cent			Number	Per cent
1909	53,475	680	1.2	1915	189,739	10,324	5.4
1910	48,160	3,399	7.0	1916	249,469	17,536	7.0
1911	869,698	13,721	1.5	1917	150,910	13,659	9.0
1912	287,499	1,443	0.5	1918	286,700	13,795	4.8
1913	650,160	11,113	1.7	1919	1,313,320	86,434	6.0
1914	81,898	1,446	1.7	1920	305,761	34,825	11.3

[1] Later figures are not available, but the number of herds infected from 1921 to 1925, inclusive, may be seen in Figure 2.

(1) The period of attack, which often lasts more than six months, during which time the infection spreads so rapidly as to be beyond control.

(2) The stationary period, during which there may be some slight increase or decrease in the spread of the disease. This may last for six months or longer; and,

(3) The period of decline of the epizootic.

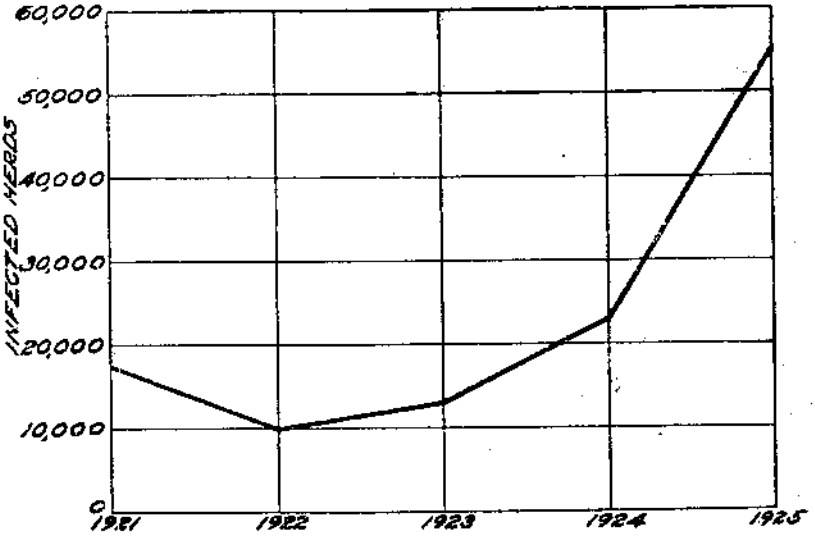

FIG. 2.—Trend of foot-and-mouth disease in Italy, 1921 to 1925, inclusive

The epizootic cycle usually does not exceed two years, unless a different type of virus is introduced. It has been noted also that the malignant type of the disease seems to be more prone to occur in six-year cycles. It is suggested that loss of immunity in the cattle and their offspring may be responsible for the malignancy of the disease.

Figure 2 shows the annual trend of foot-and-mouth disease from 1921 to 1925, inclusive. Weekly statements show that in 1921 the disease reached its peak in November, when over 4,000 herds were

reported infected in the third week. A sharp decline then took place and in 1922 the disease gradually diminished until about 100 cases a week were reported. In the fall of 1922 a gradual increase occurred until in March, 1923, when about 1,300 cases were noted for the week of March 19 to 25. In January, 1925, the disease again flared up, 2,000 cases being reported in one week. This was followed by a gradual decline until November when it again recurred, reaching its peak in the early part of December. Then more than 3,200 infected herds were reported for the week. A sharp decline followed.

But in the summer and fall of 1925 another epizootic began which reached the enormous number of 7,603 herds reported infected for the week of October 5 to 11. In the 1925 epizootic practically the whole of Italy became involved with the exception of the islands of Sardinia and Sicily. These were kept free of the disease by their geographical location and quarantine measures which prevented the introduction of live animals from the mainland. On the whole, the mild form of the disease prevailed, except in the Province of Milano, where a number of cases of the malignant type were noted.

Convalescent serum is used to advantage in the treatment of the disease.

A nervous form of the disease has been noted in Italy, the virus having been found in the brain and the cord. Vaccines prepared from the brain and cord of infected animals used in conjunction with serum, yielded fair results in the treatment of this form of the disease, according to Terni (*65*).

Much research work and scientific study of foot-and-mouth disease in the field have been done in Italy. Several commissions have been appointed to investigate the problem, but in recent years the lack of funds has seriously handicapped their investigations.

THE NETHERLANDS

Measures for controlling foot-and-mouth disease in the Netherlands include (1) slaughter of infected and exposed animals and (2) isolation and quarantine. Compensation for slaughtered animals is paid at the rate of 90 per cent of the value of the sound animal. The slaughter method is used at the beginning of an outbreak and when it is believed possible to eradicate the disease rapidly. This method was adopted frequently.

During certain years when the disease spread over large parts of Europe the Netherlands also suffered from severe epizootics, as shown in Figure 3. In the outbreak of 1924 88,930 infected herds were reported during the year. The epizootic at that time became so widespread that few of the regulations could be enforced, resulting in a further dissemination of the disease all over the country.

Convalescent and hyperimmune serums are used to advantage in the treatment of the disease.

Several commissions have been appointed at different times to investigate the subject. Research work is in progress at Utrecht and at the Rykes Serum Inrichting (government serum institute) at Rotterdam. Preparations are in progress for the manufacture of a hyperimmune foot-and-mouth-disease serum, and it is proposed to mitigate the effects of the disease by the extensive use of serum.

AUSTRIA

The disease is enzootic in Austria. Quarantine regulations are in effect in efforts to control the disease, which at times becomes widespread. Heavy losses were suffered from the malignant type of the disease in 1920 and 1921. In one community containing 400 adult cattle 33 per cent of the animals died. Cattle died as a rule within a week or two after the appearance of the disease.

Convalescent serum was used to advantage in reducing the mortality of the disease. Some practitioners used normal horse serum in the treatment of the disease and reported good results. It was stated, however, that under controlled conditions no definite value could be obtained from its use. This confirms limited laboratory tests with guinea pigs in which the writers show that normal horse serum appears

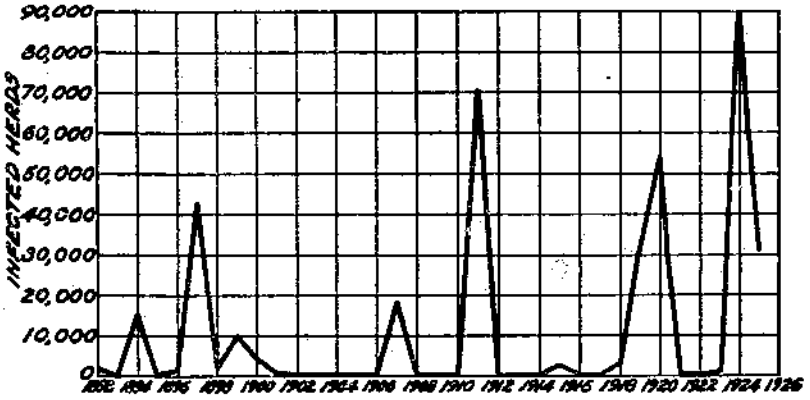

FIG. 3.—Trend of foot-and-mouth disease in the Netherlands, 1892 to 1925

to have no beneficial effect on the course of the infection. In strong contrast, convalescent cattle serum has a marked neutralizing effect on the severity of the disease.

No research work on foot-and-mouth disease was being done in Austria at the time of the commission's visit.

HUNGARY

Foot-and-mouth disease is enzootic in Hungary. Isolation and quarantine are the sanitary police methods in force. Cases are reported to the ministry of agriculture through the provincial veterinarians. Infected farms are quarantined and no one is allowed to leave the premises, except by special permission, until 30 days after the last animal recovers from the disease. All animals not infected at the onset of the outbreak are artificially inoculated by the farmer so that the disease will pass through the herd rapidly. Manure is removed from the stable, placed in a pile, and the barn disinfected, the expense being borne by the owner. The disinfection must meet with the approval of the official veterinarian.

The malignant type of the disease appeared in 1920 and 1921, when heavy losses were sustained. A mortality of 50 per cent of the adult cattle occurred in one herd. The animals died as a rule from one to two weeks after the onset of the disease, when apparently on

the road to recovery. The authorities believe that this was caused by heart failure as a result of degenerative changes in the heart-muscle fibers.

The care and condition of the cattle were not factors in the malignant type of the disease; in fact, it was noted that the better-fed animals, or those feeding during the course of the disease, were more prone to the fatal form, probably as a result of the extra work of the heart during digestion.

No research work was being done on foot-and-mouth disease at the time of the commission's visit.

BELGIUM

Two periods are recognized in epizootics of foot-and-mouth disease in Belgium and the control measures adopted are different in each period. The first period is that in which the disease has broken out in one small section of the country, its spread has been limited, and possibilities exist for its early control. In this period the disease may be combated by slaughter or stringent quarantine measures.

The second period is recognized when the disease has spread over a large area, in which case quarantine regulations are modified to reduce the heavy economic loss occurring as a result of a strict quarantine.

When the slaughter method of control is adopted compensation is paid for slaughtered animals by the government.

Belgium has had foot-and-mouth disease within its borders for many years, and at times the disease has become widespread. In 1924, for example, 37,287 cases of foot-and-mouth disease were reported. Little or no research work is being done.

CONTROL MEASURES IN THE UNITED STATES

The important features of methods for control in the United States are here presented mainly from the viewpoint of observations of the commission and of results of recent work of others.

In general these are the procedures employed:

1. Disposition of infected and exposed animals by slaughter followed by burial or burning.
2. Cleaning and disinfection.
3. Testing the infectivity of the premises, and restocking.
4. Quarantine.

The essential difference between the methods used in continental European countries (exceptions will be noted later) and in the United States is the tendency of the former to isolate and treat instead of completely disposing of infected and exposed animals by slaughter.

SLAUGHTER METHOD

A previous section stated that Germany, Belgium, Switzerland, Sweden, the United Kingdom, and the Netherlands have provisions in their laws for the slaughter of infected animals with compensation to the owner. These provisions in the laws can be put into effect whenever those in control believe that by so doing the prevention of the extension of the disease can be accomplished. The British methods now in vogue are practically the same as those in the United

States. Switzerland also depends to a great extent on the slaughter method. The other countries have not often found it practicable or economically possible to institute this procedure.

The main reason for the use of the slaughter method is to remove, as soon as possible, the greatest source of active virus. Even though the disease spreads rapidly, involving practically all cloven-footed animals, it frequently takes from one to several weeks before all susceptible animals in a herd have contracted the disease and in turn have passed through the highly infectious stage. During this entire period each animal or group of animals becomes a source of danger, in many instances, even before any evidence of the disease is seen.

The great difficulty in control work is that in spite of all the practical quarantine measures which can be enforced, active virus may be disseminated from premises before infection is suspected. It has been definitely proved not only that fluid and coverings of the vesicles contain the incitant in concentrated form, but also that the virus may be eliminated in large quantities even before fever or other indication of the disease appears. These observations indicate the necessity, in the stamping-out method, of killing all animals in an area as soon as possible after the presence of foot-and-mouth disease has been established in one of them, and, in view of the rapidity of spread, those on adjoining premises that have been exposed to the infection.

As for the question of carriers, although the commission's experiments on this point failed to reveal, definitely, carriers in appreciable numbers, the writers can not at present discard completely the striking field evidence and the positive experimental evidence of Assel, Böhm, and de Blieck (discussed more in detail in the section on carriers of foot-and-mouth disease) which indicate that recovered animals may be sources of spread of the virus. The slaughter method removes these potential reservoirs of virus.

In support of the value of the slaughter method, it may be stated that in the United States the disease has been eradicated and in continental European countries it has not been appreciably diminished.

CLEANING AND DISINFECTION

The careful methods of cleaning and disinfection have given good results in the United States. By cleaning is meant the removal, to the greatest extent, of any virus which may have been left on the premises after the destruction of the animals by slaughter.

What part disinfectants alone play is somewhat difficult to estimate in view of the recent findings. Disinfectants on foot-and-mouth-disease virus have not been studied heretofore so extensively as they have been on bacterial diseases. Livestock sanitarians have been guided by analogous work with other diseases and have recognized that disinfectants, to be of any value, should act very promptly. They have emphasized, therefore, the thorough mechanical removal of virus from all possibly contaminated materials by careful cleaning and scraping, and the destruction of articles which could not be properly cleaned.

Recent investigations, discussed briefly in this portion of the report and more in detail elsewhere, indicate that under experimental con-

ditions the most commonly used agents fail in the necessary characteristic of a desirable disinfectant, that is, ability to destroy rapidly the virus of foot-and-mouth disease. Field experience, on the other hand, indicates a high degree of efficiency for these agents. This difference may be explained possibly either by the fact that a great deal of virus is removed before the process of disinfection, that the virus which survives disinfection dies before restocking takes place, or that the laboratory virucidal tests are much more severe than the practical field tests.

The commission's work shows that a 1 or 2 per cent aqueous solution of sodium hydrate destroys, within one minute, the virus of foot-and-mouth disease as it is found in the fluid and coverings of the vesicle and also in these materials admixed with cattle urine or feces or with garden soil. The results of practical application of this agent will reveal its value.

TESTING PREMISES AND RESTOCKING

Before premises are released from quarantine and restocking is permitted, test animals are placed on previously infected premises 30 days after cleaning and disinfection, provided no active infection is present in the locality. This procedure should detect the presence of any virus which may have escaped destruction by physical and chemical means. Restocking is made gradually. As a further safeguard, inspections of new stock are made at regular intervals.

QUARANTINE MEASURES

Quarantine regulations are strict because of the highly contagious character of the disease. Quarantine measures are put into effect as soon as diagnosis of foot-and-mouth disease is established and they are maintained in force until there is reason to believe that the virus no longer exists on the premises or in the locality.

The promulgation and enforcement of effective quarantine measures should be based upon the following:

Animals are most actively contagious in the early stage of the disease.

Practically all cloven-footed animals are highly susceptible.

The greatest source of danger is removed by slaughter and proper disposition of the involved animals.

There are conditions which may exist in the field under which the virus may remain active for one or two months and perhaps longer.

Cleaning and disinfection removes most of the virus.

In spite of slaughter, cleaning, and disinfection, assurance of complete removal of every vestige of infectious material can not be given.

The testing of the premises and restocking of previously infected farms should be practiced as advocated by the Bureau of Animal Industry. This method is reasonably certain to insure freedom from active foot-and-mouth-disease virus. Some overzealous officials at times have put into force extremely unreasonable measures which have worked unnecessary hardships. Only rational quarantine measures should be used and those in keeping with facts, and antagonism toward eradication work should be avoided.

USE OF IMMUNE SERUM OR VIRUS IN THE AMERICAN METHOD

As to the advisability of using serum or virus, or a combination of the two, in the methods of control, the writers are of the opinion that virus should not be used in this scheme. It has been definitely shown that no immunity can be obtained thereby without establish-

ing at the same time a source of further spread of infection. Even in combination with serum, virus may induce a moderated or mild form of the disease.

The use of serum by itself on noninfected premises is not recommended to any great extent in Europe. It has a very limited field of usefulness, especially in view of the fact that its neutralizing effects may disappear after such a short period as eight days, as the writers and others have shown. At the expiration of this short period of resistance animals may contract the disease if active virus is present and consequently injections of serum alone have been known to prolong the duration of disease on a given farm. The use of serum alone is, however, without danger; but it is expensive and it is necessary either to import it or maintain an establishment at a very isolated point for its production, since large quantities of active virus are necessary for its manufacture.

The writers feel that at the present stage of our knowledge of foot-and-mouth disease it behooves the stockmen of this country and those more indirectly interested to realize that, except for short periods, the United States has been entirely free from foot-and-mouth disease, and that European countries, on the other hand, are saddled with it. The United States owes this freedom to the methods employed as outlined above, which in the end are the most economical.

EXPERIMENTAL STUDIES

PHYSICAL AND CHEMICAL PROPERTIES OF THE VIRUS

In the experimental studies which were made on the physical and chemical properties of foot-and-mouth-disease virus as well as in the attempts at its artificial cultivation, the writers used, except in a few instances, one strain. This virus, described below, had the advantages of causing lesions promptly and regularly, of acting powerfully, of inducing in practically all cases well-marked, secondary lesions, and of bringing about relatively few deaths in the experimental animals—a favorable condition, for it permitted a long period of observation. The animal of choice for these studies was, unless otherwise stated, the guinea pig.

STRAIN OF VIRUS EMPLOYED

On June 18, 1925, through the kindness of a local veterinarian, Doctor Fuchs, a herd of cows on a farm about 15 kilometers from Strasbourg was placed at the commission's disposal for study. The herd was affected with typical foot-and-mouth disease. Of 12 animals in the same barn, all in different stages of the disease, one cow was selected which was the last to be infected. She drooled considerably, and had broken and unbroken aphthae about the lips, tongue, and buccal mucosa, but no obvious foot lesions, and her temperature was 41° C. It was difficult to aspirate the exudate in the vesicles, so that in the end what was brought to the laboratory was an admixture of the cow's saliva and stable litter. This material was diluted 1 to 3 with Tyrode solution and then injected, unfiltered, intradermically in the hairless skin of the posterior pads of five guinea pigs.

Typical primary vesicles appeared at the sites of injection in from 24 to 48 hours in all five animals. From that time until the investigations were terminated, about a year later, the virus (Strasbourg, or "Str." strain) was propagated in the guinea pig for 261 consecutive passages in a pure state except on two occasions in late passages, when staphylococci were found in the vesicular exudate. These latter were then removed by filtration, without harming the virus. Later tests showed the failure of cross-immunity with type A; hence the Str. strain was probably identical with that of the Vallée O.

It is to be noted from the experience of transferring the impure virus from the cow to the guinea pig that the virus, when admixed with contaminating microorganisms, could free itself of ordinary bacteria in the tissues of a susceptible animal. Thus a property common to many filter-passing viruses is shown by the active agent of foot-and-mouth disease. Indeed, after the first series of transfers, disinfection of the site injected was found to be unnecessary and only twice in 261 passages did the virus become contaminated.

FREEDOM FROM ORDINARY BACTERIA

Aside from the fact that the virus tended to purify itself in the guinea-pig tissues, deliberate cultivation tests were made when it was first acquired to determine whether there might be any constant bacteria associated with this strain. Repeated cultures were attempted with the blood, with the fluid aspirated from the vesicles (the so-called lymph), with ground, infected pads, the latter filtered—all obtained on the first day of illness.

No constant, visible microorganisms were revealed by the different methods of culture, the details of which are described under "Experiments on cultivation." Hence this strain was pure and its effects could not be ascribed to any agent cultivable by ordinary means.

THE DISEASE IN THE GUINEA PIG

The clinical picture of the experimental disease in the guinea pig corresponded in the main with the comprehensive descriptions given by Waldemann and Pape (*77*), by Gins and Krause (*30*), and by the members of the British foot-and-mouth-disease research committee (*3*, *35*, *63*). Certain of the more important points are, however, worthy of recapitulation.

METHOD OF INJECTION

After a long trial with various methods of injecting the virus, the writers found that the method of choice, and the one by which practically all guinea pigs were infected, is the intradermic. The procedure consists in running a fine needle into the skin along the length of the hairless pads of the posterior extremities. Attached to this needle is a syringe containing the test fluid, and with pressure on the piston the skin tunnel thus made is filled as the needle is withdrawn. Three or four such injections are made on each posterior pad; and, in addition, the thin loose skin along the outer and inner margins of the pad is filled until it distends to a distinct, bleblike formation. If the virus is weak or diluted an additional inoculation is made subcutaneously in these pads. Inoculations by means of skin tunnels

were also found to be advantageous by the workers of the British committee (*3, 35, 63*).

Injection of the virus in sites other than the pad skin was followed by specific lesions, but certainly not so regularly as those occurring after the intradermic [8] inoculation, as the following experiment illustrates.

PROTOCOL 1

All animals were injected with the same sample of virus taken from a guinea pig in the first day of illness. The infected pads of the latter were removed, ground in sand and suspended in phosphate buffer solution (P_H 7.6) to a dilution of 1:25. One-half cubic centimeter was inoculated in each guinea pig:

(*a*) Intramuscularly in the thigh. Of 6 animals only 2 showed the typical aphthae; in 1 in all four pads, in the second in all pads and in the tongue. Incubation period, 48 to 72 hours.

(*b*) Intraperitoneally. Of 5 animals 3 exhibited vesicles in all pads and in the mouth. Incubation period three to four days.

(*c*) Subcutaneously under the abdominal skin. Of 5 guinea pigs 2 showed aphthae in all pads and in the tongue, 1 in two pads and 1 in one pad. Incubation period, three to six days.

(*d*) Intradermically in abdominal skin. Of 5 animals 4 showed lesions in all pads and in the tongue. Incubation period three to five days.

In none of these were any lesions noted at the site of the injection.

In this experiment, as in others mentioned in this report, the activity of the virus used was always determined by inoculating intradermically the posterior pads of at least two guinea pigs.

When these methods of injection are compared with the intradermic method already described, by which practically all of more than 2,000 guinea pigs were infected, it is evident that the intradermic injection of the posterior pads was more reliable. This test also illustrates the epitheliotropic nature of the infection, for, irrespective of the site of injection, the lesions appeared only in the epithelium of the pads or of the tongue or of the mouth. Furthermore, if a proper injection of the virus was given, no natural immunity to foot-and-mouth disease was determined in guinea pigs.

SECONDARY LESIONS

As already mentioned, the Str. strain induced secondary lesions regularly. These appeared in the pads or mouth or tongue, in sites other than those injected, as early as 36 hours and as late as 5 days, but as a rule from 48 to 72 hours after inoculation. For an unequivocal determination of experimental foot-and-mouth disease in the guinea pig the presence of these secondary lesions was essential. In doubtful instances, as when the primary vesicles were obscured by necrosis—a condition found frequently in experiments with antiseptics, to be mentioned later—recourse was had to transfer to normal animals for observation of primary and secondary signs before a conclusive diagnosis could be made.

PERIOD OF INCUBATION

In about 95 per cent of the cases the period of incubation was from 18 to 48 hours. In one instance the first symptoms were noted after 12 days and in three cases, after 10 days. In the remainder the primary aphthae appeared from 3 to 6 days after injection. For

[8] "Intradermic" inoculation, wherever mentioned in this report, is the particular method of injection described in the preceding paragraph.

these reasons, observations were prolonged from 10 to 12 days before an exact definition was obtained; and when necessary an injection of active material was made in animals which did not react, to determine the presence of immunity. A noteworthy fact is that the period of incubation is shortened and the severity of the disease is increased as the dose or concentration of the virus is augmented.

TIME OF GREATEST INFECTIVITY

Tests showed that the blood of the guinea pig was active when withdrawn 24 and 48 hours after intradermic inoculation. On the other hand, if this blood, in a defibrinated condition, was kept at 37° C. in vitro, it was active for 24 but not for 48 hours. The period of greatest activity of the virus in lymph aspirated from aphthae or in ground infected pads was obtained from lesions up to 24 hours in age. From this time up to 72 hours there was a gradual diminution in virulence and after 72 hours such virus was active only in exceptional cases. At about this time, the active agent, conforming to the tendency of filter-passing viruses in general, sometimes invited invasion by secondary microorganisms which was followed by purulent inflammation.

MORTALITY

The mortality rate from infection of guinea pigs with the Str. strain was about 1 per cent. As this rate corresponded to the normal death rate among the guinea pigs kept in stock, the experimental disease induced by this strain was practically nonlethal.

TRANSFER TO CATTLE

Finally, this strain after propagation in the guinea pig could induce in cattle and hogs typical foot-and-mouth disease indistinguishable from the natural affection. The material used for such transfers was derived from guinea pigs, in either the early or last passages. Further consideration is given to this subject elsewhere in this report.

TITRATION OF THE VIRUS

It was essential to determine quantitatively the activity of the virus, this estimation being important from the point of view of controlling survival or preservation, as opposed to multiplication of the virus in subplants of cultivation tests. This information also served as a guide for interpretation of the cataphoresis experiments to be reported later, and gave an idea of the relative size of the active agent.

The virus was found to be active in remarkably high dilutions as the following experiments will show.

PROTOCOL 2

Summary of 15 experiments. The virus used for these tests consisted of the aspirated lymph from 24-hour-old vesicles. The diluent consisted of phosphate buffer solution at $P_H=7.5$. Dilutions were made with pipettes. The diluted virus was injected by the intradermic method, already described, in both posterior pads of guinea pigs.

In the 15 tests the virus was found active in a dilution of 1 : 10,000,000 twice; 1 : 2,000,000 thrice; and in 1 : 1,000,000 twice. These were the final dilutions

in the foregoing trial. In four additional tests in which dilutions were made higher than that of the active range, in 1 : 4,000,000 was in one case positive, but 1 : 8 to 32,000,000 negative; in another case, 1 : 2,000 000 was active but not 1 : 4 to 80,000,000; in a third case, 1 : 1,000,000 was positive but 1 : 2,000,000 negative; in the last case, 1 : 400,000 was active but not 1 : 1,000,000.

All these experiments were made with the virus cleared of particles by filtration through filter paper. In the next four tests filtrations were made through Berkefeld V candles. Activity was shown in all four instances in a dilution of 1 : 2,000,000, the highest made.

From these titrations it is to be noted that the virus diluted to 10,000,000 times its volume may still be active. This fact leads to several considerations, the first of which is with reference to the probable size of the active agent. Since the material is not solid with virus but contains the diluent and various products usually found in an inflammatory exudate, the active agent is only a part of this quantity. Hence the agent should be still more minute. That the size of the active particles of the virus is less than 100mμ has also been confirmed in the study on molecular filtration to be described in another section.

(2) Great care should be exercised in measuring or transferring virus in glass receptacles in tests for comparative activity, such as the determination of the killing effect of antiseptics and the relative value of different media in cultivation tests. Spattering virus along the glass walls may give rise to infections which may have no bearing on the problem in hand. Whenever practicable, therefore, chemical mixing glasses or conical beakers were substituted for test tubes.

(3) In the commission's experience with cultivation of the virus, the third subplant showed activity in five different media. In this third subplant the virus was diluted by the method of seeding about 1:1,000,000. But in the fourth transplant the active agent was diluted 1:15,000,000; in this latter, however, the virus was without action. Apart from this, the writers had other and ample evidence to show that these media were not suitable for growth. Hence the conclusion derived from such experiments is that preservation or survival, but not multiplication is involved. Therefore the works of Titze (*66*) and of others who maintained virulence only in the first few "subcultures" should be interpreted as probably indicating a mere dilution of the active agent.

(4) Since one sample of the virus is active in a dilution of 1:400,000 and another in a dilution of 1:10,000,000, a factor of difference in activity between one specimen and another is 25. The investigators of the British committee (*35*) found a still greater factor to exist; one sample was inactive at 1 : 5,000, another was active at 1 : 500,000. Hence, in comparative tests, such as made in the study of the survival of the virus in different media, trials should be made with the same specimen of virus.

(5) The severity of the disease and the length of the period of incubation depended on the concentration of the virus—the more concentrated the virus the more severe was the infection and the shorter the period of incubation. For example, with one sample of a 24-hour aspirated lymph virus, a dilution of 1:1,000 induced the experimental disease of severe type in 24 hours in all of five guinea pigs; of 1:200,000 after three days in one of two animals; of 1 : 2,000,000 after four days mildly in one of two animals; and with a dilution of 1:4,000,000

one vesicle was noted after nine days on the tongue of one guinea pig out of two inoculated. Higher dilutions were negative.

The rate and energy of action, therefore, are proportional to the concentration of the active agent.

CENTRIFUGATION OF THE VIRUS

Centrifugation tests were made with two objects in view: (1) To determine the sedimentation of the virus, and (2) if the virus were not sedimented, to attempt to free by this process the supernatant fluid from a hypothetical body which may be the inhibiting factor in cultures, for example, such bodies as fragments of tissue or cells.

As for the first object, it was determined by repeated tests that with the active guinea-pig blood, aspirated lymph, or suspensions of ground, infected pad tissue, centrifugation at 2,500 to 3,000 revolutions a minute for two hours did not cause the deposition of the virus; the topmost layer of the specimen was as active as the lowest portion. Thus the writers have confirmed the prior findings of others, notably the workers of the British committee (*35*), Abe (*2*), and Gins (*28*). However, the writers could not confirm thereby Frosch and Dahmen's (*22*, *23*, *24*, *25*) first step in cultivation, namely, a concentration or sedimentation of the virus by centrifugation at 3,000 revolutions a minute for 0.5 to 1.5 hours.

The inability to sediment the virus may indicate that the active agent is very minute, but not necessarily that it is of the nature of a contagium vivum fluidum.[9] Duclaux (*16*) has demonstrated the fact that centrifugation is not a means for deposition of minute particles. For, theoretically, with a centrifugal force 40,000 times as great as the gravity force of a particle (such as is obtained in large centrifuges) a particle measuring 10mμ will settle only 1 centimeter in four hours. But, practically, in addition to this slow deposition due to size, since all machines develop a certain amount of heat, the whirling current and that due to convection operate to prevent deposition.. In the example cited the variations due to these currents should be multiplied by 40,000 to obtain the actual reading of the forces preventing sedimentation (Duclaux).

In respect to the removal of a hypothetical inhibiting body by centrifugation, the writers' repeated experiences showed that the active guinea-pig blood kept at 37° C. contained no virus in either the sediment or supernatant fluid when tested 3 days after centrifugation. But at room temperature similar ground, pad virus suspended in phosphate buffer at $P_H = 7.5$ remained alive for 9 days in the sediment and for 12 days in the supernatant fluid. With aspirated lymph under the same conditions, however, both the sediment and supernatant fluid were active for at least 14 days. No further tests were made in this instance.

To complete these experiments, three additional trials were made with the sediment after it showed inactivity by standing for 4 days at room temperature. This sediment was added to either fresh, or old, active supernatant fluids, and the mixture kept for from 18 hours to 3 days at 20° C. But no inhibition of the virus in the supernatant fluid was observable.

[9] This term was first used by Beijerinck (1899) (*7*) to denote the noncorpuscular character of the virus of tobacco-mosaic disease. In the sense of Beijerinck, the virus is present in solution, and, although a fluid, is capable of reproduction.

The commission concludes from these experiments that the hypothetical, virucidal bodies can not be deposed by centrifugation from suspensions of ground, infected pad tissues or of aspirated lymph.

At this point brief mention may be made of attempts to remove such substances which inhibit the viability or multiplication of the virus in artificial media, by precipitation of the globulins, or inactivation of complement. For this purpose carbon dioxide was used and prolonged passage of the gas was allowed through material containing the active agent. These tests likewise ended in failure.

SUMMARY

The strain of foot-and-mouth-disease virus derived from affected cattle near Strasbourg was propagated in guinea pigs for at least 261 successive passages. These rodents were uniformly susceptible to the virus after intradermic inoculation. This fact, together with the absence of natural immunity among guinea pigs and the regular production of secondary lesions, made this strain and this species of animal useful for experimental purposes. Furthermore, the virus could be readily transferred from guinea pigs to cattle and hogs and from the larger animals back to the rodents.

In the guinea pig the virus was found to show epitheliotropism, for primary lesions, and secondary vesicles were induced constantly and regularly after intradermic inoculation. The fact that inoculation of this animal in sites other than the hairless pads was often unsuccessful led to the supposition that the virus has only a limited portal of entry into the body.

The virus, similar to many other ultramicroscopic viruses, was not affected by contamination with concomitant or secondary bacteria of the ordinary species. Indeed, it could purify itself of such bacteria in the first passages in a susceptible animal.

The limit of infectiousness of the virus was found at a dilution of 1:10,000,000. There are several considerations to be given to this extraordinary activity. In the first place, it is correlated with an extremely minute size of the virus for the material used for titration is not solid with virus. Furthermore, virulence shown only in the first few subplants in cultural experiments should be regarded as a mere dilution of the incitant rather than actual multiplication. Finally, all samples of virus are not equally infectious. A considerable factor may represent the difference in activity of the weakest from that of the strongest. Therefore, comparative studies should be made with the same sample.

In addition to these considerations, it should be understood that a different technic is necessary for experimental procedures from that used with bacteria of the ordinary species, namely, a regard for spattering the virus which may give rise to infections unrelated to the problem at hand.

By means of centrifugation, it is impossible to sediment the virus. This character possessed by many other ultramicroscopic viruses may not be an indication of its fluid nature, for reasons already given. In view of the evidence presented and other tests to be reported later, failure of deposition is related to the minute size of the incitant. Finally, centrifugation has failed to remove Frosch and Dahmen's so-called "virucidal bodies."

CATAPHORESIS AND FILTRATION

CATAPHORESIS

The electric charge carried by the active agent, as well as its isoelectric range, was a criterion by which different filtration tests could be interpreted. Its determination was useful not only to explain the remarkable resistance of the virus to certain chemicals to be described elsewhere, but also to compare the charge with that characterizing known microorganisms.

The main difficulty of cataphoresis experiments with this virus was the absence of a recognizable or visible particulate body. But this was overcome by adapting to the writers' purpose the Vlès [10] apparatus. Moreover, guinea pigs could be regularly infected with even minute quantities of the active agent, so that its attraction to one or another pole could be determined by the biological test.

The apparatus (*74*) used consists essentially of horizontal U tubes containing in the curved arms a stoppered ampulla, and in each long arm a stopcock. These are connected to communicating cups by curved siphons which are joined to each other by a transverse tube carrying also a stopcock. The latter allows a prior establishment of a hydrostatic equilibrium. The orifices of the curved siphons are plugged with moist cotton to prevent hydraulic disturbances. The tubes, fixed to a board, are placed in series of four (because four determinations at different PH readings were made at one time). At both ends of the series union with the house current is made through a solution of cupric sulphate and, in the connecting cups, through physiological saline solution (0.9 per cent at PH=8). In the siphons of each tube of the series is placed the phosphate buffer [11] at the PH at which the virus is tested. In the ampulla is added the virus diluted with phosphate buffer at different PH readings, thus filling completely the respective U tubes to the stopcocks.

In the following experiments the difference of potential at the limits of the series in an open circuit was 220 volts. A milliamperemeter was then placed in the circuit, which registered from 0.8 to 4.7 milliamperes for the four tubes and the fall of potential for each tube was from 20 to 50 volts. The time of cataphoresis lasted from 20 to 70 minutes. The commission found that the best result was obtained when the virus was filtered and diluted 1:1,000 in phosphate buffer, at a milliamperage of 0.8, with a fall of potential of 20 to 30 volts and cataphoresis for 70 minutes.

This experiment, one of a series of 10, is illustrative.

PROTOCOL 3

Twenty-four-hour, aspirated, lymph virus was diluted 1:1,000 with phosphate buffer at PH=7.5 and filtered through Berkefeld V candles. This material on injection intradermically in the pads of guinea pigs showed activity in each of graded dilutions from 1:2,000 to 1:200,000.

The substance was then separated into four portions and each was adjusted to different PH readings by the colorimetric method. At the same time phos-

[10] The commission expresses its gratitude to Prof. Fred Vlès, of the University of Strasbourg, for his cordial cooperation and his valuable assistance, not only in these experiments but also in others on filtration.

[11] Phosphate buffer as mentioned here and elsewhere in this report is made by adding 2.5 grams potassium acid phosphate (KH_2PO_4) to a liter of distilled water. This is then adjusted to the desired PH by means of potassium hydrate. It is of utmost importance to readjust the material just before use, for sterilization renders it acid.

phate buffer adjusted to similar degrees was prepared. At the termination of the cataphoresis experiment the following electrometric PH measures were obtained:

Tube	Buffer	Virus	Average buffer and virus
1	6.63	6.84	6.73
2	7.68	7.62	7.65
3	8.00	7.90	7.95
4	8.17	8.05	8.11

The cataphoresis was continued for 70 minutes with a fall in potential of 20 to 30 volts with a milliamperage of 0.8.

Guinea pigs were then injected intradermically in the pads with the material removed from the negative and positive poles of each of four differently adjusted specimens with these results:

PH=6.73;
Negative pole: 2 guinea pigs; both positive after 48 hours.
Positive pole: 2 guinea pigs; both negative.

PH=7.65:
Negative pole, 1:2,000: 2 guinea pigs; both positive after 48 hours.
1:20,000: 2 guinea pigs; both positive after 48 hours.
Positive pole, 1:2,000: 2 guinea pigs; both negative.
1:20,000: 2 guinea pigs; both negative.

PH=7.95:
Negative pole, 1:2,000: 2 guinea pigs; both positive after 48 hours.
1:20,000: 2 guinea pigs; both positive after 48 hours.
Positive pole, 1:2,000: 2 guinea pigs; both negative.
1:20,000: 2 guinea pigs; both negative.

PH=8.11:
Negative pole: 2 guinea pigs; both negative.
Positive pole: 2 guinea pigs; positive after 48 hours.

Results of other tests at PH readings different from those mentioned showed:

PH=6.6:
Negative pole, 1:500: 2 guinea pigs; both positive after 48 hours.
Positive pole, 1:500: 2 guinea pigs; negative.

PH=7.0:
Negative pole, 1:500: 2 guinea pigs; both positive after 48 hours.
Positive pole, 1:500: 2 guinea pigs; both negative.

PH=7.4:
Negative pole, 1:500 to 1:100,000: In all dilutions, all guinea pigs positive after 48 hours.
Positive pole, 1:500 to 1:100,000: In all dilutions, all guinea pigs negative.

PH=7.5:
Negative pole, 1:250 to 1:250,000: In all dilutions, all guinea pigs positive after 2 to 4 days.
Positive pole, 1:250 to 1:250,000: In all dilutions, all guinea pigs negative.

PH=8.0:
Negative pole, 1:150 to 1:30,000: In all dilutions, all guinea pigs positive after 48 to 72 hours.
Positive pole, 1:150 to 1:30,000: In all dilutions, all guinea pigs positive after 24 to 48 hours.

PH=8.5:
Negative pole, 1:500: 2 guinea pigs; both negative.
Positive pole, 1:500: 2 guinea pigs; both positive after 48 hours.

It appears, therefore, that the foot-and-mouth-disease virus is capable of wandering in an electric field and then, if the active agent is at a hydrogen-ion concentration below PH=8, the direction of motion is toward the negative pole (electropositive); if above PH=8, toward the positive pole (electronegative). At about PH=8, there

is a tendency to move in either direction (isoelectric range). Hence it may be stated that under ordinary conditions the active agent has an electropositive charge, and that its isoelectric range is at P_H = about 8. It should be remembered that, in view of the absence of a visible, particulate body and the slight changes in hydrogen-ion concentration during cataphoresis, this figure is relative; hence the term "range" is employed rather than the word "point."

It was noted that, as a rule, there was clouding at the pole opposite to that which the virus was attracted. For example, if the fluid at the positive pole appeared hazy and at the negative limpid, then the material at the negative pole contained the active agent. Whether the force of the electric current is sufficient to tear the virus from the electronegative protein particles to which it may be attached [12] it is not possible to say, but these findings are suggestive. The possible objection that the virus wanders along with protein does not hold, for the incitant moved in an opposite direction from the protein, ordinarily electronegative.

The fact that the virus has an unusually high isoelectric range and, under ordinary conditions, is electropositive does not speak against its possible protein or living nature. For, although bacteria are generally electronegative in charge, spirochetes, as a genus, carry electropositive charges (with the exception of *Treponema pallidum*); so do trypanosomes, such as the species of brucei, equiperdum, gambiense, and rhodesiense. Among protein substances, fibrin and gliadin (at $P_H = 9.3$) are known to possess a high isoelectric point. The outstanding fact is that the virus, in respect to its charge, is different from ordinary bacteria.

The determination of the charge, however, was of greatest importance in interpreting the results of ordinary and molecular filtration experiments, and therefore gave indirectly some indication of the relative dimensions of the causal agent.

FILTRATION

The filters employed in the following experiments comprised the Seitz asbestos disks, Berkefeld V and N candles, Chamberland's L 1, L 2, L 3, L 5 (F), L 7 (B), L 9, L 11, and L 13 bougies, various thicknesses of collodion membranes, and, finally, Bechhold's ultrafilter prepared with different percentages of acetic collodion.

It is important to note that the foot-and-mouth-disease virus, as employed in the following tests, may contain the incitant in a free state, as well as adsorbed to particles. That the virus can be free and that there is a lack of complete adsorption to a number of different substances has been observed by several investigators (Dahmen, Gins, Bedson, Burbury and Maitland, and Stockman and Minett). Furthermore, there is practically no loss in activity of the virus after filtration through coarse and fine filters. In addition, filtrations proceed in a definite, regular manner. For these reasons, and from the results of cataphoresis experiments showing a separation of the virus from protein particles, one can not regard the whole of the incitant as adsorbed to minute particles of the same size.

[12] In another section will be described the adsorption of the virus to large protein coagula.

Experience with the Seitz filter [13] showed that the virus filtered easily through one disk of asbestos. The writers did not employ this method for study of filtration phenomena but merely to purify the virus from contaminating microorganisms. For this purpose the Seitz filter is effective.

In regard to the other methods of filtration, a general principle was involved in all cases, namely, all the filters carry an electronegative charge (*58*). As the virus has an electropositive charge, considerable adsorption occurs during the passage of the active agent through the candles, bougies, or membranes. In some filters in which the pore size is minutely small and the charge strongly negative, complete adsorption takes place with the consequence that the filtrates contain no virus.

FILTRATION THROUGH BERKEFELD V AND N CANDLES

The material for these tests consisted of aspirated lymph or of ground, infected pads suspended in phosphate buffer at $P_H = 7.5$ or 7.6. In any event, preliminary filtration through one layer of filter paper was resorted to. This was done to prevent clogging of the small candles with particles of tissue, fibrin, or cells.

To test the charge and relative pore size of these filters, there were passed through them suspensions containing micellae of either charge and of different sizes. Electropositive particles such as methylene blue, basic fuchsin, and night blue (bleu de nuit) regularly failed to pass, but electronegative particles, such as acid eosin and Prussian blue, readily traversed the candles. Hence these filters carried an electronegative charge.

It was therefore necessary, in determining the size of particles capable of passing through the V and N filters, to eliminate adsorption caused by filtering oppositely charged substances. Accordingly, collargol, the mean diameter of its micellae being 20mμ and colloidal arsenic trisulphide, 100mμ, were tested. Both passed rapidly through V, and definitely, but less speedily, through N candles. Both substances have an electronegative charge. Hence these Berkefeld filters, with the adsorption action counteracted, allow the passage of particles of at least 100mμ in diameter.

In respect to the virus of foot-and-mouth disease a standard molecule for comparison should be one with a positive charge and of a definite known size. For this purpose pure crystallized hemoglobin in solution was used. Its isoelectric range is at $P_H = 6.75$, and its size, as computed by Bechhold (*6*), is 3.6mμ in diameter. Suspended in phosphate buffer (employed also in suspensions of the virus) at $P_H = 6$, hemoglobin is electropositive, and at $P_H = 7.5$, electronegative. In the former condition this substance passes through V and N candles; in an electronegative condition, the passage is more free and completely unobstructed. Since the virus with an electropositive charge also passes regularly through both these filters, showing at times activity in at least a 1:2,000,000 dilution of the filtrate, the conclusion derived from these observations is that the active agent is at least larger than the particle of hemoglobin. This has been confirmed by the commission's experiences with filtrations through Chamberland bougies.

[13] For more extensive studies on this type of filter, the reports of the investigators of the British committee (*55*) should be consulted.

FILTRATION THROUGH CHAMBERLAND BOUGIES

The Chamberland bougies of all types are similar to the Berkefeld candles in that they are charged electronegatively. Electropositive particles such as methylene blue basic fuchsin, and night-blue dye do not pass through their walls, but the electronegative micellae of acid eosin, Prussian blue, collargol, and colloidal arsenic trisulphide do. In regard to hemoglobin, at $P_H = 6$, with an electropositive charge, this substance passes these filters only slightly—the first few drops of filtrate are clear, but after a time traces of hemoglobin are revealed by spectroscopic examinations. At $P_H = 7.5$, with an electronegative charge, hemoglobin traverses freely through all the types of Chamberland filters.

In other words, as an electropositively charged molecule, hemoglobin first satisfies the avidity of the electronegative material of the bougie and later its minute size enables it to pass through the barrier.

A similar experience was obtained with the electropositive virus. With bougies of graded series to L 5, the active agent traversed through their walls. But with more dense types the passage became more difficult until with the L-11 type[14] the filtrates were free from virus. For example, 5 trials with 5 different new L-7 type bougies yielded in only one case a positive filtrate; 3 filtrations through 3 different, new, L-9 bougies resulted in 1 positive; and 7 tests with 7 different L-11 type bougies, 6 of which were new, the seventh having been used more than once failed in all instances. In these experiments the virus was suspended in phosphate buffer at $P_H = 7.5$ (electropositive). Diluted in similar material at $P_H = 8.5$ the virus, then in an electronegative state, passed freely through these bougies.

It appears therefore that the foot-and-mouth-disease virus, by elimination of adsorption due to opposite electric charges, can traverse the small spaces of L-11 filters. On the other hand, if adsorption due to opposite charges is unhindered, the virus fails to pass. But, in the latter relation hemoglobin under similar conditions can traverse this filter. Hence the active agent is larger than the hemoglobin particle of 3.6mμ diameter. Experiments with filtration through collodion membranes confirm this relative measurement.

FILTRATION THROUGH COLLODION MEMBRANES

For a determination which might give a measurement of the virus between narrower limits, and for a more extended study of the filtration phenomena, a still tighter filter was employed, namely, the collodion membrane.

Collodion membranes are negatively charged. Indeed, the individual particles of pure collodion remain so charged, or become even more strongly negative, in the presence of either acid or alkali, as measured by the cataphoretic potential difference between these particles and water (*48*).

The collodion membranes, usually in the form of small sacs, were prepared with French codex collodion (5 per cent). In all, 68 sacs were made with different proportions and conditions of collodion, so that 18 different experiments were performed. The concentra-

[14] Several trials with a number of the L-13 type bougie, supposed to be the tightest of all of the series, resulted in irregular filtrations of titrating reagents. Hence work with this type was discontinued.

tion of collodion varied from 5 to 12 per cent (12 per cent corresponding to 15 seconds' reading on the apparatus devised by Gates (*26*) for measuring its relative viscosity). From one to three layers were used, and dipping in 95 per cent alcohol was resorted to in some instances to increase the permeability of the sacs. Negative pressure from 10 to 25 centimeters of mercury was applied. The virus comprised 24-hour aspirated lymph diluted 1 : 50 or 1 : 100 in phosphate buffer at $P_H = 7.5$ or 7.6 to $P_H = 8.5$ which in all tests, as measured by control inoculations in guinea pigs, was very active.

Six experiments were also made with sacs prepared after the manner of Levaditi, Nicolau, and Galloway (*47*) who used three layers of 5 per cent collodion (codex), without alcohol immersion.

To summarize the results of these experiments: The commission found that the collodion membranes prepared in the manner stated are not suitable for the filtration of the virus of foot-and-mouth disease. More than half of the number of membranes showed macroscopic holes before they were put to test. In the remaining ones, the writers could not convince themselves that microscopic holes were absent. In the absence of the standardization of the membrane, it was necessary that each sac be tested separately by titration with known substances of definite charge and size. Otherwise the results were valueless. Membranes which the writers thought were hole-free and at the same time made so as to withstand a negative pressure of from 10 to 25 centimeters mercury (without this negative pressure, filtration was impossible), failed, as a rule, to allow passage of methylene blue, eosin, collargol, hemoglobin, etc.—the materials employed for titration of the Berkefeld V and N and all types of Chamberland filters. They did allow the passage of peroxidase (which was found to be more easily filtrable than blue litmus), of blue litmus itself, the size of which is computed by Bechhold (*6*) to be 1.8mμ in diameter, and of crystalloids. The crystalloids which traversed the membranes were, in order of molecular weights from strychnine, molecular weight, 397, acid quinine sulphate, to basic quinine sulphate, molecular weight, 890.[15] The virus, on the other hand, failed to pass through these membranes.

It may, therefore, be stated that collodion membranes of the types mentioned are permeable to colloids containing particles in size of the order of blue litmus, and to crystalloids, but not to the virus carrying positive or negative charges. Furthermore, in the absence of standardized membranes, they are unsuitable for titration experiments. The writers, however, confirmed the observation of Stockman and Minett (*63*) that the virus does not traverse two layers of 5 per cent collodion. One conclusion may be derived from these experiments—the active agent is not comparable to crystalloids in respect to filtration through collodion membranes.

It was then found necessary to seek a more permeable membrane, by which the size of the virus could be delimited more precisely and, at the same time, be free from the variables in the methods already mentioned. For these purposes the commission found the Bechhold ultrafilter membranes to be very satisfactory.

[15] Strychnine was tested for its presence in the filtrates by inoculation of frogs; quinine, by opalescence in a strong beam of light, after acidulation with sulphuric acid.

FILTRATION THROUGH BECHHOLD ULTRAFILTER MEMBRANES[16]

The prepared Bechhold disks are made by impregnating in a vacuum Schleicher & Schüll filter paper with different concentrations, from 1½ to 7½ per cent, of acetic collodion. By this method unevenness of the layer and microscopic holes are eliminated; the membrane is strong enough to withstand the necessary negative pressure and a concentration of as little as 1½ per cent of the collodion can be used with, as a rule, unequivocal results.

The commission adapted these disks to its purposes by cutting each membrane to a circumference corresponding to that of the lower part of a Seitz [Manteufel (*51*), E. K. model] filter. The membrane was placed over the wire-gauze support and a smooth, well-fitting, firm, rubber washer was superposed. The cup end was then tightly screwed in and the mechanism was tested for leakage at the negative pressure employed in each test.

The Bechhold disk is electronegative. To eliminate the absorption of substances oppositely charged, both the virus and the test materials for titration were employed in a condition of the same, that is, negative charge. Hence for virus the writers prepared 24-hour-old lymph aspirated from fresh, unbroken vesicles, diluted to 1:100 in phosphate buffer at $P_H=8.5$. The substances for titration consisted of blue litmus and hemoglobin each suspended in the phosphate buffer at the same hydrogen-ion concentration. The collargol and the colloidal arsenic trisulphide, both electronegative, were prepared fresh after the directions of Ostwald (*61*). As a test for the charge of the membrane and its ability to absorb an oppositely charged substance, a suspension of electropositive night-blue (bleu de nuit) dye was used. Complete absorption of this dye occurred in every case.

In Table 6 are summarized the experiments made with membranes of different permeability, that is, of different thicknesses, containing from 1½ to 7½ per cent acetic collodion. The virus under electronegative conditions is compared with the electronegative test substances, blue litmus, hemoglobin, collargol, and colloidal arsenic trisulphide. These varied in size from 1.8mμ to 100mμ in diameter.

The negative pressure required for each type of membrane differed with the thickness of the layer. For example, with the 7.5 per cent membrane, −30 centimeters mercury pressure was employed; with the 6 per cent, −10 centimeters; with the 4.5 per cent, from −5 to −10 centimeters; with the 3 per cent, −5 centimeters; with the 1.5 per cent, −5 centimeters. In all cases the filtrates were tested for the presence of virus by intradermic inoculation of the hairless pads of guinea pigs, and for the titrating substances by colorimetric spectroscopic, and chemical tests. The virus taken from the apparatus, before filtration and at $P_H=8.5$, was injected intradermically into three or four guinea pigs in each instance. After 24 hours all animals showed the typical experimental disease. Finally, in these, as in all filtrations, only from 5 to 10 cubic centimeters of material were filtered, and the time regulated. Results therefore can not be ascribed to employment of large quantities filtered during a long period.

[16] The membranes are manufactured by Carl Schleicher and Schüll, Düren, Rhine Province, Germany.

TABLE 6.—*Results of filtration through Bechhold's ultrafilter membranes*

Substance	Charge	Size of particle	Percentage acetic collodion in membrane [1]											
			7.5		6		4.5		3				1.5	
			Membranes used	Results	Membranes used	Results	Membranes used	Results	Membranes used	Results in each case (membrane No.) 1 2 3 4			Membranes used	Results in each case (membrane No.) 1 2 3 4
		mμ	No.		No.		No.		No.				No.	
Virus	Negative		4	−	4	−	4	−	4	− − − −			4	+ + + +
Blue litmus	do	1.8	4	+	4	+	4	+	4	+ + + +			4	+ + + +
Hemoglobin, 1 per cent	do	3.6	4	−	4	−	4	−	4	+ + + −			4	+ + + +
Collargol	do	20	4	−	4	−	4	−	4	+ − − −			4	+ + + +
Colloidal arsenic trisulphide	do	100	4	−	4	−	4	−	4	− − − −			4	+ − − −

[1] A minus sign, −, indicates the nonfilterability, and +, the filterability of the substance tested. With the 4.5 to 7.5 per cent collodion membranes, the results were uniform. With the 1.5 and 3 per cent membranes, there were slight variations, so that the tests with each are tabulated.

An examination of the tabulated, summarized results reveals that, in general, there are only a few irregularities in filtration through the single-layer Bechhold membranes, indeed, fewer than were expected. But in view of the fact that each membrane was titrated and its porosity delimited, the results of the so-called molecular filtrations appear to be conclusive. Furthermore the selective passage of the titrating substances which the writers used is identical with that reported by Bechhold (*6*).

Only the minute particles of blue litmus could traverse the 4.5 to 7.5 per cent membranes, which they did with considerable regularity. The virus uniformly failed to pass through them. The virus, however, did traverse one of four membranes of the 3 per cent type. In that instance collargol also passed. In the three others of this series, two allowed the passage of hemoglobin and one the blue litmus only. On the other hand, the virus filtered uniformly through all membranes of the 1.5 per cent type. In these the porosity was of such an order of magnitude that in all of them collargol traversed the membranes as well, and in one case, the colloidal arsenic trisulphide.

It may be concluded, therefore, that the order of magnitude of the active agent of foot-and-mouth disease, after elimination of interference by adsorption due to unlike charge, is in relative terms correspondingly between that of collargol and of colloidal arsenic trisulphide particles. In other words, the relative size of the virus is between 20mμ and 100mμ.[17]

These tests have not only served to delimit the relative size of the active agent but have also brought to light a valid refutation of the notion that the virus is a fluid or a contagium vivum fluidum after the meaning of Beijerinck (*7*), of which more will be said later. Furthermore, the same comparative ease with which the virus and collargol particles filtered through the 1.5 per cent membranes and the difficulty with which colloidal arsenic trisulphide traversed similar

[17] These tests supply a basis of only a relative calculation. Hence it was decided, especially in view of the definite limitation of time for experimental work in Europe, to forego further trials in an attempt to narrow the range of relative size to closer approximations.

disks indicate that the size of the active agent may be nearer the minimal reading than the maximal. The minuteness of size may explain the invisibility of the virus; the difficulty of its artificial cultivation and its resistance to alcohol and other chemicals, to be described later; its noncentrifugability; and its activity in very high dilutions. Particles of these relative dimensions are governed by physical laws different from those influencing larger structures (*75*). Finally, the question arises as to whether these measurements preclude the notion that the active agent is living. In the absence of a standard for comparison, one may compare the virus to bacteria in respect to their chief function, the enzymatic, and their composition, which is mainly protein. The writers have determined that peroxidase can pass freely through spaces which do not permit the passage of litmus, the particles of which are estimated at 1.8mμ in size (Bechhold, *6*). Assuming the virus to be spherical and of a diameter of 50mμ, it should be more than 2,500 times the volume $\left(V=\frac{4}{3}\pi r^3\right)$ of the protein molecule hemoglobin. Furthermore Vlès [18] has calculated that spherical bodies of the order of magnitude of 30mμ may contain 360 protein molecules and those of 90mμ, 9,000.

SUMMARY

The writers have found that the electric charge carried by the virus is positive and by cataphoresis tests have shown that its isoelectric range is at the high point of P_H = about 8. With regard to these properties, it may be said that the virus differs from ordinary bacteria. The common genera of bacteria carry an electronegative charge and have a much lower isoelectric point. The fact, however, that the virus has an unusually high isoelectric range, and is, under ordinary conditions, electropositive does not speak against its possible protein or living nature. Certain filtration phenomena can be explained by this charge, as well as the remarkable resistance which the virus shows to some chemicals—a subject to be dealt with in the next section of this report. Finally, cataphoresis indicates the possible separation of the virus from protein particles.

Filtration through Seitz disks, Berkefeld V and N candles, Chamberland bougies of practically all sizes, collodion membranes, and Bechhold's ultrafilter confirms the findings not only of the minuteness of the size of the virus but also its electropositive charge. With respect to filtration through Chamberland bougies, the active agent traversed L 11 filters only when its charge was shifted to negative; under ordinary conditions, carrying an electropositive charge, it failed to pass this denser wall, and was completely adsorbed in the oppositely charged barrier. Filtration through collodion was successful only when the thinnest and most permeable membranes were used, such as the most porous of Bechhold's ultrafilter membranes. With coarser collodion filters, prepared in different ways and of varying thicknesses, the presence of microscopic holes as a source of error in positive results had to be frequently considered. The use of standardized, thin, collodion membranes of Bechhold's ultrafilter type enabled the writers, by means of so-called molecular filtrations, to delimit the size

[18] Personal communication.

of the active particles of the virus. This was found to be, in relation to other particles of like charge, between 20mμ and 100mμ in diameter.

The filtration phenomena of the foot-and-mouth-disease virus can be accounted for on the basis of minute size of the particles of the incitant, which carry an electropositive charge, and no evidence can be deduced therefrom that the virus is a fluid character. The relative size of the particles is constant and the invariability of the limits of measurement contradicts the notion that the incitant may be a solute varying in different solvents.

RESISTANCE OF THE VIRUS TO CHEMICALS

Although ultramicroscopic viruses are generally resistant to certain chemicals, the active agent of foot-and-mouth disease is exceptionally so. In this remarkable resistance a parallel has been found thus far in the virus of mosaic disease and also in bacteriophage. For example, Abe, (*2*) found that from 70 to 75 per cent alcohol precipitates the virus of foot-and-mouth disease, with the albumins of infective blood, which can then retain its activity from 2 to 3 days in a dried state. Stockman and Minett (*63*) reported that from 25 to 50 per cent alcohol does not destroy it after at least 3 days nor does 10 per cent, after at least 20 days. These writers state that 10 drops of chloroform to 5 cubic centimeters of 1:100 virus do not inhibit activity for at least 27 days; and 10 drops of ether to 3 cubic centimeters of filtrate do not destroy it after more than 10 days. In similar proportions of acetone the active agent survives for at least 4 days, and in 50 per cent glycerol, as is the case with many filter-passing viruses, indefinitely. These remarkable conditions have also been confirmed in general by Bedson and Maitland (*3*).

The results of the American commission's experiments are in agreement with those of Abe (*2*) and the British investigators, (*35*, *37*) for the writers found repeatedly that in 20, 40, 50, and 60 per cent alcohol the virus either in ground, infected, pad tissue or in aspirated lymph remained alive for at least 26 hours. In five experiments the virus admixed with practically undiluted acetone maintained its viability for 20, but not for 30 minutes. Nor was it rapidly killed by supersaturation with ammonium sulphate; the active agent was viable in the coagulum for at least 2 hours.

In view of the fact that bacteria generally are destroyed very rapidly by these agents (staphylococci in one minute by 60 per cent alcohol), the writers believed that it was sufficiently important to undertake a series of experiments which might indicate whether the resistance to chemicals was real or merely masked. For if the virus actually withstood these reagents, it might reveal the nature of the active agent rather as an inanimate chemical than as a living protoplasmic body.

The writers were aware that under the methods used by earlier experimenters a comparison between the resistance of the virus and that of bacteria, such as staphylococci, could not be made. In the case of the latter the reagents were added to a pure culture or a material which was presumed to contain nothing but bacteria and the diluent; in that of the virus the chemicals were added to tissues or the solid and fluid constituents, mainly protein, of an inflammatory exu-

date, of which the virus occupied only a small part. Furthermore all the chemicals to which the active agent is resistant are capable of coagulating proteins. That the virus is readily adsorbed to these large coagula will be shown in the following protocol:

PROTOCOL 4.—CONTROL

Infected guinea-pig pads 24 hours after injection were ground with sand and a quantity of 90 per cent alcohol was added to make the final dilution of the latter 60 per cent. A heavy coagulum resulted. After two hours' centrifugation at 2,500 revolutions per minute, the clear, supernatant fluid and the sedimented material were injected intradermically into three guinea pigs, respectively, for each of the two portions. After 24 hours all six showed typical primary lesions and after 48 hours, secondary vesicles in the mouth and pads.

Test

At the same time, the coagulum of similar alcoholized material, which was active for at least 26 hours, was allowed to settle for 1 hour. The topmost and lowest portions were injected into guinea pigs with the result that all of four animals exhibited primary lesions 24 hours after inoculation and secondary lesions 24 hours later. The supernatant fluid was filtered through Berkefeld candles. The filtrate was free from virus.

This experiment is typical of seven others. In the remaining tests the contact with alcohol before filtration covered a period of from two to three hours and the virus used consisted of infective blood, ground, pad tissue, or aspirated lymph.

To summarize the results of all these tests it may be stated that alcohol, when added to blood, ground pad tissues, or aspirated lymph containing the virus, causes the formation of a more or less dense coagulum. Centrifugation of these agglomerations leaves a supernatant fluid which is still active. The writers have already demonstrated that the virus as it occurs in the tissues of the guinea pig is not centrifugable and have discussed the inability of the centrifuge to depose or sediment completely minute particles. On the other hand, filtration is a more effective means of separating particles from a fluid. Hence by the use of Berkefeld filters, which, owing to their relatively larger size pores, permit the passage of the active agent irrespective of electric charge, these particles are withheld from the filtrate. The filtrate then is free from virus. In other words, the active agent is closely attached or adsorbed to the coagula formed after the addition of alcohol.

Similar results were obtained with acetone. In pure acetone, which forms a heavy, soft coagulum, the active agent survived for 20 but not for 30 minutes. If the material was filtered immediately after complete coagulation occurred, the Berkefeld filtrate was inactive.

With ammonium sulphate there was the same tendency. In this case, materials containing the virus after supersaturation with ammonium sulphate for one hour at 37° C. exhibited a heavy coagulum. The latter was active, but on filtration of the substance through Berkefeld candles the filtrates were inactive. In two instances the clear filtrate obtained by using two layers of Schleicher & Schüll No. 589 filter paper was also free from virus.

Hence it appears that the active agent in guinea-pig tissues is completely adsorbed and protected, as will soon be demonstrated, by the precipitates formed after the addition of alcohol or acetone or ammonium sulphate.

The next step to suggest itself in a further study of this phenomenon was the relation of the amount of protein in the material containing the virus to its capacity to resist the reagents.

By filtration of vesicular fluid through Berkefeld candles, it was possible to remove a considerable amount of protein substances, especially cells, fibrin, and larger miscellaneous particles. Filtrates, however, usually showed the Biuret reaction, so that not all proteins were thus eliminated. However, when alcohol or acetone was added to filtrates, only a faint haze of micellae resulted. How this influences the resistance of the virus to the reagents is illustrated in the following experiment:

PROTOCOL 5

One cubic centimeter of aspirated lymph from pads injected 24 hours previously was added to 20 cubic centimeters of phosphate buffer at PH=7.5. This was then filtered through a Berkefeld N candle and of the filtrate 5.4 cubic centimeters were taken. To this quantity, 10 cubic centimeters of 90 per cent alcohol were added which made the concentration of the latter equal to 60 per cent. To test its activity 0.1 cubic centimeter of the remainder of the filtrate, nonalcoholized, was injected into each of two guinea pigs, as controls. In 48 hours both animals exhibited the typical experimental disease.

A series of guinea pigs was now inoculated intradermically with the alcoholized filtrate after varying intervals. After 5 minutes, only one of two animals injected was positive, with an incubation period of 4 days; after 15 minutes the same result occurred with an incubation of 5 days; after 30 minutes and 1 hour, the filtrate was inactive.

This experiment is typical of several others in some of which 50 per cent alcohol as well as filtrates of ground, infected pad tissues were also used.

The conclusions to be derived from all these tests are that although the unfiltered virus remained viable in 50 or 60 per cent alcohol for at least 26 hours, filtered material showed no such resistance. When 50 per cent alcohol was added to active filtrates of aspirated lymph or ground, infected pad tissues, the virus was killed within 15 to 20 minutes, and with 60 per cent alcohol within 1 to 15 minutes, depending on the concentration of the active agent. The unfiltered material contained more protein substance than the filtered and hence a greater amount of coagulum which displays a greater adsorptive function. The consequence was that the virus in filtrates was more open to the direct attack by the reagent.

As a corollary, the immediate refiltration of the active coagulum formed by adding alcohol to filtrates removed the virus. This is not due to double, or repeated filtration of the virus materials, for control tests showed no such tendency.

From these observations further proof is adduced that there is a relationship between the amount of virus adsorbed to large coagula and the killing effect of alcohol. There is an indication here which points to the possibility of increasing the destructive action of alcohol, if the latter could be placed in contact with free or unadsorbed virus.

The writers believe that they succeeded in bringing this about by taking into consideration the fact that coagulation of proteins by alcohol is a periodic phenomenon, depending on hydrogen-ion concentration (*42*, *52*). For example, a basic experiment was performed with ascitic fluid as the protein material. It was found that the heaviest coagulation by 60 per cent alcohol occurred between PH=

7 and 7.6, maximum at $P_H=7.5$. At $P_H=8.2$ there was absolute clearing and at $P_H=6.5$, only a slight, micellar haze was noted. Hence at a certain point, reached by adding a definite, minute quantity of 0.5 per cent NaOH to protein material, alcohol does not coagulate. Furthermore, the quantity of alkali necessary to prevent this coagulation is insufficient in itself to kill the virus. Since it is impossible to make determinations of the hydrogen-ion concentration of alcohol, the writers proceeded in an empiric manner, after the method of Loeb (*48*) in his studies on solutions of protein in alcohol-water mixtures, to determine precisely the amount of alkali necessary to cause inhibition of coagulation.

PROTOCOL 6

A large number of experiments were made during a period of three months and several hundred guinea pigs were used before unequivocal results could be obtained. It is possible to state here only a résumé of results.

The commission found these technical operations to be important in carrying out the tests:

(*a*) All material should be measured in conical beakers or chemical mixing glasses and not in test tubes, for the spattering of the virus or insufficient mixing of the substances may give irregular results.

(*b*) The active virus, whether in aspirated lymph or ground, infected pad tissues should be filtered first through filter paper to remove clots and small particles. The dilution employed in tests and controls is 1:50 to 1:100. In each experiment, the paper filtrate is injected intradermically into guinea pigs to test its activity.

(*c*) The phosphate buffer at $P_H=7.5$ should be adjusted to this point just before use, since sterilization or standing in glass utensils may cause a change in hydrogen-ion concentration. The buffer is used only for making suspensions of the virus. For controls (see Table 7) distilled water is substituted for the alcohol or NaOH, as the case requires, so that the buffer effect remains constant throughout each series.

(*d*) In the test the alkali should be added first, then the alcohol (or water in controls), and lastly the virus. Immediately thereafter these ingredients are thoroughly mixed, and after the time set for contact, fluid is removed from about the center of the mixture.

(*e*) Since the measure of the killing effect of alcohol is not cultural but biological, great care should be observed in the injection of the test materials intradermically into guinea pigs to differentiate the dull-white alcoholic or the dirty-yellow alkali inoculation necroses from the lesions of experimental foot-and-mouth disease. As safeguards it is necessary (1) to have a strain of virus which produced regularly secondary vesicles, (2) to transfer suspected tissues to normal guinea pigs for the observation of uncomplicated effects, and (3) whenever possible to subject recovered animals to an immunity test.

In the tests 60, 40, and 20 per cent alcohol dilutions were used. These percentages indicate the final concentrations which were obtained by using stock alcohol of 90 per cent strength. Of this a sufficient amount was taken to make the required concentration, in a total volume of test or control materials of 7.7 cubic centimeters.

It was found that in this volume and with these percentages of alcohol the amount required of the NaOH, 0.5 per cent solution, was 0.33 cubic centimeter.

In Table 7 the different amounts of the materials employed are tabulated, as well as the general manner of the procedure, both in regard to tests and their corresponding controls.

TABLE 7.—*Effect of alcohol on foot-and-mouth-disease virus*

60 PER CENT ALCOHOL [1]

Time	Control: Virus (2.7 c. c.) + Alcohol 90% (5 c. c.) + H_2O (0.33 c. c.)	Control: Virus (2.7 c. c.) + H_2O (5 c. c.) + NaOH 0.5% (0.33 c. c.)	Test: Virus (2.7 c. c.) + Alcohol 90% (5 c. c.) + NaOH 0.5% (0.33 c. c.)
1 minute	+	+	−
2½ hours	+	+	----
6 hours	+	+	----
24 hours	+	----	----
26 hours	+	----	----
30 hours	−	----	----

40 PER CENT ALCOHOL

Time	Control: Virus (4.4 c. c.) + Alcohol 90% (3.3 c. c.) + H_2O (0.33 c. c.)	Control: Virus (4.4 c. c.) + H_2O (3.3 c. c.) + NaOH 0.5% (0.33 c. c.)	Test: Virus (4.4 c. c.) + Alcohol 90% (3.3 c. c.) + NaOH 0.5% (0.33 c. c.)
1 minute	+	+	−
2½ hours	+	+	----
6 hours	+	+	----
24 hours	+	----	----
26 hours	+	----	----
30 hours	----	----	----

20 PER CENT ALCOHOL

Time	Control: Virus (6.0 c. c.) + Alcohol 90% (1.7 c. c.) + H_2O (0.33 c. c.)	Control: Virus (6.0 c. c.) + H_2O (1.7 c. c.) + NaOH 0.5% (0.33 c. c.)	Test: Virus (6.0 c. c.) + Alcohol 90% (1.7 c. c.) + NaOH 0.5% (0.33 c. c.)
1 minute	+	+	+
2½ hours	+	+	−
6 hours	+	+	----
24 hours	+	----	----
26 hours	+	----	----
30 hours	----	----	----

[1] The plus sign (+) signifies virus active, the minus sign (−) signifies virus killed. [2] Longest time tested. [3] Result of five experiments.

From this tabulated résumé it is to be noted that the active agent as it exists in the tissues or exudates of the guinea pig resists the action of alcohol in dilutions of 60, 40, or 20 per cent. But the resistance is due to the adsorbing coagulum which results from the interaction of alcohol on the proteins of the milieu in which the virus resides. The latter is also unharmed by the presence of NaOH 0.5 per cent, in the small quantity used, that of 0.33 to 8.03 cubic centimeters (or about 1 in 5,000) of the medium. But in the presence of the alkali, alcohol fails to coagulate the protein; the resultant mixture is clear. Then the alcohol comes into direct contact with the unprotected virus and kills it quickly, the 40 per cent and 60 per cent dilutions within one mintue, and the 20 per cent within an average time of 2½ hours.[19]

The writers could make no comparison with other filter-passing viruses in respect to sensitiveness to alcohol under similar conditions. Hence the commission turned its attention to a definite, living microorganism, the staphylococcus, for a relative study.

COMPARISON WITH STAPHYLOCOCCI

In the following experiments cultures of *Staphylococcus aureus*, obtained from a patient with osteomyelitis, and grown on plain agar slants, were substituted for the virus materials, and then subjected to alcohol action under similar conditions.

PROTOCOL 7

Suspensions of staphylococci were made in phosphate buffer at $P_H=7.5$. Proportional amounts of this and of the stock 90 per cent alcohol were taken to make final concentrations of 60, 40, and 20 per cent of the latter, with the same quantities outlined in Table 7 for the virus-alcohol tests. This constitued one series, to check the next in which again 0.33 cubic centimeter of NaOH, 0.5 per cent, was added to the volume of 7.7 cubic centimeters of staphylococcus-alcohol suspensions. A tabulation of the results is given in Table 8.

TABLE 8.—*Action of alcohol on staphylococci* [1]

Time	Staphylococci+alcohol			Staphylococci+alcohol+NaOH 0.5 per cent 0.33 c.c.		
	60 per cent alcohol	40 per cent alcohol	20 per cent alcohol	60 per cent alcohol	40 per cent alcohol	20 per cent alcohol
Minutes	*Colonies*	*Colonies*	*Colonies*	*Colonies*	*Colonies*	*Colonies*
1	—		Inf.[2]	—	10	Inf.
1.5		110	Inf.			Inf.
3.5			Inf.		5	Inf.
5.5			Inf.		2	Inf.
6		2	Inf.		—	Inf.
6.5		—	Inf.			Inf.
60			Inf.			Inf.
Hours						
2.5			Inf.			Inf.
4 to 7			Inf.			P. G.
8 to 22			P. G.[3]			P. G.
23			P. G.			10
24			P. G.			—
Days						
2			75			
3			8			
4			—			

[1] The minus (—) sign signifies no growth in subculture of 0.2 c. c.
[2] Inf. signifies prodigious, normal growth as compared with controls.
[3] P. G. signifies profuse, confesced growth, making it impossible to count individual colonies. Control cultures of the staphylococcus suspension showed the usual growth after four days.

[19] In four instances the lethal point was 1.5 hours, 1 hour, or less, but in one instance, which the writers can not explain, the virus resisted 20 per cent alcohol plus NaOH for 7 hours. Stockman and Minett (*65*), however, found that 25 per cent alcohol of itself kills the virus after 3 days and 10 per cent after 20 days.

In respect to a suspension of staphylococci containing only microorganisms which are not admixed with proteins as is the case with the virus, Table 8 shows that 60 per cent alcohol was capable of inhibiting growth completely within 1 minute, 40 per cent alcohol killed staphylococci after 6 minutes, and 20 per cent after 3 days. But with this microorganism as well, the addition of the alkali increased somewhat its sensitiveness to the reagent—in 40 and 60 per cent alcohol the lethal action was too short to make a definite difference, but in 20 per cent alcohol the killing time was shortened 2 days.

It is now possible to make a comparison between the virus and staphylococci in regard to this phenomenon.

In 60 per cent alcohol the virus in its protein milieu remains alive for at least 26 hours—for a much longer time according to Abe and the workers of the British committee. But, freed from the protective coagulum, the virus is killed within 1 minute—so are staphylococci.

In 40 per cent alcohol the virus in the guinea-pig tissues or exudates is resistant for at least 26 hours, or even several days. In its free state, however, it is killed also within 1 minute. The growth of staphylococci is inhibited, on the other hand, after from 5½ to 6 minutes.

In 20 per cent alcohol the virus in its natural state can remain viable for at least 3 days. But when it is not adsorbed to coagula and there exposed directly to the action of this reagent it is killed in less than 1 hour, though in an exceptional case it remained alive 7 hours. Staphylococci, on the other hand, are killed after 23 hours (with alkali) and after 3 days (without alkali).

When one is dealing with the virus admixed with proteins, one encounters in antiseptic reactions complications caused by the protective action of the protein coagula. When coagulation is prevented by taking advantage of the periodic phenomenon, and the virus is exposed completely to the action of the reagent, it is then even more sensitive to destruction than a culture of microorganisms, such as staphylococci. The opinion, therefore, that the active agent of foot-and-mouth-disease, by itself, is more resistant to alcohol than living bodies, such as bacteria, is untenable.

ALCOHOLIZED VIRUS IN CULTURES

It was believed that concentration of the virus could be effected by its adsorption to the coagulum which is formed by adding 60 per cent alcohol to the active agent in a protein milieu. By employing such alcoholized virus the writers also had in mind the possibility of removing ordinary, contaminating bacteria and, at the same time, the inhibiting substances which prevent growth in vitro. Results of experiments show that the virus prepared in this manner can not be used for culture. For example, in buffered gelatin (described under the heading "Experiments on cultivation" control virus survived at 34° C. for at least nine days but no activity was noted with alcoholized virus on the fifth day of incubation.

VIRUCIDAL CHEMICALS

It may be concluded from the observations already stated that chemicals which cause coagulation of proteins and consequent protection of virus adsorbed to the formed particles may not be active as virucides. On the contrary, such substances which do not coag-

ulate the proteins of the material containing the virus, thus placing one in more direct contact with the other, may act more powerfully as destructive agents. As the sequel will show, the writers were justified in this point of view.

The scope of this problem was extended to include chemicals which are coagulating and those which are not, both being employed with the same sample of virus materials and their powers of destruction compared. Accordingly the commission chose as examples of the first group such substances as bichloride of mercury and two samples of cresol. Later a new preparation advanced as an effective antiseptic against foot-and-mouth-disease virus, chloronal (*67*), was added.[20] As specimens of the second group of noncoagulating substances the writers selected antiformin and sodium hydrate.

In view of the fact that under actual field conditions the coagulating substances are generally used as virucides, most often cresol and bichloride of mercury, the results of the following experiments assume greater importance.

The first experiment dealt with the virucidal properties of bichloride of mercury and two different samples of cresol, one labelled as such, and the other as "compound cresol." Protocol 8 illustrates one such test.

PROTOCOL 8

Aspirated lymph virus was diluted in phosphate buffer at P_H=7.5. This form of virus was employed instead of fragments of infected tissue so as to favor any destructive action of the chemicals. To 2 cubic centimeters of 1:100 of the suspension of the active agent were added 2 cubic centimeters of 1:500 bichloride of mercury and to other respective lots, 2 cubic centimeters of 6 per cent cresol and the compound cresol. In the end the dilution of the virus was 1:200, of the bichloride 1:1,000, and of the cresol preparations 3 per cent. After periods of contact varying from 5 minutes to 6 hours (the longest time of testing), the mixtures were injected intradermically into guinea pigs. The results with all these substances were practically identical. Guinea pigs injected with material after from 5 minutes to 2 hours contact showed primary vesicles in 24 hours and secondary lesions in 48 hours. Those animals injected with material after 4 to 6 hours' contact exhibited primary lesions in 48 hours and secondary vesicles after 72 hours.

All the precautions mentioned in protocol 6 were employed and great care was taken not to confuse chemical necroses with the lesions of experimental foot-and-mouth disease.

Hence bichloride of mercury, cresol or the cresol compound—all forming large coagula with virus materials—acted as alcohol did. They did not destroy the virus quickly, at least not after six hours, the longest period tested. On the other hand, in two other experiments chloronal in a 5 per cent solution, which forms much lesser and finer coagula, showed no destructive action within from 30 to 32 minutes, but after 1 hour inactivated the virus.

The next step was to test the action of antiformin and sodium hydrate, substances which do not coagulate the proteins of the medium containing the virus.

PROTOCOL 9

Of antiformin, 1 per cent was used, of sodium hydrate one-fourth, one-half, 1, and 2 per cent. The virus was diluted in phosphate buffer to 1:40 and, as a more severe test of the action of these reagents, consisted of ground, infected,

[20] According to Trautwein (*67*), chloronal contains 25 to 30 per cent chlorine, mainly in a dissociable state, but it is stated that it coagulates albumin.

pad tissue, unfiltered, and containing particles up to about 3 millimeters in size. In other experiments in this series, aspirated lymph was also used as a source for virus; in still others the latter was diluted only 1:10. In all cases, however, the results were identical, and are given below.

All guinea pigs injected intradermically with antiformin 1 per cent in contact with virus for 1, 2, 3½, 5, 15, and 60 minutes failed to show signs of the experimental disease.

In two of four guinea pigs injected intradermically with sodium hydrate, one-fourth per cent, there were, after three days, primary lesions of the experimental disease, with specimens in which the alkali and virus were in contact for one minute. The virus, however, after exposure for three minutes was inactive.

One of four guinea pigs injected intradermically with sodium hydrate, one-half per cent, showed, after two days, primary vesicles and after five days, secondary lesions. The virus was exposed to the alkali one minute. After three minutes' contact all the injected animals remained well.

None of four guinea pigs injected with sodium hydrate 1 per cent in contact with the virus for one and three minutes were affected.

Of 10 guinea pigs injected intradermically with sodium hydrate 2 per cent in contact with the virus (1:20 to 1:40 final dilution) for one to three minutes all remained normal. Observations were also extended to cover a period af contact from three minutes to one hour. Again none of the animals inoculated with virus after more prolonged contact with alkali were affected.

It is to be noted that variations in the technic outlined in protocols 6 and 8 may lead to errors of interpretation. With antiformin and sodium hydrate, especially in higher concentrations, chemical necroses are apt to occur at the site of injection which may mask the primary lesions. Hence attention should be given to the appearance of secondary lesions, and, in addition, material from all suspected pads should be removed and suspensions of them injected into a series of normal animals for control of the specificity of the reaction.

These experiments demonstrate that chemicals such as antiformin and sodium hydrate, which do not coagulate the proteins of the medium containing the virus and therefore come in direct contact with the active agent, were powerfully destructive to the incitant. Antiformin, 1 per cent, killed the virus within one minute.[21] Sodium hydrate, 0.25 to 0.5 per cent, destroyed the active agent within three minutes but not in one minute. At this concentration, however, the destructive effect was weak and irregular. But in 1 to 2 per cent solutions the inhibiting action occurred within one minute and was complete and regular. It may be stated, in favor of the completeness of this neutralizing effect, that the virus was penetrated and killed even when present in fragments of tissue, as in ground, infected, pad tissues containing particles about 3 millimeters long.

The question arose then whether soda could replace sodium hydrate in respect to the latter's powerful action. But the writers' experience with 5 per cent anhydrous sodium carbonate has shown that this can not be done.

The rapid, regular, and energetic destruction of the virus by sodium hydrate in low concentrations led the commission to seek a practical application. Field conditions were imitated by admixing virus in bits of tissue with cattle urine, manure, and with garden earth, and then exposing such mixed materials to the action of sodium hydrate. An illustrative experiment, one of several, follows.

PROTOCOL 10

Twenty-four hours after injection, four infected pads were removed from guinea pigs and ground in 20 cubic centimeters of phosphate buffer at $P_H=7.6$. To this were added 20 cubic centimeters of aspirated lymph virus of a dilution

[21] Stockman and Minett (*63*) have also found that the virus (in comparison with its great resistance to alcohol, ether, etc.) is easily destroyed, in "less than one hour," by antiformin, 1 per cent.

of 1:10. The material was unfiltered and contained a number of small particles of tissue. Two cubic centimeters of this virus suspension were added to 2 cubic centimeters of urine from a normal cow. The probable dilution of virus in this instance was 1:20. Two cubic centimeters were also added to about 5 grams of manure from normal cattle and to a similar amount of earth obtained from an area near the stalls. In all instances the virus was thoroughly mixed with the substances to which it was added and the mixtures were allowed to stand at room temperature for one hour.

Then to the virus suspension itself, and to the urine, the manure, and the earth containing the virus, a quantity of sodium hydrate 4 per cent solution was added to make the final concentration of the latter 2 per cent. Guinea pigs were injected intradermically with the mixtures exactly 1, 3, and 5 minutes after the alkali had been added. Observations extending over 12 days showed that none of these animals were affected.

At the end of an hour or after the completion of the test, portions of the virus suspension itself, and the urine, the manure, and the earth containing the virus, were injected intradermically into respective guinea pigs, which served as controls for activity of the different materials. In all animals typical, primary lesions were observed 24 hours after injection and secondary vesicles 12 to 24 hours later.[22]

It appears, therefore, that sodium hydrate in a 2 per cent solution is capable of destroying, within one minute, not only the virus, even when contained in fragments of tissue, but also such virus admixed with cattle urine, with manure, and with earth.

As a further application of the virucidal value of this reagent to field conditions, the test was extended to include cattle as experimental animals. The results are shown in the following protocol.

PROTOCOL 11

The materials used in this experiment were prepared in a manner exactly similar to that mentioned in protocol 10. To portions of a suspension of virus, and virus mixed with cow's urine, with manure and with earth—in all cases thoroughly incorporated—was added sodium hydrate in a sufficient quantity so that the final concentration of the latter was 2 per cent (see protocol 10). One minute later 9 cattle were inoculated by scarifying the mucous membrane of the mouth and then rubbing in the substances briskly; 3 cattle were inoculated with the NaOH-virus material, and 2 each with the remaining three mixtures, respectively.

One hour later 3 normal cattle and 4 guinea pigs were inoculated, the former by the same scarification method used in the test animals, with the suspension of virus, and the latter intradermically, with the portions of the remainder of the four different virus mixtures. These animals served as controls for the activity of the virus and the latter admixed with urine, manure, or soil. In addition to these controls, a hog was injected intravenously, and 2 convalescent heifers were inoculated by scarification of the gum and dental pad.

None of the cattle inoculated with the virus mixtures exposed to the action of sodium hydrate for one minute were affected, while all the control animals, except the recovered heifers, showed within 48 hours the symptoms of experimental foot-and-mouth disease.

The nine cattle, the first group, used in the test series, were inoculated, after 28 days, by the same scarification method, with active virus. All became ill with the typical experimental disease, thus demonstrating that the absence of effect after the first inoculation was not due to a natural resistance.

It may be concluded from the foregoing that the destructive effect of 2 per cent sodium hydrate on the active agent is complete and rapid—it occurs within one minute—as tested not only on guinea pigs but on cattle as well. This leads to the consideration of this reagent as an effective virucide in practice.[23]

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[22] Care should be taken not to confuse chemical necroses with the true, typical lesions of experimental foot-and-mouth disease. (See protocol 6, par. c.)

[23] The limited time and space at the disposal of the commission prevented similar tests on cattle with 1 per cent sodium hydrate. But the results with guinea pigs demonstrate that this concentration of the chemical may be equally effective.

COMPARISON OF ACTION OF REAGENTS ON STAPHYLOCOCCI

Attention was now directed to the action of the different chemicals used as virucides upon definite, living microorganisms, such as staphylococci. The object in such determinations was to note whether the active agent of foot-and-mouth disease is by virtue of these reactions in a class by itself, or whether it can be correlated with living bacteria.

PROTOCOL 12

Suspensions of *Staphylococcus aureus* were prepared as in the experiments cited in protocol 7. To these were added, respectively, bichloride of mercury in 1:1,000, cresol or the compound cresol in 3 per cent, chloronal in 5 per cent, antiformin in 1 per cent, and sodium hydrate in 2 per cent, final concentrations. After varying periods of time at room temperature, 0.1 cubic centimeter was subcultured on agar. The results are tabulated in Table 9.

TABLE 9.—*Effect of certain chemicals on Staphylococcus aureus*[1]

Time	Bichloride of mercury, 1:1,000	Cresol, 3 per cent	Chloronal, 5 per cent	Antiformin, 1 per cent	NaOH, 2 per cent	Control suspension of staphylococci
Minutes	*Colonies*	*Colonies*	*Colonies*	*Colonies*	*Colonies*	*Colonies*
1	1,000	Inf.[2]	4	14	200	Inf.[2]
3	100	Inf.	1	—	30	Inf.
5	5	Inf.	—	—	5	Inf.
7	—[1]	P. G.[3]	—	—	—	Inf.
10	—	1,000	—	—	—	Inf.
15	—	300	—	—	—	Inf.
60	—	28	—	—	—	Inf.
120	—	—	—	—	—	Inf.

[1] The *minus* (—) sign signifies no growth in subculture of 0.2 cubic centimeter.
[2] Inf. signifies prodigious, normal growth as compared with controls.
[3] P. G. signifies profuse, coalesced growth, making it impossible to count individual colonies. Control cultures of the staphylococcus suspension showed the usual growth after four days.

The results summarized in Table 9 reveal that pure cultures of staphylococci were killed by contact with bichloride of mercury 1:1,000 after 5 minutes; by cresol 3 per cent after 1 hour; by chloronal 5 per cent after 3 minutes; by antiformin 1 per cent after 1 minute; and by sodium hydrate 2 per cent after 5 minutes.

The destructive action of these substances on pure staphylococci may now be compared with that on the virus as it is ordinarily used in a protein medium. For greater ease of comparison the results of the latter experiments are summarized in Table 10.

Table 10 shows that the virus resisted the chemicals which caused protective coagulation of the proteins of its medium, but was killed within 1 minute by those which did not form a coagulum. By comparing the results given in Table 9 with those in Table 10 it will be noted that the active agent when not protected by coagula is still more sensitive to destruction by the same reagents, sodium hydrate and antiformin, than are staphylococci. Hence these tests confirm the prior conclusions with respect to the action of alcohol on the virus and on staphylococci. Furthermore, none of the effects of these reagents on the virus can be interpreted as an indication of its inanimate character.

SUMMARY

The virus of foot-and-mouth disease exhibits a remarkable resistance to such bactericidal agents as the narcotic solvents (alcohol, ether, and chloroform, or such antiseptics as phenol, bichloride of mercury, or cresol), as shown by tests made by others and the writers.

TABLE 10.—*Effect of certain chemicals on foot-and-mouth-disease virus* [1]

[Plus sign (+) signifies that virus was alive; minus sign (−) signifies that virus was dead]

Time	Bichloride of mercury 1:1,000	Cresol, 3 per cent	Chloronal, 5 per cent	NaOH, 2 per cent	Antiformin, 1 per cent	Control of virus
Minutes						
1	+	+	+	−	−	+
3	+	+	+	−	−	+
5	+	+	+	−	−	+
15	+	+	+	−	−	+
20	+	+	+	−	−	+
30	+	+	+	−	−	+
32	+	+	+	−	−	+
Hours						
2	+	+	−	−	−	+
4	+	+	−	−	−	+
6	+	+	−	−	−	+

[1] The virus was either in the form of lymph, diluted 1: 40, or admixed with, adherent to, or incorporated in small fragments of tissue. Six hours was the longest period tested.

The resistance of the incitant to these chemicals operates only under certain conditions. For example, some reagents coagulate the proteins of the medium in which the virus is, as a rule, suspended; one does not apply the chemicals to a pure culture of the virus, as is done in the case of cultivable microorganisms. As a consequence of this coagulation the virus is protected by the large coagula, owing to its minute size or to its positive charge, or to both factors. Hence the virus is held from direct contact with the chemicals. The writers showed that this coagulation can be prevented (as, in the case of alcohol, by adding a trace of sodium hydroxide). The virus is, under these conditions, acted upon directly by the antiseptics. The incitant is then as sensitive to destruction by the reagents as is the living staphylococcus. As a corollary, the active agent can be destroyed as quickly as staphylococci by substances which do not form coagula. Among the latter may be mentioned antiformin or sodium hydroxide in 1 per cent solutions. Either reagent kills the virus within 1 minute. It can not be stated definitely, therefore, that the resistance of the virus to chemicals is an indication of its inanimate nature.

EXPERIMENTS ON CULTIVATION

Mention has already been made of the fact that the lesions induced by foot-and-mouth-disease virus are free from ordinary bacteria. But before undertaking an extensive study on cultivation, it was believed desirable to add to the routine, morphological studies, and to the use of some ordinary means of culture, a large variety of media under different atmospheric conditions, different temperatures, and with different sources of the active agent. In this way the writers

thought that there might be revealed any constant, secondary, or concomitant microorganism.

Accordingly, a large number of experiments were made with the virus in the blood of actively infected guinea pigs, with filtered and unfiltered lymph, and with filtered and unfiltered, ground pad from guinea pigs and material from the mouths and feet of cattle. The materials were cultured in media at room temperature, also at 34° and 37° C. The atmospheric conditions varied from aerobic to anaerobic and in either condition plus the addition of carbon dioxide up to 25 per cent concentration. A variety of media, both fluid and solid, was used. Ascitic fluid with 5 per cent defibrinated, guinea-pig blood; ascitic fluid, calf-tongue broth; 5 per cent sheep blood, 1 per cent dextrose calf-tongue broth; and these media with 2 per cent agar. Agar plates with rabbit or sheep blood were also used.

No constant, visible microorganisms were seen in these artificial media, even after repeated subplants. Nor, in the absence of visible growth, were the writers able to obtain a pathogenic culture sufficiently diluted to eliminate actual transfer of the virus. These preliminary tests showed, therefore, that the ordinary methods appeared to be unsuitable for the cultivation of the virus. Another conclusion was that only the filtered pad material, or the blood, was useful for cultivation. The unfiltered, ground, infected, pad tissues or aspirated lymph were often admixed with the different bacteria normally present in the pads of guinea pigs or in the oral cavities of cattle, and the usual method of searing the surface was ineffective.

The commission's plan of study from this point was to make a deductive inquiry into the conditions which were best suited for the viability of the active agent in vitro. It was thought that this way of approach would yield more satisfactory results in the limited time available than that of applying at once special methods of cultivation. But before this could be done it was essential to investigate the findings of Frosch and Dahmen (*21*, *22*, *23*, *24*, *25*).

A widespread interest was aroused in 1924 when these writers announced the cultivation, by special methods of minute, bacillary bodies, *Loeffleria nevermanni*, which they maintained were the inciting agents of foot-and-mouth disease. Their colonies, as well as the bodies themselves, were visible only by means of short-wave length, ultra-violet photomicrography. Their method of culture consisted in centrifuging the virus materials (lymph) during a long period to concentrate the virus, and then washing the sediment in changes of salt solution to free the virus from inhibiting substances. The sediment was seeded on a solid, slope medium containing Martin broth, 3 per cent agar, to which was added, just before use, 20 to 50 per cent of horse or ox serum. The medium was adjusted to $P_H = 7.8$. Growth occurred aerobically after 7 to 10 days at 33° to 34° C. Suspensions of these subvisible colonies, even to the twenty-fifth generation, induced in guinea pigs experimental foot-and-mouth disease, albeit at times the reactions were rather feeble.

According to the statements of Frosch and Dahmen, prolonged centrifugation of the virus materials was a prerequisite for successful cultivation. The writers have already shown that this step in the technic does not concentrate the virus, nor can the so-called inhibiting substances be thereby removed. Furthermore, in repeated tests the virus failed to survive in the Frosch and Dahmen medium

after 3 days, although a sample of the same specimen of virus, seeded at the same time, remained viable for 7 days in buffered 10 per cent gelatin at $P_H=7.6$, and for 9 days in buffered phosphate at $P_H=7.6$. The writers will show later that broth is an unsuitable medium; this applies with equal force to the serum. In addition, the hydrogen-ion concentration and the aerobic conditions which are employed in this method were also found to be unsatisfactory.

The American commission, therefore, could not confirm the conclusions of Frosch and Dahmen, nor did it find in them, on analysis, any clew which might lead to a lengthening of the life of the virus under artificial conditions. In respect to this lack of confirmation of Frosch and Dahmen's results the American commission is in agreement with the German commission directed by Doctor Gins (*28*), who also found that in Martin broth serum-agar the virus dies in three days. The writers are also in accord with the work of the British committee (*3*, *35*, *63*). These investigators have shown that the so-called colonies and their constituents could be imitated by materials other than the virus of foot-and-mouth disease.

In view of the importance generally ascribed to the hydrogen-ion concentration of media in affecting growth or viability of microorganisms, the writers' earlier studies were directed to the optimum range required for the virus.

OPTIMUM HYDROGEN-ION CONCENTRATION

As the following illustrative protocol shows, variation in the hydrogen-ion concentration of the media containing the virus affected its viability.

PROTOCOL 13

The same specimen of virus, 24-hour-old aspirated lymph in phosphate buffer and filtered through a Berkefeld candle, was employed in all these tests; one-half cubic centimeter was inoculated into each 10 cubic centimeter of the medium to be stated.

(*a*) Buffered 10 per cent gelatin at $P_H=6.9$ after 5 days' incubation at 34° C; two guinea pigs injected intradermically. Both negative.

Buffered 10 per cent gelatin at $P_H=7.5$ after 5 and 7 days' incubation at 34° C; four guinea pigs injected, two each respectively with the 5 and 7 day incubated materials. All positive.

(*b*) Buffered agar one-fourth per cent at $P_H=8$ after 3 days' incubation; two guinea pigs injected intradermically. Both negative.

Buffered agar one-fourth per cent at $P_H=7.6$ after 3, 5, 7, and 9 days' incubation; eight guinea pigs injected, two each respectively with a portion of each specimen. All positive.

These instances do not show the extreme effect of variations of hydrogen-ion concentration on the preservation of the virus. In one case in 2 per cent dextrose, 5 per cent sheep blood, calf-tongue extract medium the latter survived 25 days longer at room temperature at $P_H=7.4$ than at $P_H=6$. In general, a large experience demonstrated that the optimum hydrogen-ion concentration best suited for the viability of the active agent in vitro is at $P_H=7.5$ to 7.6. Variations above or below this narrow range exhibit an unfavorable influence. The writers are thus in agreement with the findings of Stockman and Minett (*63*) and of Bedson and Maitland (*3*) of the British committee. Conversely, it became apparent from a number of tests, which need not be quoted here, that the phosphate buffer solution at $P_H=7.5$ or 7.6 could be used to greater advantage

as a base for the preparation of a wide variety of media used in tests of their preserving action than media not prepared on this base.

TEMPERATURE

The active agent is very sensitive to higher temperatures. The writers have already stated that the virus in guinea-pig blood survives after 24 but not 48 hours at 37° C. The following protocol exemplifies the advantageous effect of lower temperatures.

PROTOCOL 14

Virus material consisted of a 1 : 100 dilution of aspirated lymph in phosphate buffer, adjusted to $P_H=7.6$ and filtered through a Berkefeld candle. One portion was kept at 37° C., another at 32° to 34° and a third at 18° to 20° (room temperature). After varying intervals of time the materials were injected intradermically into guinea pigs. The virus retained at 37° survived for 3 days but not for 5; at 32° to 34° for 9 days but not for 11; and at 18° to 20° for at least 25 days. In addition, in this medium plus 50 per cent glycerol the virus remained alive indefinitely in the ice box (2° to 5°).

In another test with a more complex medium, the dextrose, sheep-blood, calf-tongue extract mentioned kept under anaerobic conditions, the virus survived therein for 6 days at 37° C., and for 25 but not for 32 days at from 18° to 20°.

From these and other observations to be described later it appeared that the virus of foot-and-mouth disease, like that of mosaic disease (*41*) and other filter passers, is sensitive to the higher but is quite resistant to the lower temperatures. At 37° C. the writers were unable to keep the active agent alive for more than 6 days. At 32° to 34° viability was maintained for 10 days; at 18° to 20° for from 2 weeks to over 2 months (in an instance to be described later), and at ice-box temperature, indefinitely. These observations concur in the main with those of the workers of the British committee.

OXYGEN TENSION

The importance of the question whether the life of the virus is favored by aerobic or anaerobic conditions was sufficiently realized. Hence a number of repeated tests in a variety of media were made, some of which are exemplified in the following.

PROTOCOL 15

Twenty-four-hour-old virus in the form of aspirated lymph, 1 to 20 cubic centimeters phosphate buffer at $P_H=7.6$, was filtered through Berkefeld candles. Of the filtrate 1 cubic centimeter was added to each 10 cubic centimeters of the medium which was always placed in Petri dishes so as to give it as much surface exposure as possible either to the aerobic or to the anerobic condition.

A medium of 10 per cent gelatin was prepared by Loeb's method (to be described later). After 5 days' incubation of this medium, guinea pigs were injected respectively with the aerobic and anaerobic materials. Those injected with aerobic subplant showed after 48 to 72 hours mild, primary lesions and 24 to 48 hours later similar, secondary vesicles. The others, injected with the anaerobic material exhibited within 24 hours severe primary vesicles and 24 hours later many secondary lesions. After 7 days' incubation, the aerobic subplants were inactive; the anaerobic could induce the typical experimental disease after 48 hours. After 10 days' incubation, the anaerobic material still showed activity in one of three guinea pigs.

The same general results were obtained when other media were employed.

These experiments reveal that under strict anaerobic conditions the virus retained its viability at least twice the length of time beyond that in an aerobic condition. The investigators of the British commit-

tee (*63*) report, however, that the state of air tension is of no importance from the standpoint of survival. The discrepancy in results can be ascribed to a difference of technic. The writers used not only Petri dishes for the tests but also the Boëz apparatus for producing anaerobic conditions (*10*). This mechanism, employing vacuum, consists of a central-post catalyzing station for the combustion of oxygen. It is leak proof and establishes a perfect and permanent anaerobic condition within five minutes. None of the methods employed by the British, such as capping tubes, or using a vacuum (Geryk) pump, or exhausting air with or without hydrogen, or boiling, then quickly cooling the medium, and later adding vaseline, are comparable to the Boëz apparatus [24] in promptness and effectiveness [25]. Indeed, in practically all their methods it is questionable whether a strictly anaerobic condition prevailed.

INFLUENCE OF DIFFERENT MEDIA

In the following experiments efforts were made to study the effect of different media on the duration of life of the virus. The work began with a series of substances which comprised the Frosch and Dahmen medium, namely broth, serum, agar, and also Witte and Martin peptones.

BROTH

The writers have already stated that the virus dies within three days in Martin's broth. No greater success was obtained after repeated trials with plain broth, buffered broth, tryptic broth, broth prepared with calf tongue instead of beef infusion, and beef infusion itself. In view of the fact that in other media, such as simple buffer phosphate or 10 per cent gelatin, the virus survived much longer under the same conditions of temperature (34° C.) and in parallel experiments, it is apparent that broth is noxious to the active agent. Furthermore, when materials—for example, 10 per cent gelatin—which in themselves were capable of maintaining the life of the virus for a considerable period, were added to broth, not only was the latter unimproved but the favorable action of the intended adjuvant was also lost. For example, in 10 per cent gelatin broth the virus did not survive after three days, but in the gelatin itself it remained viable after nine days.

Hence broth including the Martin variety may be considered not only an unfavorable medium but, moreover, a rapidly destructive agent to the virus.

The question naturally arose as to what rôle peptones played in this regard. They were therefore put to test.

PEPTONES

Two samples of peptone were available for study, Martin's and Witte's. The following protocol, presenting one of many experiments, exemplifies their action.

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[24] Further improvements of this apparatus such as were made use of in these tests are described by Boëz (*10, 11*).

[25] It was determined, for example, that *Bacterium pneumosintes*, a strict anaerobe, failed to grow by methods depending on the mechanical extraction of air per se. When the vaseline seal method is used the cooling of the medium permits a prompt reabsorption of air (*27*).

PORTOCOL 16

Aspirated lymph, 24-hour-old virus was diluted 1:50 in phosphate buffer at PH=7.5. This was filtered through a Berkefeld candle and 1 cubic centimeter of the filtrate was added to each 10 cubic centimeters of each of the media to be stated. All were incubated at 34° C.

Medium A comprised phosphate buffer at PH=7.5; medium B, the same plus 1 per cent Martin's peptone, and medium C, the phosphate buffer plus 1 per cent Witte's peptone.

After three days' incubation portions of each were inoculated intradermically into each of two guinea pigs, respectively. Within 48 hours all animals showed the typical primary and secondary lesions.

After five days' incubation, medium A could induce primary lesions within 24 hours; media B and C much weaker reactions after 48 to 72 hours.

After seven and nine days' incubation, medium A was still active in both of the two guinea pigs; media B and C inactive.

In addition to this experiment, another was made in which 1 per cent Martin's or 1 per cent Witte's peptone was added to buffered agar or buffered washed 10 per cent gelatin, thus making four distinct combinations.

The virus remained alive in the agar (0.25 per cent) for 9 days, but in the agar with Witte's peptone only 5 days, and in the agar with Martin's peptone only 3 days. The virus survived in the 10 per cent washed gelatin for 7 days, but in the gelatin with Martin's or Witte's peptone for 5 days. In the latter instances only one of two guinea pigs showed feeble reactions after injection.

This experiment demonstrates that Martin's and Witte's peptones were not only unfavorable for the preservation or viability of the virus, but that they also tended to exert a harmful effect when added to media which by themselves are favorable to preservation. In this action a counterpart is found in broth. Hence peptone is one of its constituents which is noxious. Since, however, the period of survival in peptone is somewhat longer than in broth, it appears then that there may be present therein another harmful agent. The writers have not yet been able to identify this element.

SERUM

What has been stated regarding the unfavorable effects of broth or peptone on the survival of the virus applies with equal weight to fresh, animal serum and also to human ascitic fluid. For example, the virus remained alive in buffered 10 per cent washed gelatin for seven days at 34° C., but in this medium with 50 per cent fresh, sheep serum, the same sample of virus was inactive after five days of incubation. Repeated tests have shown that fresh sheep or horse serum, or ascitic fluid added to substances such as agar or gelatin, which by themselves are favorable to the preservation of the virus, exert a virucidal effect. Serum or ascitic fluid also does not counteract the injurious action of broth. Hence these materials are valueless as adjutants in artificial cultures.

AGAR

Three per cent agar is the final ingredient of the Frosch and Dahmen medium. The writers have found that 2 to 3 per cent agar exerts a harmful influence on the virus, as the latter does not survive in this medium longer than three days whereas other media under the same conditions can maintain it for a much longer period. However, the authors' studies on the influence on lower concentrations of agar were extended with the hope that perhaps a semifluid, rather than a

solid medium, might be favorable. The next protocol illustrates the results obtained.

PROTOCOL 17

The virus employed in these tests consisted of aspirated lymph diluted 1:30 in phosphate buffer at $P_H=7.6$ and filtered; 1 cubic centimeter of the filtrate was added to specimens of agar of 10 cubic centimeters each. The agar was thoroughly washed in distilled water, buffered by the phosphate buffer (*11*) to $P_H=7.6$, and prepared in concentrations of 0.1, 0.25, and 0.5 per cent, respectively. After 5, 7, 9, and 11 days of incubation at 34° C., a portion of each of the inoculated agars was injected intradermically in at least two guinea pigs respectively for each test. The virus survived in the 0.1 and the 0.5 per cent agar for 7 but not for 9 days, and in the 0.25 per cent agar for 9 but not for 11 days.

The optimum was at 0.25 per cent of the concentrations of buffered, washed agar used. In this simple medium, the virus survived for nine days. But as already stated, the addition of 1 per cent, Witte's or Martin's peptone, 50 per cent beef maceration, 50 per cent fresh sheep serum, or 50 per cent ascitic fluid, shortened the viability period to five days or less. In addition further tests were made with 1 per cent lecithin, 1 per cent dextrose, 4.6 per cent glycerophosphate, and 2 per cent sodium citrate. When these latter substances were added respectively to the 0.25 per cent agar, the life of the virus was again shortened to less than five days.

It appears from the foregoing, furthermore, that the survival of the active agent depends on the simplicity of the medium in which it is contained—a principle amply confirmed by later tests.

The next step was to test the survival of the virus in 0.25 per cent agar at a lower temperature, namely 18° to 20° C.

PROTOCOL 18

The virus consisted of a filtrate of infected pads, removed 24 hours after injection, ground in phosphate buffer at $P_H=7.5$. The filtrate, the final dilution of which was 1:100, was injected intradermically into two guinea pigs, which promptly reacted with the typical primary and secondary vesicles. Thoroughly washed agar, 0.25 per cent, buffered with the phosphate buffer to $P_H=7.5$ comprised the medium. To each 10 cubic centimeters 1 cubic centimeter of the filtrate was added, and the mixtures were kept at 18° to 20° C. At intervals of 7, 12, 18, 24, 35, 52, and 76 days portions of the semisolid medium were injected intradermically into at least two guinea pigs. It was found that the specimens incubated for from 7 to 52 days were active in the animals, but that of the seventy-second day was inactive.

Subplants were made from the first series of inoculated media after seven days' incubation into fresh agar. This second transfer also showed activity. The second transfer after six days' incubation was subplanted to fresh, similar medium. The third was still active. The fourth subplant, however, failed to affect guinea pigs. This test of subplanting the virus was repeated after different periods of incubation and the results were always the same. Activity was shown in the first three transfers but not in the fourth, provided that the third transfer was within 52 days after the original seeding of the medium.

In these tests the commission also encountered a minute bacilloid microorganism which was filter-passing through Berkefeld V filters in the first but not in later generations. It also grew in peptone and beef maceration, semifluid agar. In transferring this microorganism, an admixture of it with some of the virus medium was employed, as it was impossible to obtain a pure culture. Material containing this microbe was also infectious in the third subplant but not in the fourth.

From the foregoing one may conclude that 0.25 per cent agar is a favorable medium for the preservation of the virus; at least, it is not toxic to the active agent, for it can survive therein for 52 days at 18° to 20° C. In view of later experiences with gelatin, which

acted still more favorably than agar, it appears that the beneficial effect of the latter is due to its physical property of providing a semisolid structure to the medium.

This experiment also demonstrates that activity in three successive subplants was obtained not only with the virus but also with a microorganism for which there was sufficient evidence to indicate that it was merely concurrent with the particular sample of virus studied, and that it could not be identified as the active agent itself. But the fourth subplant in both these cases failed. Since the fourth transfer is the critical one (see "Titration of the virus"), for in this the virus is diluted beyond its original limit of activity, the infectivity of both the virus and the microorganism—which was maintained not in pure culture but admixed with the virus in the medium—can be interpreted as an expression of preservation but not of multiplication.

GELATIN

The advantage of 10 per cent gelatin, which forms a semisolid medium, over other substances as a preservative of the virus soon became apparent and the writers were thus led to an extensive study of this material.[26]

It is well known that gelatin contains, as impurities, metals such as arsenic and copper and a considerable concentration of acids. A comparative test was made first with the same sample of virus added to the ordinary Poulenc 10 per cent gelatin and this substance thoroughly washed in distilled water. Both media were buffered at $P_H=7.6$. The active agent survived in the unwashed gelatin for about three days but in the washed, seven days.

The next effort concerned the probability of prolonging the preserving action of the washed gelatin by adding to it a number of organic or protein substances:

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To different lots of 10 per cent washed gelatin prepared on the phosphate buffer base at $P_H=7.6$ were added, respectively, an equal part of calf-tongue broth; or 1 per cent Martin's tryptic, or Witte's peptones; or 0.5 per cent aspargin; or 2 per cent dextrose, or an equal part of fresh sheep serum. As a control medium, buffered, washed agar 0.25 per cent was employed. To each of these substances was added 24-hour-old aspirated lymph virus diluted 1:30 in the phosphate buffer, $P_H=7.5$, and filtered. After varying periods of incubation at 34° C., portions from each were removed and injected intradermically in guinea pigs. The virus survived in the agar for 9 days, in the washed gelatin for at least 7 days, but in the mixture of organic and protein substances with the washed gelatin not longer than 3 to 5 days.

Thus, as in the case of agar, the addition of protein or other organic substances has a distinctly deleterious effect; the virus requires for its life only the simplest material. From this point studies were therefore made on the effect of the gelatin alone.

The writers then used a more thoroughly purified gelatin, prepared after the manner of Loeb for making isoelectric gelatin (*48*). The method of preparation was as follows:

Fifty grams of Poulenc powdered gelatin were added to 3 liters of M/128 acetic acid at 10° C. and stirred frequently. After 30 minutes the supernatant liquid

[26] The protein gelatin also is favored by its simplicity of composition. Its minimal molecular weight is estimated at 10,300 and that of hemoglobin at 50,000. For comparison, furthermore, the minimal molecular weight of egg albumen is stated as 33,800, of fibrin 42,000, of serum globulin 81,000, and of casein 192,000 (*12*).

was decanted, and fresh M/128 acetic acid at 10° (or in the cold room) was added to equal the original volume. The mass was frequently stirred and after a half hour the acid was again decanted and replaced by an equal volume of distilled water at 5°. The gelatin was well stirred and then filtered by suction in a Büchner funnel. It was then washed in the funnel five times with 5 liters of cold water. After all the water was drained off the gelatin was removed and suspended in the buffer phosphate to a concentration of 10 per cent. It was then heated in the autoclave for sterilization on three successive days for one hour at 100° and the PH was adjusted to 7.6 between the heatings and after the final sterilization.

The writers then compared the effect of this buffered Loeb gelatin with buffered 0.25 per cent agar and found that the former permitted the survival of the virus for a much longer time than the agar. For example, in one experiment the same sample of virus remained alive in the gelatin for 11 days at 34° C., but in the agar for less than 5 days. Hence the Loeb gelatin was not only superior to that washed by ordinary methods, but it also surpassed agar.

The Loeb gelatin was simplified still further by omitting the buffer phosphate solution and adjusting the material to PH=7.5 or 7.6 merely with potassium hydrate. Comparative tests revealed that the "adjusted" gelatin maintained the virus for longer periods than the buffered, at either 34° or 18° to 20° C., and under either aerobic or anaerobic conditions.

In the adjusted Loeb gelatin the virus was kept alive for a longer period than in any of the other media tested. This period was longer than 69, but shorter than 100 days, when the material was kept at room temperature (18° to 20° C.). In a parallel experiment, the active agent remained viable in 0.25 per cent agar for 45 days.

In this medium three successive subplants showed activity, as in the case with the agar. But this fact is regarded as merely indicative of preservation of the virus rather than of its multiplication, for the fourth subplants—the critical ones, the ones in which the virus is diluted beyond its original limit of activity—were uniformly negative.[27]

At this point, when the writers had developed the optimum medium for the preservation of virus, namely, the adjusted Loeb gelatin, the limitation of time compelled the discontinuance of the tests.

While these experiments were in progress the commission attempted the cultivation of the active agent in a variety of other special media and by different methods. These are reported here, because of their general interest.

MISCELLANEOUS CULTIVATION TESTS

In Smith-Noguchi medium the virus was incubated at 32° to 34° C., and subplants were made after three days. Material of the first subplant induced the typical, experimental disease in all of three guinea pigs injected, but that of the second and of the third successive transfers was inactive. In a similar medium without the vaseline seal and incubated at 32° in an atmosphere of 10 per cent carbon dioxide, combined with complete anaerobiosis, identical results were obtained. In the Smith-Noguchi medium, therefore, the virus is preserved for about three days, or in the first subplant. But no evidence has been obtained to indicate that the active agent can reproduce itself therein.

Tissue cultures

In tissue cultures made after the manner of Borrel, somewhat better results were obtained. These cultures were made with chicken plasma, chicken or guinea-

[27] The third subplants were maintained at a temperature of 34° C., as well as at 18° to 20° C. In each case the medium preserved the virus.

pig embryonic juice, and with fragments of embryonic epithelium. In these experiments it was possible to maintain the life of the virus through the second subplant, or for four days. The third and subsequent successive transfers, however, were uniformly negative. In the later transplants the writers frequently encountered secondary contaminations, so that the results of tissue culture are inconclusive.

Adjusted Loeb 10 per cent gelatin: In this medium still better results were obtained. At from 32° to 34° C. the virus was found in three successive subplants—in other words, in a period of nine days from the time of seeding. But later transfers were negative. Hence no multiplication was noted here as well. Efforts were made to employ this medium in a 10 per cent carbon-dioxide atmosphere but this method proved less favorable—the first subplant was the only one positive. The addition of a fragment of fresh, rabbit kidney to this gelatin showed no better results, but this is additional proof that the requirements of the virus for life postulate the simplest structure of the medium. Whether the authors' experiences with this medium can be interpreted to indicate that the conditions of preservation are distinct from those of multiplication, or whether the medium can be still further improved to the point of making it a basic material for successful artificial cultivation, remains an open question.

CONCLUSIONS

No multiplication of the virus in vitro was observed. The commission found, however, that the optimum conditions necessary for the preservation of the virus in artificial media are as follows:

The hydrogen-ion concentration of the medium should be 7.5 to 7.6 not only at the beginning but, and more important, at the conclusion of the period of observation. A strict anaerobic condition is also favorable, as is a temperature below 37° C. A semisolid structure of the medium appears to be advantageous and this can be effected by the use of one-fourth per cent agar or 10 per cent gelatin. Of the two, gelatin is more desirable, and the most effective preparation is gelatin prepared after the manner of Loeb and adjusted to the proper hydrogen-ion concentration with potassium hydroxide. Gelatin is the simplest of protein media available and its employment is in keeping with the principle that the virus requires only a simple material for life. The addition of organic substances or of proteins causes a destruction of the active agent.

From the standpoint of technic, it was found necessary when comparing two or more media for their effectiveness to employ all of them in a parallel experiment with the same sample of virus, since the factors of potency of the active agent, contamination, and changes in the hydrogen-ion concentration, if variable, may give rise to faulty interpretations. Furthermore, activity in three successive subplants may be regarded as mere preservation but not multiplication of the virus.

Finally, the commission consistently failed to confirm the results of Frosch and Dahmen in respect to their reported artificial cultivation of virus.

MISCELLANEOUS EXPERIMENTS

The writers now report experiments of a diverse nature, some more or less fragmentary, thus completing the laboratory investigations of the virus.

RESPIRATION OF THE VIRUS

A study of this problem was undertaken with the idea that the living nature of the virus could be confirmed. In one test an attempt was made to measure the respiration of the active agent in the blood or tissues by changes in hydrogen-ion concentration; in another, by

changes in the oxygen, carbon dioxide, and nitrogen volumes during its infective periods. In the end these experiments were discontinued for want of a suitable control; the virus could not be separated in pure state from respiring living tissues.

INTRADERMIC REACTIONS

An effort was made to concentrate the virus by adsorbing it to coagula which are formed by 50 and 60 per cent alcohol, and in undiluted acetone. It was believed that by so doing a sufficiently potent material could be obtained which on injection intradermically might yield a difference of reaction between animals with the active disease and those normal or recovered from the experimental disease. It was thought that perhaps ultimately a diagnostic skin test might be evolved.

To summarize a large number of experiments: The virus after immersion in 50 per cent alcohol for 26 hours was still active. The coagulum which formed was centrifuged at the end of that time, washed, and suspended in physiological, saline solution to opacity to a beam of light through 3 centimeters of the suspension. After injection intradermically into a shaved area of the abdomen 8 guinea pigs with the active, experimental disease showed a somewhat greater induration and discoloration than 6 normal and 6 recovered animals. These reactions began after 48 hours and persisted for two to three days. This experiment was then performed on heifers; 3 actively diseased, 3 recovered, and 1 normal of these animals were injected intradermically with the same material and in similar amounts (0.2 cubic centimeter). There were practically no reactions. It is to be noted that the dose employed in the heifers is not in the same proportion to body weight as that in guinea pigs.[28]

With 60 per cent alcohol and 26 hours' contact precisely similar results were obtained with active material in the same proportion of guinea pigs. Cattle were not used in this test.

On the other hand, in undiluted acetone, immersion of the virus for one-half hour led to its destruction. The coagulum prepared as in the case with the 50 per cent alcohol showed practically no definite reaction in 10 actively diseased, 6 normal, and 6 recovered guinea pigs.

The virus after immersion in 60 per cent alcohol for three days was also inactive. Intradermic injections of this inactive material yielded practically no reactions in 4 actively diseased, 4 normal, and 4 recovered guinea pigs.

In conclusion it may be stated that if the material employed for the skin tests is active, there is, as a rule, a slightly more prominent reaction in guinea pigs at the height of the experimental disease than in those which are normal or recovered. But in a few instances the reactions in the normal or recovered animals were as pronounced as in some of those in the active stages of the disease. On the other hand, when the material for injection is inactive practically no reactions are exhibited. Hence, while these findings tend to show a certain sensitiveness of the skin of guinea pigs with active experimental foot-and-mouth disease to the living virus, there is no practical value in these tests as a diagnostic aid.

[28] The normal heifer, although injected intradermically with 0.2 cubic centimeter of active virus was not thereby immunized against a later injection in the mucosa of the mouth with a larger dose of virus.

EARTHWORMS AS CARRIERS

In a single experiment 12 earthworms from 4 to 12 centimeters in length were placed in garden earth kept in a clay vessel. To the dried earth, contained in an area of a 16-centimeter cube, were added aspirated, lymph virus taken 24 hours after inoculation, diluted 1 to 10 in phosphate buffer at $P_H = 7.5$. Of this diluted virus 200 cubic centimeters were used. It is to be noted, therefore, that the worms were in a pabulum of strongly concentrated virus.

Evaporation was prevented by covering the container, which was kept at 9° C. so as to favor the preservation of the virus. After 5 and 10 day intervals living worms were removed and washed thoroughly. Thick suspensions of worms ground in sterile sand, their washings in distilled water, and suspensions of the earth were injected intradermically in the hairless pads of guinea pigs. Only the suspensions of the earth but not the other materials induced the typical, experimental disease. At this point the test was discontinued with the inference that earthworms probably do not harbor the virus.

SURVIVAL OF THE VIRUS IN EARTH

Dried, garden soil was saturated with virus in a dilution of 1 : 10. The latter consisted of aspirated lymph from 24-hour-old vesicles and was free from tissue particles. The mixture was kept at 9° C. for 10 days and thereafter at 20° C. For 15 days the earth was moist and thence it was permitted to dry so that from the twentieth day it was sensibly dry.

Five grams of this material were removed and suspended in 25 cubic centimeters of phosphate buffer at $P_H = 7.5$, at intervals of 5 days, and of this, 0.4 cubic centimeter was injected intradermically and subcutaneously into the hairless pads of guinea pigs. The suspensions of earth after 5, 10, 15, 20, and 25 days induced in the animals typical, experimental foot-and-mouth disease. At this point the experiment was discontinued.

The commission found, therefore, no evidence to sustain the earlier experimenters in their opinions that the virus dies very rapidly outside of the animal body. Under the varying conditions described, the active agent was found to be viable for at least 25 days, and if conditions in nature could be more closely imitated the period of preservation in earth might prove to be much longer. In another experiment described elsewhere in this report the virus in a fragment of bovine tissue admixed with hay and kept in a stall survived for at least 30 days. Further tests in this case were not made because of exhaustion of material.

THE PARTICULATE AND LIVING CHARACTER OF THE VIRUS

The commission considered the questions which have often been discussed as to whether the filter-passing virus of foot-and-mouth disease is an inanimate chemical material or a multiplying, living body, or whether it is fluid, as postulated by the theory of contagium vivum fluidum (Beijerinck) (7) or particulate. A justification of experiments on artificial cultivation rests on the assumption that the virus may be particulate and living.

With reference to the theory of contagium vivum fluidum, it appears that the definition of this term is not in accord with the

modern concept of the particulate structure of matter. The burden of proof that fluids, in general, are not corpuscular rests, then, on the partisans of this theory. Apart from this the commission has presented what it believes to be sufficient evidence that the active agent of foot-and-mouth disease is particulate, for by repeated and controlled filtration tests it has not only delimited measurement of individual, active masses as relatively between 20mμ to 100mμ but also found that this relative size is constant. The invariability of the limits of size contradicts, therefore, the notion that the incitant may be a solute varying in size in different solvents. The electric charge carried by these particles, has, moreover, been determined by cataphoresis which can effect their separation from the protein substances in the medium containing virus.

The activity of the living character of the virus in extraordinarily high dilutions, its inability to sediment by means of centrifugation, and its remarkable resistance to such bactericidal agents as the narcotic solvents (alcohol, ether, and chloroform), or such antiseptics as phenol, cresol, and bichloride of mercury, tend to support the idea that it is inanimate, or a chemical substance.

The commission believes, nevertheless, that nothing has yet been brought forward in these respects to prove conclusively that the active agent is inanimate. The factors just mentioned may represent reactions consequent upon its minuteness of size or its electropositive charge, or both. It should be remembered that minute particles react to physical laws in a manner different from that of larger structures (*73*). For example, internal pressure, surface tension, and charge exert profound effects. However this may be, if each of the factors mentioned as opposed to the notion of a living nature of the incitant is analyzed, as will be done immediately, it will be noted that none are incompatible with this idea.

The commission found that active materials are still infective in a dilution of 1:10,000,000. This can be interpreted as merely indicating the minute size of the active agent although it is sufficiently large in volume to contain from several hundred to several thousand protein molecules. On the other hand, the action of the virus stops at a definite dilution and is directly proportional in respect to the length of incubation period and severity of symptoms upon its concentration. The writers have not encountered any effect similar to that noted in certain enzymatic actions, of nullifying antibodies in low dilutions.

As for the inability to depose the virus in a fluid medium by centrifugation, it has already been explained that this method is not useful for sedimenting minute particles—whirling and convection currents and other forces hinder the deposition. This property is, therefore, also a consequence of its size and does not indicate, by itself, a fluid material.

The resistance to chemical reagents is really illusory, since these reagents coagulate the proteins of the medium in which the virus is as a rule suspended. As a result the virus is protected by the coagulum and direct contact with the reagent becomes impossible. If coagulation is prevented, which can be done, the virus is then acted upon directly by the antiseptics. The incitant is then more rapidly destroyed by the chemicals than are living bodies, such as staphylococci.

Finally, among analogies to living microorganisms, may be mentioned the epidemiological factors, the existence of at least two distinct types of virus, and the more or less solid immunity which is induced, and a period of incubation which can last, at times, 12 days. Studies of these conditions are described in detail in another section of the report. On the other hand, if the virus is a living body, it appears to be of an order wholly different from that of known, cultivable microorganisms. For, aside from its electropositive charge, deductive study of its requirements for artificial life indicates that the metabolism of the incitant is of a very simple kind and is quite limited to definite and inflexible conditions.

The commission, therefore, concludes that nothing has yet been presented to prove that the virus of foot-and-mouth disease is of a fluid character or inanimate nature.[29]

SUMMARY OF PHYSICAL AND CHEMICAL PROPERTIES

This section of the report deals with the results of studies on the physical and chemical properties of the virus of foot-and-mouth disease and includes a description of efforts at cultivation. Of the commission's personnel, Doctors Olitsky and Boëz were mainly interested in these problems and Doctors Schoening and Traum in the work described in the remainder of the report. However, there was a free interchange of ideas and suggestions and a hearty cooperation among all the colleagues, so that the entire report may be regarded as the result of collaboration as a unit.

A strain of foot-and-mouth-disease virus was recovered from a cow at the height of the disease and was propagated through at least 261 passages in the guinea pig. Considerably more than 2,000 of these animals were sensitive to the virus, which could be transferred at will back to cattle and hogs and then again returned to guinea pigs. No natural immunity in guinea pigs could be determined. Secondary lesions were easily and regularly induced, thus making this strain particularly favorable for experimental purposes. In general, the guinea pig, therefore, may be regarded as the animal of choice for laboratory studies. The virus was shown in these passage experiments to be identical with the Vallée O type, and was characterized by its freedom from ordinary cultivable microorganisms and its tendency to purify itself of chance concomitant bacteria in the first passages through a susceptible animal, a character generally possessed by filter-passing viruses.

The guinea pig can be infected by different methods of injection in different sites, but constant and regular production of primary and secondary lesions follows intradermic "tunneling" combined with subcutaneous inoculation of the posterior hairless pads of full-grown animals.

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[29] This problem is still being studied by one of the writers (Olitsky), but with the virus of vesicular stomatitis of horses. This virus is similar in clinical manifestations and in many biological reactions, to be reported later, to those of foot-and-mouth disease. In a recent collaboration with F. L. Gates, of the Rockefeller Institute, it was found that the virus of vesicular stomatitis is destroyed by the same limits of energy and wave length of ultra-violet light which can kill staphylococci. "Since the absorption of specific energies is one index of chemical character, and in this instance results in similar effects, these parallel reactions are indirect but suggestive evidence that the substance of the virus is similar in character and chemical constitution to bacterial protoplasm" (*59*).

The virus can be active in a dilution of 1:10,000,000. This indicates not only the minuteness of the active agent but also the necessity for a change of technic from that used with larger-sized infectious agents. Apart from this, the dilution factor is important in interpreting mere preservation of the virus rather than multiplication when only early successive subplants in culture experiments are positive. On the other hand, some samples of virus are not so active—among 15 specimens titrated, the weakest showed one-twenty-fifth of the activity of the strongest. This demonstrates that comparative tests—as, for example, of survival in different media—should be made with the same sample. In any case the rate and energy of action of the virus are proportional to its concentration.

The active agent is not sedimented by centrifugation. This may not indicate its fluid nature, but, rather, in view of other evidence presented, its minute size. This method has also failed to remove virucidal bodies in the sense used by Frosch and Dahmen.

Cataphoresis experiments demonstrate that the virus carries an electropositive charge and that its isoelectric range centers at the high point of P_H = about 8. Although cultivable bacteria are as a rule electronegative, yet certain protozoa, such as trypanosomes and spirochetes, are also electropositive. However, a knowledge of the charge carried by the virus is important among other reactions in interpreting filtration phenomena and in delimiting its relative size. Moreover, cataphoresis can effect the separation of active material from the protein particles in the medium containing virus.

Filtration experiments were made with different types of filters—Seitz, Berkefeld V and N, and Chamberland, of practically all sizes. The results confirm the electropositive charge of the virus as well as the minuteness of its size. Filtrations were positive through all filters except Chamberland L 11. In this bougie, however, the virus will pass through when its charge is shifted to a negative condition. Filtrations through electronegative, collodion membranes prepared in different ways and of different thicknesses resulted, as a rule, in failure, unless the thinnest and most permeable membranes were employed; but in these, the complication of microscopic holes was to be considered. Hence, this method was regarded as impracticable. Success, however, was obtained with Bechhold's ultrafilter membranes of the most permeable type. With these it was possible to measure, by a system of molecular filtrations, the relative size of the active masses. This was determined to be between 20mμ and 100mμ in diameter.

The commission confirmed previous investigations with regard to the remarkable resistance of the virus to chemicals, but showed that this resistance is merely masked. The virus is not employed in these tests as a pure culture but admixed with the animals' proteins. The addition of those chemicals to which the active agent is resistant causes a coagulation of the proteins, with the consequence that the virus is fully protected by the large coagula. But if this coagulation is prevented by taking advantage of the periodic type of this process, the virus can be placed directly in contact with the reagents. Then the active agent is destroyed as rapidly as staphylococci. Chemicals, such as sodium hydroxide and antiformin, which do not cause

coagulation, are very destructive, and these also are as active on the virus as on staphylococci. The commission can not subscribe therefore to the opinion that this remarkable resistance of the virus admixed with proteins is an indication of its inanimate nature.

A large series of experiments have led to the conclusion that of a number of antiseptics employed, the simple sodium hydroxide in 1 to 2 per cent solutions is an effective virucide. It is capable of killing the virus within one minute, as shown by tests on cattle and guinea pigs. Furthermore, its effectiveness is not diminished even when the infective material is admixed with cattle urine, manure, or earth. The experimental evidence suggests its use in field practice.

It was found that the skin of guinea pigs in the active stages of the experimental disease is slightly more sensitive to the living active agent than is that of normal or immune animals. But no practical, diagnostic, skin test for detection of the disease, or immunity thereto, could be developed.

The commission could not implicate earthworms as carriers of the virus.

The causal factor of foot-and-mouth disease can survive in earth or hay for at least 25 to 30 days—longer periods were not tested. Thus, contrary to the opinion prevailing in some laboratories, it appears that the virus does not die rapidly outside the animal body.

The commission's efforts at cultivation were unsuccessful, but a deductive study of the prerequisites for the survival of the virus in artificial media has demonstrated several noteworthy conditions: The hydrogen-ion concentration of the medium is at its optimum at $P_H = 7.5$ to 7.6. A strict anaerobic condition favors survival. The temperature requirement is less than 37° C. A semisolid structure of the medium is also favorable and for this purpose either 0.25 per cent agar or 10 per cent gelatin can be used, but the latter is the more effective. Of the preparations of gelatin, the one of choice is that from which the impurities have been most thoroughly removed, namely, the gelatin employed by Loeb for his isoelectric determinations. The commission found also that the requirements for life of the active agent are of the simplest. The addition of organic or protein substances such as dextrose, broth, serum, lipoids, etc., to a simple, basic medium interferes with the effectiveness of the latter. It is thus not surprising that the writers were unable to confirm the cultural results of Frosch and Dahmen, for neither their medium and its components, nor their method satisfies the essential conditions necessary to maintain artificially the life of the virus.

PLURALITY OF TYPES OF FOOT-AND-MOUTH-DISEASE VIRUS

In 1922 Vallée and Carré (*69*) reported that cattle recovered from natural or artificial infection with foot-and-mouth-disease virus, type O, derived from the French Department Oise, were immune to this same type. They were, however, susceptible to reinfection either by contact with or by injection of virus originating in Germany (Allemand type A). Vallée and Carré (*70*) also demonstrated later that cattle recovered from infection with type A virus were immune to that virus but were susceptible to type O virus. Here were two viruses, each of which could protect against its own effects but not

against those of the other. This finding explained, at least in part, the many cases of reinfection, and the failure to immunize by methods which have hitherto had certain value in procedures of immunization.

The existence of more than one type of foot-and-mouth-disease virus was not at first generally accepted (*76*). Plurality of virus has an important bearing on the interpretation of results of experimental work and also on the control of the disease.

Investigation of the correctness of Vallée and Carré's work was therefore one of the first subjects to receive the commission's attention. At that time there were available cattle, swine, and guinea pigs which had recovered from experimental infection with the Strasbourg virus. Professor Vallée furnished the commission with samples of type A and O viruses in the form of defibrinated blood. The writers started with the assumption that the Strasbourg virus would react in the same manner as Vallée's type O. This was soon found to be correct, for early experiments indicated that the Strasbourg virus was not like Vallée's type A in the immunity it produced—animals recovered from infection with Strasbourg virus were susceptible to type A. The commission did not use Vallée's strain of type O in its subsequent studies but substituted for it the Strasbourg strain. This virus, therefore, was classed as type O after comparison with type A.

Either type of virus readily induces experimental foot-and-mouth disease in cattle, swine, and guinea pigs. The reactions of these animals to the two types show no distinguishing features. Neither induces the disease in the horse. The determination of plurality of foot-and-mouth-disease viruses is based on cross-immunity tests. In addition to Vallée's type A and the writers' Strasbourg virus, a sample from the island of Riems was also used in the tests. This sample was obtained through the courtesy of Professor Waldmann and when received had been passed in series through 1,271 guinea pigs. The strain was highly virulent for guinea pigs; extensive lesions were produced with a high mortality. It served a useful purpose in the following experiments as a rigid test for the completeness of immunity, since its enhanced virulence might break down any feeble resistance.

CROSS-IMMUNITY TESTS IN CATTLE, SWINE, AND GUINEA PIGS

CATTLE

The cross-immunity tests in cattle are recorded in Table 11. For convenience of presentation the data in that table are further tabulated and discussed under two groups, (1) the resistance of animals recovered from each virus to later exposure to one or more viruses, and (2) the effect of each virus on animals recovered from infections with one or more viruses.

TABLE 11.—*Results of inoculations of cattle and hogs bearing on plurality of types of foot-and-mouth-disease virus*

Kind and No. of animal	Date of inoculation	Type of virus	Source of virus	Method of exposure	Result [1]
Heifer 1	Sept. 30, 1925	Strasbourg	Guinea pig, 79th passage.	Intramuscular	Positive.
	Oct. 26, 1925	Vallée A	Vesicle coverings from heifer 2.	Local—scarification [2]	Do.
	Nov. 20, 1925	Strasbourg	Guinea pig, 123d passage.	do	Negative.
	Jan. 4, 1926	Vallée A	Guinea pig	do	Do.
Heifer 2	Sept. 20, 1925	Strasbourg	Heifer 10	Natural (heifer 10)	Positive.
	Oct. 16, 1925	Vallée A	Blood of cow 368, received from Vallée.	Intramuscular	Do.
	Nov. 26, 1925	Strasbourg	Guinea pig, 123d passage.	do	Negative.
Heifer 3	Sept. 17, 1925	do	Guinea pig, 67th passage.	Local—scarification	Positive.
	Nov. 25, 1925	do	Guinea pig, 123d passage.	do	Negative.
	Feb. 2, 1926	do	Guinea pig, 178th passage.	do	Do.
Heifer 4	Sept. 30, 1925	do	Guinea pig, 79th passage.	Intradermic—gums	Positive.
	Dec. 9, 1925	Rioms	Guinea pig	Local—scarification	Negative.
	Jan. 13, 1926	Strasbourg	do	do	Do.
	Feb. 3, 1926	Vallée A	do	do	Positive.
Heifer 5	Oct. 26, 1925	do	Heifer 2	do	Do.
	Nov. 26, 1925	Strasbourg	Guinea pig	do	Do.
	Jan 4, 1926	Vallée A	do	do	Negative.
Heifer 6	Sept. 17, 1925	Strasbourg	do	Intradermic—pad and lip.	Positive.
	Oct. 31, 1925	Vallée A	Heifers 1, 2, and 5; and hog 3.	Local—scarification	Do.
	Nov. 26, 1925	Strasbourg	Guinea pig	do	Negative.
	Jan. 4, 1926	Vallée A	do	do	Do.
Heifer 7	Sept. 17, 1925	Strasbourg	Guinea pig, 67th passage.	do	Positive.
	Dec. 9, 1925	Rioms	Guinea pig	Intramuscular	Negative.
	Jan. 8, 1926	Vallée A	Heifers 2 and 12; and hogs 4 and 5.	Local—scarification	Positive.
Heifer 8	Sept. 30, 1925	Strasbourg	Guinea pig	do	Do.
	Dec. 9, 1925	Rioms	do	do	Negative.
	Jan. 13, 1926	Strasbourg	do	do	Do.
	Feb. 2, 1926	do	do	do	Do.
Bull 9	Sept. 17, 1925	do	Guinea pig, 67th passage.	Intramuscular	Positive.
	Nov. 26, 1925	do	Guinea pig	Local—scarification	Negative.
Heifer 10	Sept. 25, 1925	do	Bull 9	Natural (bull 9)	Positive.
	Sept. 28, 1925	do	Guinea pig	Intramuscular	Negative.
	Jan. 8, 1926	Vallée A	Heifers 2 and 12, and hogs 4 and 5.	Local—scarification	Positive.
Heifer 11	Nov. 26, 1925	Strasbourg	Guinea pig	do	Do.
	Jan. 4, 1926	Vallée A	do	do	Do.
Heifer 12	Nov. 26, 1925	Strasbourg	do	Intramuscular	Do.
	Jan. 4, 1926	Vallée A	do	do	Do.
Heifer 13	Dec. 9, 1925	Rioms	do	Local—scarification	Do.
	Jan. 7, 1926	Vallée A	Heifers 11 12	Natural (heifers 11 and 12).	Do.
Heifer 14	Jan. 15, 1926	do	Guinea pig	Local—scarification	Do.
	Feb. 3, 1926	do	do	do	Negative.
	Apr. 14, 1926	Strasbourg	do	Intramuscular	Positive.
Heifer 15	Jan. 15, 1926	Vallée A	do	Local—scarification	Do.
	Feb. 2, 1926	Strasbourg	do	do	Do.
Heifer 16	Mar. 3, 1926	Vallée A	do	do	Do.
Heifer 28	May 7, 1926	Strasbourg	Heifers 37 and 38	Natural (heifers 37 and 38).	Do.
	June 10, 1926	Vallée A	Guinea pig	Local—scarification	Do.
Hog 1	Sept. 30, 1925	Strasbourg	Guinea pig, 79th passage.	do	Do.
	Nov. 9, 1925	Rioms	Guinea pig	Intravenous	Negative.
	Jan. 8, 1926	Vallée A	Heifers 11 and 12; and hogs 4 and 5.	do	Do.
	Jan. 15, 1926	do	Guinea pig	do	Do.
Hog 2	Sept. 30, 1925	Strasbourg	Guinea pig, 79th passage.	do	Positive.
	Nov. 9, 1925	Rioms	Guinea pig	do	Negative.
	Jan. 8, 1926	Vallée A	Heifers 11 and 12; and hogs 4 and 5.	do	Do.
	Jan. 15, 1926	do	Guinea pig	do	Do.

[1] Normal cattle and hogs were used to control the activity of the virus.
[2] The virus was applied to a scarified area on the gum and dental pad.

TABLE 11.—*Results of inoculations of cattle and hogs bearing on plurality of types of foot-and-mouth-disease virus*—Continued

Kind and No. of animal	Date of inoculation	Type of virus	Source of virus	Method of exposure	Result
Hog 3	Oct. 26, 1925	Vallée A	Heifer 2	Intravenous	Positive.
	Nov. 26, 1925	Strasbourg	Guinea pig	do	Do.
	Jan. 4, 1926	Vallée A	do	do	Negative.
Hog. 4	Nov. 26, 1925	Strasbourg	do	do	Positive.
	Jan. 4, 1926	Vallée A	do	do	Do.
Hog 5	Nov. 26, 1925	Strasbourg	do	do	Do.
	Jan. 4, 1926	Vallée A	do	do	Do.
Hog 6	Jan. 15, 1926	do	do	do	Do.
	Feb. 3, 1926	do	do	do	Negative.
Hog 7	Jan. 15, 1926	do	do	do	Positive.
	Feb. 3, 1926	do	do	do	Negative.
Hog. 8	Jan. 15, 1926	do	do	do	Positive.
	Feb. 2, 1926	Strasbourg	do	do	Do.
Hog. 9	Feb. 3, 1926	Vallée A	do	do	Do.
	Apr. 14, 1926	Strasbourg	do	Local and intravenous	Do.

It appears from Table 12 that the Strasbourg virus can induce immunity against reexposure to itself and also to Riems virus, but not to Vallée type A.

TABLE 12.—*Results of exposing cattle recovered from Strasbourg-virus infection to that virus, Vallée A, and Riems virus*

Positive results	Virus used	Negative results	Virus used
Heifer 1	Vallée A	Heifer 3	Strasbourg.
Heifer 2	Do.	Heifer 4	Riems.
Heifer 6	Do.	Heifer 7	Do.
Heifer 11	Do	Heifer 8	Do.
Heifer 12	Do.	Heifer 9	Strasbourg.
Heifer 28	Do.	Heifer 10	Do.

TABLE 13.—*Results of exposure to Strasbourg virus of animals recovered from infection with that and with other viruses*

Positive results	Previous exposure to—	Negative results	Previous exposure to—
Heifer 5	Vallée A virus.	Heifer 1	Strasbourg and Vallée A viruses.
Heifer 14	Do.	Heifer 2	Do.
Heifer 15	Do.	Heifer 3	Strasbourg virus.
		Heifer 4	Strasbourg and Riems viruses.
		Heifer 5	Vallée A and Riems viruses.
		Heifer 6	Strasbourg and Vallée A viruses.
		Heifer 8	Strasbourg and Riems viruses.
		Heifer 9	Strasbourg virus.
		Heifer 10	Do.

Table 13 shows that the Strasbourg virus caused infection in three animals which had first received only Vallée A. On the other hand, Strasbourg virus did not induce disease in animals recovered from either Strasbourg or Riems strains. Thus either Strasbourg or Riems virus is capable of producing immunity against Strasbourg virus. Animals recovered from Vallée A infection, on the contrary, exhibit no protection from later injections of the Strasbourg strain.

TABLE 14.—*Results of using Vallée A virus on cattle which had been infected with and recovered from one or more of the viruses*

Positive cases	Previous exposure to—	Negative cases	Previous exposure to—
Heifer 1	Strasbourg virus.	Heifer 1	Strasbourg and Vallée A viruses.
Heifer 2	Do.	Heifer 5	Vallée A and Strasbourg viruses.
Heifer 4	Strasbourg and Riems viruses.	Heifer 6	Strasbourg and Vallée A. viruses.
Heifer 6	Strasbourg virus.	Heifer 14	Vallée A virus.
Heifer 7	Strasbourg and Riems virus.		
Heifer 10	Strasbourg and Strasbourg viruses.		
Heifer 11	Strasbourg virus.		
Heifer 13	Riems virus.		
Heifer 28	Strasbourg virus.		

The tests recorded in Table 14 demonstrate that Vallée A virus induced foot-and-mouth disease in animals which had been infected with and recovered from either Strasbourg or Riems types, or both viruses. None of the nine animals reacting positively to Vallée A virus were previously infected with this type. On the other hand, Vallée A virus did not induce foot-and-mouth disease in animals recovered from type A infection.

Riems virus was used on cattle (hiefers 4, 7, and 8) recovered from Strasbourg-virus infection. None were infected, thus indicating that Strasbourg virus induced immunity against Riems virus. Heifer 13, recovered from Riems-virus infection, developed foot-and-mouth disease when exposed to Vallée A strain.

On the basis of these tests in cattle it may be concluded that Strasbourg and Riems strains are of the same type, and differ in this respect from the Vallée A virus.

SWINE

Table 11 contains a record of cross-immunity tests in hogs. Four hogs (1, 2, 4, and 5) recovered from Strasbourg-virus infection were exposed to either Riems or Vallée A virus. Those which received Riems virus (1 and 2) were unaffected, but the ones injected with Vallée A virus (4 and 5) showed typical foot-and-mouth disease. Five hogs (3, 6, 7, 8, and 9) recovered from infection with Vallée A virus were given either Strasbourg or Vallée A virus. Those injected with Strasbourg virus (3, 8, and 9) developed foot-and-mouth desease, while the ones injected with Vallée A virus (6 and 7) remained unaffected.

Hogs 1 and 2 were first infected with Strasbourg virus. They then showed resistance to Riems and also to Vallée A viruses. No explanation is offered for these untoward results. Lack of time and accommodations did not permit a repetition of this series of tests. Attention is directed, however, to the case of cattle 4, 7, and 8, which were infected with Strasbourg virus and later were refractory to Riems virus but not to later injection of Vallée A virus. The case of heifer 13 may also be considered at this time. It recovered from a Riems-virus infection, then contracted a Vallée A infection by experimental contact exposure to cattle 11 and 12.

The tests indicate in the main that Strasbourg virus induces in hogs an immunity against Riems virus, but not against Vallée A; and

Vallée A protects from effects of itself, but not from those of Strasbourg virus[30]

GUINEA PIGS

Cross-immunity tests in guinea pigs are summarized in Tables 15 and 16.

TABLE 15.—*Results of guinea-pig cross-immunity tests with Strasbourg and Vallée A viruses*

Test No.	Number of guinea pigs in tests	First injection: Virus used	First injection: Number of days before second injection	Second injection, virus used	Results [1]
1	10	Strasbourg	26 to 51	Vallée A	10 Pr.+Sec.
	6	Vallée A	43 to 56	do	3 M. P.—Sec.; 3 negative.
2	12	Strasbourg	20 to 84	Strasbourg	2 M. P.—Sec.; 10 negative.
	7	Vallée A	47 to 59	do	7 Pr.+Sec.
3	7	Strasbourg	23 to 52	do	7 negative.
	9	Vallée A	20 to 27	do	9 Pr.+Sec.
4	8	Strasbourg	26 to 48	Vallée A	5 Pr.+Sec.; 1 Pr.+Sec.
	7	Vallée A	27 to 34	do	7 negative.
5	5	Strasbourg	21 to 50	Strasbourg	5 negative.
	6	Vallée A	35 to 45	do	6 Pr.+Sec.
6	6	do	54 to 62	Swedish [2]	6 negative.
	5	Strasbourg	31 to 38	do	5 Pr.+Sec.
7	6	Vallée A	25 to 30	Denmark 4 [2]	5 negative; 1 M. P.—Sec.
	3	Swedish [2]	26 to 32	do	2 negative; 1 M. P.—Sec.
	5	Strasbourg	25 to 48	do	5 Pr.+Sec.
8	17	do	28 to 50	Strasbourg	16 negative; 1 M. P.—Sec.
	10	Vallée A	22 to 39	do	10 Pr.+Sec.

[1] In all tests normal guinea pigs were used to control the activity of the virus. "Pr.+Sec."=Primary and secondary vesicles. "Pr.—Sec."=Primary vesicles only. "M. P". (mild primary) is the designation given to a slight primary lesion. The guinea pigs in the groups giving an "M. P"—"Sec." (mild primary only) reaction are considered immune to the virus used. See footnote on p. 118, under "Comparative studies of vesicular stomatitis and foot-and-mouth disease."

[2] Swedish and Denmark 4 viruses are of the Vallée type A.

TABLE 16.—*Results of guinea-pig cross-immunity tests with Strasbourg, Vallée A, and Riems viruses*

Test	Number of guinea pigs in tests	First injection: Virus used	First injection: Number of days before second injection	Second injection, virus used	Results
9	10	Vallée A	38 to 42	Vallée A	4 M. P.—Sec.[1]; 6 negative.
	2	Riems	38 to 44	do	2 Pr.+Sec.
10	8	Vallée A	36 to 44	Riems	8 Pr.+Sec.
	3	Riems	24 to 26	do	3 negative.
11	7	Strasbourg	23 to 52	Strasbourg	7 negative.
	4	Riems	20	do	4 negative.
12	4	do	16 to 20	Vallée A	2—Pr.+Sec; 2 negative.
	7	Vallée A	22 to 34	do	7 negative.
13	17	Strasbourg	28 to 50	Strasbourg	16 negative; 1 M. P.—Sec.
	6	Riems	28 to 50	do	5 negative; 1 M. P.
14	8	Strasbourg	25 to 53	Riems	6 negative; 2 M. P.—Sec.
	5	Riems	20 to 24	do	5 negative.
	8	Vallée A	17 to 24	do	8 Pr.+Sec.
15	8	Strasbourg	25	Strasbourg	7 negative; 1 Pr.—Sec.
	10	Riems	25	do	9 negative; 1 Pr.—Sec.
16	8	Strasbourg	25	Riems	8 negative.
	12	Riems	25	do	12 negative.

[1] See footnote, Table 15.

[30] Additional data on the plurality of types of the virus may be found under "Carriers of foot-and-mouth-disease virus." There it is shown that 4 hogs and 11 out of 13 cattle recovered from type O virus were again readily infected with type A virus.

The principal results of the cross-immunity experimental work may be stated briefly as follows:

Of 73 guinea pigs recovered from Strasbourg virus infection and then exposed again to the same virus, all proved to be immune. Twelve of these guinea pigs were in test 2, 7 in test 3, 5 in test 5, 17 in test 8, 7 in test 11, 17 in test 13, and 8 in test 15.

Of 45 guinea pigs recovered from Vallée A infection and then again exposed to the same virus, all proved to be immune. Six of these guinea pigs were in test 1, 7 in test 4, 6 in test 6, 6 in test 7, 3 in test 11, 10 in test 9, and 7 in test 12.

Of 20 guinea pigs recovered from Riems virus infection and then again exposed to the same virus, all proved to be immune. Three of these guinea pigs were in test 10, 5 in test 14, and 12 in test 16.

Of 26 guinea pigs recovered from Strasbourg virus infection, all proved to be susceptible to infection with Vallée A virus. Ten of these guinea pigs were in test 1, 6 in test 4, 5 in test 6, and 5 in test 7.

Of 16 guinea pigs recovered from Strasbourg virus infection and then exposed to Riems virus, all proved to be immune. Eight of these guinea pigs were in test 14, and 8 in test 16.

Of 32 guinea pigs recovered from Vallée A virus infection, all proved to be susceptible to infection when inoculated with Strasbourg virus. Seven of these guinea pigs were in test 2, 9 in test 3, 6 in test 5, and 10 in test 8.

Of 16 guinea pigs recovered from Vallée A type infection, all proved to be susceptible to infection when inoculated with Riems virus. Eight of these guinea pigs were in test 10, and 8 in test 14.

Of 20 guinea pigs recovered from Riems virus infection, all proved to be immune when subsequently exposed to Strasbourg virus. Four of these guinea pigs were in test 11, 6 in test 13, and 10 in test 15.

Of 6 guinea pigs recovered from Riems virus infection, 4 were found susceptible and 2 immune when exposed later to Vallée A virus. Two of these guinea pigs were in test 9, and 4 in test 12.

The combined results show that of 258 guinea pigs recovered from infection with any of the three viruses (Strasbourg, Riems, and Vallée A) 256 reacted in a manner similar to that of cattle; that is, guinea pigs recovered from either Strasbourg or Riems virus infection were immune to the effect of either of these two viruses, but were susceptible to infection with Vallée A virus. On the other hand, the guinea pigs recovered from infection induced by Vallée A virus were rendered immune to Vallée A, but not to either the Strasbourg or Riems virus.

Two exceptions to this usual occurrence were noted in a group of four guinea pigs recovered from Riems virus infection (test 12). So far as could be determined none of the four showed visible, primary vesicles; two, nevertheless, exhibited unequivocal, secondary vesicles. Results such as these in guinea pigs might be expected from an injection of virus in sites other than in the pads, although only pad inoculations were made in these instances. The general principle deduced from these large series of tests in cattle, hogs, and guinea pigs, is that foot-and-mouth disease can be caused by at least two immunologically distinct types of virus.

PLURALITY OF VIRUSES DETERMINED BY SERUM TESTS

The serum from guinea pigs, recovered from the experimental disease,[31] offers further evidence to confirm the plurality of foot-and-mouth-disease virus. Such serum contains protective properties against the homologous, but not against a heterologous type of virus.

TABLE 17.—*Plurality of viruses demonstrated by serum tests*

Guinea pig No.	Serum injected	Quantity of serum	Virus to which exposed	Results[1]
		c. c.		
1	Guinea pig type O	0.1	Vallée A	Pr.+Sec.
2	do	.1	do	Do.
3	do	.5	do	Do.
4	do	.5	do	Do.
5	do	1.0	do	Do.
6	do	1.0	do	Do.
7	do	3.0	do	Do.
8	do	3.0	do	Do.
9	Guinea pig type A	.1	do	Do.
10	do	.1	do	Do.
11	do	.5	do	Pr.—Sec.
12	do	.5	do	Do.
13	do	1.0	do	Do.
14	do	1.0	do	Do.
15	do	3.0	do	Do.
16	do	3.0	do	Do.
17	Control		do	Pr.+Sec.
18	do		do	Do.
19	Guinea pig type O	.1	Strasbourg type O	Do.
20	do	.1	do	Do.
21	do	.5	do	Pr.—Sec.
22	do	.5	do	Do.
23	do	1.0	do	Do.
24	do	1.0	do	Do.
25	do	3.0	do	Do.
26	do	3.0	do	Do.
27	Guinea pig type A	.1	do	Pr.+Sec.
28	do	.1	do	Do.
29	do	.5	do	Do.
30	do	.5	do	Do.
31	do	1.0	do	Do.
32	do	1.0	do	Do.
33	do	3.0	do	Do.
34	do	3.0	do	Do.
35	Control		do	Do.
36	do		do	Do.

[1] See footnote 1, Table 15.

Table 17 shows that guinea pigs injected with as much as 3 cubic centimeters of type O serum were not protected against generalization [32] induced by type A virus, whereas guinea pigs injected with as small a quantity as 0.5 cubic centimeter of type A serum were protected against generalization caused by this same type. Guinea pigs which received 0.5 cubic centimeter of type O serum were resistant to generalization induced by type O virus, but those injected with 3 cubic centimeters of type A serum were not resistant to the secondary lesions of type O virus. Additional tests are mentioned in the discussion of typing viruses, under the heading "Danish and Swedish samples."

These findings indicate that the antibodies in recovered animals are type specific. The practical significance is that serum used in treatment and prevention should be derived from the same type of virus which causes the disease.

[31] For a discussion of this test the reader is referred to the chapter on "Serum tests in guinea pigs," p. 86.
[32] The term is used to denote a generalization of the virus as shown by the appearance of secondary vesicles.

TYPING VIRUSES

A study of the viruses responsible for outbreaks of the disease in the various countries where foot-and-mouth disease is enzootic should furnish information of great interest to epizoology, to laboratory investigations, and to the preparation of immune serum for treatment and prevention.

In addition to the writers' studies with Vallée type A and Strasbourg type O, there was a limited opportunity to type several other viruses.

SWISS SAMPLE

Through the courtesy of M. Bürgi, chief federal veterinarian of Switzerland, a sample was obtained of foot-and-mouth-disease virus contained in the coverings of a ruptured tongue vesicle of a cow slaughtered at the municipal abattoir at Berne, Switzerland. No difficulty was found in propagating this virus in guinea pigs. It was carried through 55 passages and then discontinued. This sample was designated "Berne" virus. Cross-immunity tests on guinea pigs, using Strasbourg type O virus, revealed this strain as type O.

TABLE 18.—*Guinea pig cross-immunity tests, Strasbourg and Berne viruses*

Test No.	Number of guinea pigs in test	First exposure		Second exposure		Result [1]	Remarks
		Virus used	Number of days before second exposure	Virus used	Date		
1A	6	Strasbourg type O.	10 to 20	Strasbourg type O.	Aug. 6, 1925	6 negative	
	6	do	10 to 20	Berne	do	do	
2A	3	do	33 to 41	Strasbourg type O.	Oct. 2, 1925	3 negative	
	9	Berne	24 to 45	do	do	6 negative; 3 M. P.—Sec. [2]	24, 31, and 29 days. [3]
3A	8	Strasbourg type O.	22 to 46	Berne	Oct. 3, 1925	8 negative	
	8	Berne	25 to 46	do	do	7 negative; 1 M. P.	38 days. [3]

[1] Normal animals were used to control the activity of the virus.
[2] See footnote [1], Table 15.
[3] The number of days between the first and second exposures in animals developing mild, primary lesions.

As will be seen in Table 18, 14 guinea pigs recovered from Strasbourg (type O) infection were entirely resistant to reinfection with Berne virus. As a control, 9 guinea pigs recovered from Strasbourg-type infection were also completely resistant to reinoculation with the homologous type. Of 9 animals recovered from infection with Berne virus, 6 were completely resistant to reinoculation with Strasbourg-type virus while 3 showed only mild, primary lesions. Of 8 guinea pigs recovered from the effects of the Berne virus, 7 were completely resistant to the same strain, and 1 reacted with only a mild, primary lesion. Type A virus was not at hand at the time so that cross-immunity tests could not be made with that virus.

OTHER STRASBOURG SAMPLES

Two samples obtained at different times from animals slaughtered at the Strasbourg abattoir were easily transferred to guinea pigs. After several passages active material from both sources was injected into two series each of six guinea pigs which had recovered from Strasbourg type O infection. None of the animals were affected, indicating that both of these viruses were of the type O. No test was made with type A.

DANISH AND SWEDISH SAMPLES

In March and April, 1926, specimens of virus were received from C. O. Jensen, chief federal veterinarian of Denmark, and H. Magnusson, director of the veterinary bacteriological laboratory of the Län of Malmöhus, Sweden, with the requests that the type of these viruses be determined. In connection with these samples the following conditions prevailed in Denmark and Sweden.

In 1924 foot-and-mouth disease became very extensive in Denmark. The Län of Malmöhus, Sweden, owing to its close proximity to Denmark, also became involved and the disease rapidly spread to a large number of herds.

In Denmark the epizootic reached its peak in January, 1925, when about 62,000 farms and 50 per cent of the whole number of animals were affected. The epizootic gradually abated, but in February, 1926, a recurrence took place in the districts which were involved the previous year. It is noteworthy that animals which had had the disease the previous year again contracted the malady, in many cases in a much more severe form than in the first attack. In Denmark convalescent serum was used extensively in the 1924–25 outbreak in the treatment of the disease, as a result of which the severity of the disease was moderated and the number of deaths considerably reduced. However, the convalescent serum obtained from affected animals of the 1924–25 outbreak had no influence whatsoever on the course of the 1926 disease, but convalescent serum from animals affected in 1926 acted with beneficial results.

The same conditions appeared in Sweden. In 1926 the disease attacked animals which were affected in 1925. Convalescent serum from 1925 cases was of no avail in treatment of animals affected in 1926, but similar serum obtained from the latter animals proved to be useful.

The fact that the second epizootic swept through herds affected the previous year, and the inefficacy of the convalescent serum collected the previous year and the favorable action of convalescent serum obtained during the 1926 outbreak seemed to indicate that this outbreak was caused by a type of virus different from that causing the 1924–25 outbreak.

Samples of virus from both Denmark and Sweden were tested by the writers on guinea pigs since no large animals were available. The results follow.

DANISH SAMPLES

Five separate samples of virus in the form of coverings of vesicles were received in glycerol-saline solution. Only one sample was easily transferred to guinea pigs and this was propagated through successive passages. Three samples were very irregular in their action in guinea pigs; difficulty was experienced in adapting them to this animal. One sample could not be transferred. The cross-immunity tests could therefore be made with only one specimen designated Denmark 4. After the ninth passage through guinea pigs this virus was inoculated into 5 guinea pigs recovered from type O infection with the result that all 5 developed promptly primary and secondary vesicles. Of 9 guinea pigs recovered from type A infection, 7 were unaffected by the Danish virus and 2 showed only mild, primary vesicles. These results clearly indicated that this sample was of type A.

These results were confirmed by the guinea-pig serum test, which revealed that the 1925 outbreak in Denmark was due to type O virus. For example, a sample of Danish convalescent serum which had been received in 1925 was then tested for its neutralization of type O, and of the Danish type A virus.

TABLE 19.—*Danish serum prepared in 1925 tested against type O and type A viruses*

Guinea pig No.	Quantity of Danish convalescent serum	Virus	Result [1]
1	2 c. c.	Type O, Strasbourg	Pr.—Sec.[1]
2	2 c. c.	do	Pr.+Sec.
3	5 c. c.	do	Pr.—Sec.
4	5 c. c.	do	Pr.+Sec.
5	10 c. c.	do	Pr.—Sec.
6	10 c. c.	do	Do.
7	Control	do	Pr.+Sec.
8	do	do	Do.
10	2 c. c.	Type A, Denmark 4	Do.
11	2 c. c.	do	Do.
12	5 c. c.	do	Do.
13	5 c. c.	do	Do.
14	10 c. c.	do	Do.
15	10 c. c.	do	Do.
16	Control	do	Do.
17	do	do	Do.

[1] Pr.—Sec. = Primary vesicle only. Pr.+Sec. = Primary and secondary vesicles.

As will be seen in Table 19, the Danish convalescent serum of 1925, in quantities as small as 2 cubic centimeters, protected guinea pigs against generalization of type O virus. While one guinea pig which had received 2 cubic centimeters, and another which had received 5 cubic centimeters of serum developed secondary vesicles, these results are in marked contrast to those in which the serum-treated guinea pigs were exposed to the Denmark 4 (1926) virus. In this series of guinea pigs all developed secondary vesicles; amounts of serum as large as 10 cubic centimeters failed to arrest generalization of the Denmark 4 (1926) virus.

These results show, therefore, that convalescent serum obtained in 1925 in Denmark had a distinct retarding influence on type O virus, but had no effect on the course of the disease produced by the Denmark 4 (1926 type A) virus. Hence the 1926 outbreak of foot-and-mouth disease in Denmark, at least in a certain locality, was caused by type A and the 1925 outbreak by type O virus.

SWEDISH SAMPLES

Two samples of foot-and-mouth-disease virus were received from Sweden. One consisted of vesicular coverings obtained from a guinea pig in the sixteenth passage, derived originally from a cow with a second attack of the disease within a year. The second sample consisted of fresh, vesicular coverings of a cow affected one year after the first attack. Both materials were suspended in glycerol.

The first sample could not be transferred to guinea pigs. The second, from the cow, was easily transferred to the guinea pig. Active material from the first passage was injected into guinea pigs recovered from type A and type O infections with the following results:

Of 6 guinea pigs recovered from type A infection, 5 failed to reveal any evidence of disease and 1 showed only a mild primary vesicle. Of 5 guinea pigs recovered from type O infection, 4 developed primary and secondary vesicles and 1 showed only a primary vesicle. These results indicated that the Swedish virus was also of type A.

Tests with Swedish convalescent serum prepared in 1925 gave results similar to those obtained with Danish convalescent serum. In brief, the serum showed protective value against type O but none against the Swedish 1926 virus and the commission's Vallée A virus. Hence, here again the 1925 outbreak was caused by type O and the 1926 outbreak by type A virus.

IMMUNITY—IMMUNE AND HYPERIMMUNE SERUM

SERUM TESTS IN GUINEA PIGS

The discovery that foot-and-mouth disease could readily be transferred to guinea pigs brought this species into prominence as a test animal for a study not only of the virus itself but also of various immunological problems.

Waldmann and Pape in 1921 (*78*) reported on the use of guinea pigs as test animals for titrating hyperimmune foot-and-mouth-disease serum (Loeffler serum). Their method was to inject a series of guinea pigs subcutaneously with graded doses of serum and at the same time to infect the animals with foot-and-mouth-disease virus by scarifying the hairless pads of the hind legs and then rubbing the virus into the scarified area. Controls of the activity of the virus were also used. They found that the serum failed to arrest the development of the disease at the site of inoculation, for primary vesicles formed in all the serum-injected animals as well as in the controls. Generalization of the disease occurred in all control animals and in some test animals which received small quantities of serum. A certain amount of serum, depending on its potency, prevented generalization of the virus—pigs receiving that or larger amounts failed to yield vesicles at sites other than the point of inoculation. In animals which had received smaller amounts, however, the disease did become generalized, as shown by the formation of secondary vesicles. The amount of serum which prevented secondary manifestations was considered to be the unit of neutralization. Normal horse and cattle serum, even in large amounts, did not prevent generalization. A sample of convalescent serum, on the other hand, protected against secondary lesions, but the amount required for that purpose was twenty times greater than that of hyperimmune serum.

EXPERIMENTAL TECHNIC

A large number of tests were made with normal, convalescent, and hyperimmune serums. The technic employed was in general similar to that of Waldmann and Pape. Guinea pigs were injected subcutaneously with the serum and at the same time were exposed to active virus by scarification of both pads of the hind legs. Control guinea pigs were inoculated simultaneously to check the activity of the virus. It was found essential to use an active virus—one that would produce primary vesicles in 24 hours and secondary lesions 24 to 48 hours later.

The method of inoculation of the virus was as follows: Lymph and coverings of vesicles of guinea pigs inoculated 24 hours previously were ground in a mortar with a small quantity of physiological salt solution. A drop of this material was placed on the hairless pad of each hind foot and numerous punctures made in the skin with a fine, sharp knife or needle. The virus was then gently rubbed into the injured skin. No attempt was made to measure the activity of the virus. All pigs in the test were injected in a like manner. Readings were made every 24 hours for 10 days. Animals which failed to show secondary vesicles within that time were considered protected.

NORMAL SERUM

The effect of normal serum from cattle, swine, horses, guinea pigs, and rabbits on the course of foot-and-mouth disease in guinea pigs was first studied.

PROTOCOL 20

Normal serum from 45 cattle was injected subcutaneously into guinea pigs in quantities of 5 and 10 cubic centimeters. The animals were then exposed to 24-hour, guinea-pig virus by scarification. Control animals to check the activity of the virus were inoculated at the same time. Primary lesions appeared in 24 hours at the site of inoculation in all animals, followed in every case by generalization of the disease as shown by secondary vesicles 24 to 48 hours later. That the cattle from which this serum was obtained were susceptible to foot-and-mouth disease was later proved when they contracted the disease following artificial inoculation. Likewise, numerous tests of normal swine, horse, rabbit, and guinea-pig serums used in quantities from 1 to 10 cubic centimeters showed that the serum from these different species invariably failed to protect guinea pigs against the generalization.

It may, therefore, be stated that no substances, specific or nonspecific, could be demonstrated in normal cattle, swine, horse, rabbit, or guinea-pig serum which were capable of protecting guinea pigs against secondary manifestations of foot-and-mouth-disease virus. Indeed it was often noted that the disease assumed a more severe form in the normal serum-treated animals than in the controls.

IMMUNE OR CONVALESCENT SERUM

It has been found from field experience that convalescent serum when used in large quantities has considerable value in lessening the severity of the disease in naturally susceptible animals, and when the malignant type prevails the mortality is reduced by its prompt use. Protective power in such serum can be readily demonstrated by the guinea-pig test, and when a sufficient quantity of serum is used, guinea pigs will be protected against the generalization of the disease. This is in strong contrast to the ineffective action of normal serum. The following experiment, which has been repeated many times, illustrates the action of convalescent serum.

PROTOCOL 21

Each of two guinea pigs was inoculated subcutaneously with the following quantities of convalescent cattle serum: 0.5, 1, 1.5, 2, and 5 cubic centimeters. The same number of guinea pigs was inoculated with the same quantity of normal cattle serum. The animals were then exposed to 24-hour, guinea-pig virus in the usual manner. Two normal guinea pigs were also inoculated with the virus alone as controls for its activity. All showed primary vesicles in 24 hours. The pigs injected with normal serum, those injected with 0.5 cubic centimeter of convalescent serum, and the two controls yielded secondary vesicles from 24 to 48 hours later. Those receiving 1, 1.5, 2, and 5 cubic centimeters of convalescent serum failed to develop secondary lesions. This test demonstrates that 1 cubic centimeter of convalescent serum was sufficient to protect guinea pigs against generalization. Five cubic centimeters of normal serum, however, failed to protect.

The potency of convalescent serum varies. It depends on the individual from which it is taken and on the length of time between recovery from the disease and the drawing of the blood. Reference to Table 20 shows that within 23 days after infection, some serum will prevent generalization in guinea pigs in doses of 0.5 cubic centimeter, whereas between 1 and 3 cubic centimeters of other serums are required to accomplish the same result. Serum collected from guinea pigs 10 days to 2 weeks after infection with foot-and-mouth-disease virus usually showed sufficient protective properties in quantities of 0.5 cubic centimeter to prevent secondary lesions. Limited time prevented any special study of the duration of protective bodies in the serum after recovery, but it was observed that serums 1 to 2 months after infection were not so potent as those obtained 2 or 3 weeks after. It is generally held that the maximum amount of antibodies is present from 10 to 14 days after infection. After a certain length of time it may be impossible to demonstrate protective bodies in convalescent serum, as the following test shows:

Serum from eight cattle, which was drawn from 80 to 210 days after natural infection with virus, failed to arrest the development of secondary lesions in guinea pigs although as much as 5 cubic centimeters was employed. The animals from which these serums were obtained could not be tested for immunity, as they were not available for further use. As a rule, immunity in cattle, however, persists for a longer period than 210 days. It is believed, therefore, that all or most of these cattle were still immune to the disease even though protective bodies demonstrable by the guinea-pig test had disappeared from their serum.

The application of the serum guinea-pig test as a means of determining the susceptibility of cattle to foot-and-mouth disease or for diagnosis of their prior infection is, therefore, of limited value.

Data on the length of time that the serum retains its protective activity are furnished in the following experiment.

PROTOCOL 22

The titers of various convalescent serums tested varied from 0.5 to 2 cubic centimeters. Two lots of serums,[33] A and B, were tested after being held in a cold chamber approximately one year. In June, 1925, 1 cubic centimeter of serum A and 2 cubic centimeters of serum B were found to prevent generalization of the disease in guinea pigs. These two samples were again tested in May,

[33] Through the courtesy of Dr. H. Magnusson, Malmö, Sweden, and Doctor Schmit-Jansen, Copenhagen, Denmark, several bottles of convalescent serum were obtained, as prepared for use in those countries in combating foot-and-mouth disease.

1926. During this interval the bottles were kept in the cold chamber. Serum B was found to be just as potent, but serum A was less active, its titer having fallen to between 2 and 5 cubic centimeters. Serum A was found to contain a very heavy precipitate which filled half the bottle, obviously a result of contamination. Serum B was free from precipitate and was in good condition.

It appears, therefore, that convalescent serum can retain its potency for at least one year if kept in the cold chamber and kept free from contamination. It is also evident that bacterial contamination may cause a reduction in potency.

The next step was to study the possible usefulness of convalescent serum in preventing the formation of the primary vesicle at the site of inoculation.

PROTOCOL 23

Three guinea pigs were inoculated subcutaneously with 1, 2, and 5 cubic centimeters respectively, of convalescent cattle serum A. The pads of the hind feet of a fourth guinea pig were scarified, a drop of the convalescent serum was rubbed into the scarified area, and a small quantity of the serum was also injected intradermically into the skin of the pad. Four additional guinea pigs were treated in a similar manner with convalescent cattle serum B. In addition, two guinea pigs were injected with normal cattle serum. One received 5 cubic centimeters subcutaneously while the other was inoculated by scarification of the pad in a manner similar to that given to the test animals.

Forty-eight hours after the injection of the serum the 10 guinea pigs were inoculated on both pads of the hind legs with 24-hour guinea-pig virus by the usual puncture-scarification method. Two normal guinea pigs were inoculated at the same time to serve as controls on the activity of the virus. As a result of this inoculation all of the 12 guinea pigs showed after 24 hours well-marked primary vesicles at the site of inoculation. Between 24 and 72 hours later the following guinea pigs developed secondary vesicles: The two controls, the two treated with normal serum, both animals given convalescent A and B serums locally and the guinea pig which received 1 cubic centimeter of convalescent B serum.

Convalescent serum injected 48 hours prior to artificial exposure to the virus failed, therefore, to prevent infection. Primary vesicles occurred at the site of inoculation within the usual time of 24 hours. Direct application of the serum to and into the skin of the pads on the hind legs had no retarding effect on the development of vesicles when the virus was inoculated 48 hours later into these sites.

HYPERIMMUNE SERUM

Numerous tests were made on the action of hyperimmune serum in guinea pigs. The sources of the serums were as follows: (1) Loeffler serum, purchased from the Staatliche Forschungsanstalt (the government research station), island of Riems, Germany; and (2) serums obtained from various species of animals which received numerous injections of foot-and-mouth-disease virus for the purpose of hyperimmunizing. The Loeffler serum was received in original, sealed flasks of 1,000 cubic centimeters. It was kept in the cold chamber from the time of its receipt and was used well within the expiration date marked on the label.

PROTOCOL 24

A comparative test was made with the following serums: 1 of Loeffler hyperimmune, 8 from convalescent cattle, 1 from a normal hog, and 1 from a normal cow. Guinea pigs were injected subcutaneously with 0.05, 0.1, 0.5, 1, and 3 cubic centimeters of the hyperimmune and convalescent serums, and 5 cubic centimeters

of normal serum. All animals were then exposed to 24-hour, guinea-pig virus by the usual puncture method on the pads of the hind legs. Control animals were also inoculated at the same time.

Three convalescent serums proved to be as potent as the Loeffler hyperimmune serum. The titers were between 0.5 and 1 cubic centimeters. Two convalescent serums showed a higher titer, that is, between 0.1 and 0.5 cubic centimeter, and three a lower titer, between 1 and 3 cubic centimeters. Five cubic centimeters of both normal serums failed to arrest generalization of the disease. These results are given in Table 20.

TABLE 20.—*Comparative titers of hyperimmune, convalescent, and normal serums*

Serum	Days[1]	Date of test	Titer	Serum	Days[1]	Date of test	Titer
			Cubic centimeters				*Cubic centimeters*
Hyperimmune (Loeffler) serum	------	Oct. 20, 1925	0.5–1	Convalescent serum—heifer 7	20	Oct. 20, 1925	0.5–1
Convalescent serum—heifer 11	23	----do----------	0.1–0.5	Convalescent serum—heifer 8	20	----do----------	3
Convalescent serum—heifer 3	18	----do----------	0.5–1	Convalescent serum—bull 9	17	----do----------	1 –3
Convalescent serum—heifer 4	20	----do----------	0.1–0.5	Convalescent serum—heifer 10	12	----do----------	1 –3
Normal serum—heifer 5	------	----do----------	[2] 5	Normal serum—hog 4	------	----do----------	[2] 5
Convalescent serum—heifer 6	18	----do----------	0.5–1				

[1] The number of days between infection and the collection of serum.
[2] No protection was obtained.

These results are not in accord with those of Waldmann and Pape, who found that Loeffler hyperimmune serum by the guinea-pig test gave protection with 0.08 cubic centimeter and convalescent serum with 1.6 cubic centimeters. Through the courtesy of M. Bürgi, chief federal veterinarian of Switzerland, a sealed liter bottle of Loeffler hyperimmune serum of a different serial number was obtained. A comparison of this serum with the original lot tested showed no practical difference between the two.

From the foregoing test it may be concluded that two different serial lots of hyperimmune (Loeffler) serum showed no greater activity than three convalescent cattle serums. On the other hand, two other convalescent serums showed a higher measure of protection, and two additional ones, a lower.

Loeffler hyperimmune serum also failed to prevent the appearance of primary or inoculation vesicles when used as described in protocol 23 for convalescent serum.

The writers' efforts to make a hyperimmune serum are illustrated by the following protocol:

PROTOCOL 25

In attempts to produce a hyperimmune serum, 5 guinea pigs, 4 rabbits, 3 cattle, and 1 horse were used. These animals were given repeated injections of foot-and-mouth-disease virus; most of the injections were made intradermically. As the virus of foot-and-mouth disease is of an epitheliotropic nature, it was thought that virus injected into the skin might produce a more potent serum than virus injected by other routes. The virus for inoculation was obtained from the lymph and epithelial coverings of vesicles from guinea pigs which had been inoculated 24 hours previously. A few injections were also made with virulent guinea-pig blood. The method of injection for each species was as follows:

Guinea pigs.—Five guinea pigs which had recovered from foot-and-mouth disease were used. The dates of inoculation and method used are given in Table

21. From 0.25 to 0.5 cubic centimeter of a heavy suspension of virus was injected intradermically in the skin of the thigh or abdomen. When the virus was injected intraperitoneally 0.5 cubic centimeter was given. Blood was collected from the animals five days after the last injection by severing the large blood vessels in the throat. The serum was collected, preserved with phenol (0.5 per cent), and stored in the cold chamber.

Rabbits.—Four rabbits were injected with foot-and-mouth-disease virus as indicated by the method shown in Table 21. No evidence of foot-and-mouth disease in any of these animals was observed subsequently. For intravenous injections, 1 cubic centimeter of a heavy suspension of virus was given, and for the intradermic in the abdominal region, 0.5 cubic centimeter. Following intradermic injection of the virus, abscesses were noted at the site of inoculation. The same virus injected intradermically in the guinea pigs, cattle, and the horse did not cause such abscess formation. Suppuration following the intradermic injection of foot-and-mouth-disease virus in rabbits has also been observed by others. Five days after the last injection of virus, blood was drawn from the ear, the serum collected, preserved in phenol 0.5 per cent, and stored in the cold chamber.

Cattle.—Three cattle, recovered from foot-and-mouth disease, were injected with the virus as indicated in Table 21. Intradermic injections of the virus were made into the skin of the fold of the tail and into the mucous membrane of the lower lip. One cubic centimeter of a heavy suspension was the amount usually injected. For intravenous injections, 10 cubic centimeters of virulent guinea-pig blood were used. Five days after the last injection the animals were bled, the serum preserved with 0.5 per cent phenol and stored in the cold chamber.

Horse.—One horse received a number of injections of foot-and-mouth-disease virus as indicated in Table 21. Although a large number of injections of active virus were given, the animal never reacted with symptoms of foot-and-mouth disease. Intradermic injections were made in the skin of the lower eyelid. The amount usually given was 1 cubic centimeter. The subcutaneous dose was 2 cubic centimeters. The intravenous injection consisted of 5 cubic centimeters of virulent guinea-pig blood. Serum was collected five days after the last injection, preserved with phenol (0.5 per cent), and stored in a cold chamber.

TABLE 21—*Preparation of hyperimmune serum*

HYPERIMMUNIZATION OF GUINEA PIGS

Date of inoculation	Nov. 26	Nov. 27	Dec. 2	Dec. 3	Dec. 4	Dec. 6	Dec. 7	Dec. 8	Dec. 9	Dec. 10	Dec. 11	Dec. 12	Dec. 19	Dec. 21	Dec. 22	Dec. 23	Dec. 26	Dec. 27	Dec. 28	Dec. 29	Dec. 30
Method of inoculation[1]	I. D.	I. D.	I. D.	I. P.	I. D.	I. D.	I. D.	I. P.	I. D.	I. P.	I. D.	I. D.	I. D.	I. D.	I. P.	I. D.	I. D.	I. D.	I. D.	I. D.	I. P.

HYPERIMMUNIZATION OF RABBITS

Date of inoculation	Nov. 27	Dec. 2	Dec. 3	Dec. 4	Dec. 6	Dec. 7	Dec. 8	Dec. 9	Dec. 10	Dec. 11	Dec. 12	Dec 19	Dec. 21	Dec. 22	Dec. 23	Dec. 26	Dec. 27	Dec. 28	Dec. 29	Dec. 30
Method of inoculation[1]	I. V.	I. V.	I. D.	I. V.	I. D.	I. D.	I. V.	I. D.	I. V.	I. V.	I. D.	I. V.	I. V.	I. V.	I. V.	I. V.	I. D.	I. D.	I. D.	I. V.

HYPERIMMUNIZATION OF CATTLE

Date of inoculation	Dec. 7	Dec. 8	Dec. 9	Dec. 10	Dec. 11	Dec. 12	Dec. 19	Dec. 21	Dec. 22	Dec. 23	Dec. 26	Dec. 27	Dec. 28	Dec. 29	Dec. 30	Dec. 31	1926 Jan. 1	Jan. 2	Jan. 3
Method of inoculation[1]	I. D.	I. D.	I. D.	I. D.	I. D.	I. D.	I. D.	I. D.	I. D.	I. D.	I. D.	I. D.	I. D.	I. D. I. V.	I. D. I. V.	I. D. I. V.	I. D. I. V.	I. D. I. V.	I. D. I. V.

HYPERIMMUNIZATION OF HORSE

Date of inoculation	Dec. 7	Dec. 8	Dec. 9	Dec. 10	Dec. 11	Dec. 12	Dec. 19	Dec. 21	Dec. 22	Dec. 23	Dec. 26	Dec. 27	Dec. 28	Dec. 29	Dec. 30	Dec. 31	1926 Jan. 1
Method of inoculation[1]	I. D.	I. D.	I. D. S.	I. D. S.	I. D. S.	I. D. S.	I. D. S.	I. D. S.	I. D. S.	I. D. I.[2]	I. D. S.	I. D. S.	I. D. S.	I. D. S. I. V.[2]	I. V.[2]	I. D. I. V.[2]	I. D. S.

[1] I. D.=Intradermic; I. P.=Intraperitoneal; I. V.=Intravenous; S.=Subcutaneous.
[2] Virus consisted of virulent guinea-pig blood.

The protective action of these various serums was then tested on guinea pigs as follows:

PROTOCOL 26

Five guinea pigs were used in testing each serum. Four were injected subcutaneously with 0.005, 0.01, and 1 cubic centimeter of hyperimmune serum respectively, and one guinea pig was injected with 1 cubic centimeter of normal serum taken from each species before immunization. The animals, after the injection of the serum, were exposed to active virus by the usual puncture-scarification method. Two control animals were employed as checks on the virus. The results of the test are summarized in Table 22.

TABLE 22.—*Result of tests of serums of animals hyperimmunized*

Source of serum.	Titer of serum	Source of serum	Titer of serum
Heifer 3	Between 0.1 and 1 c. c.	Rabbit 1	Between 0.01 and 0.1 c. c.
Heifer 10	Greater than 1 c. c.	Rabbit 2	Do.
Bull 9	Do.	Rabbit 4	Do.
Pooled serum of five guinea pigs.	Between 0.01 and 0.1 c. c.	Horse 1	Greater than 1 c. c.

The titration of the serum of the cattle gave results similar to those obtained with convalescent serum. In the serum of one animal a quantity between 0.1 and 1 cubic centimeter protected, and in that of the other two, 1 cubic centimeter failed to prevent generalization. Decidedly better results were obtained with the guinea-pig and rabbit serums. The guinea-pig serum and the three rabbit serums all showed a titer between 0.01 and 0.1 cubic centimeter. In respect to the horse serum the largest quantity used, 1 cubic centimeter, failed to show any protective property whatsoever against generalization. All the guinea pigs injected with 1 cubic centimeter of the normal serum from each species showed generalization of the disease, as did the two virus control animals.

Serum from rabbits and guinea pigs after numerous injections of virus showed distinct, protective properties in small quantities, when more than 10 times these amounts of normal serum failed.

From the experience gained from a large number of tests certain conditions were found necessary to insure generally satisfactory results. The guinea pigs should be of healthy stock in good condition, of medium or large size, and uniform weight. A sufficient number should be used to overcome the factor of individual differences in reaction. It was frequently observed that guinea pigs suffering from malnutrition as a result of faulty diet or intercurrent diseases were more resistant to infection when inoculated with the virus of foot-and-mouth disease than were normal, healthy animals. This was manifested by the delayed appearance of the primary and secondary vesicles. The failure of the secondary vesicles to appear in some instances was so frequent that the serum of similarly sick animals was tested for the presence of antibodies. In no case, however, did the serum of such animals in 1 to 2 cubic centimeter quantities prevent the generalization of the disease.

Owing to the large number of tests made it was at times impossible to select the animals properly and in some of the tests paradoxical results were occasionally obtained. For example, a guinea pig that had received a certain amount of a serum showed secondary vesicles whereas an animal that had received a smaller amount of the same serum was protected. Such irregularities may be a consequence of disproportion of the dose used to the body weight.

Lack of time prevented special study on other methods of testing serums, but it was felt that the guinea-pig test could be considerably improved by other methods of inoculation of the virus. A more ideal method might include a standard dose of virus and a method of injection whereby the primary lesion would be eliminated.

ACTION OF HYPERIMMUNE SERUM IN CATTLE

For several years guinea pigs have been used to test the potency of hyperimmune serum. The method employed is described elsewhere in this report under the heading "Serum tests in guinea pigs" (p. 86). Although many reports have been issued concerning the value of hyperimmune serum in preventing foot-and-mouth disease in cattle under field conditions, there has been little direct experimental work on the subject. Loeffler and his coworkers tested each lot of hyperimmune serum by the injection of two cattle, each weighing between 400 and 500 pounds, with 100 cubic centimeters of the serum in one case and 200 cubic centimeters in the other. Twenty-four hours later each animal, as well as a normal bovine animal, was injected intravenously with 0.1 cubic centimeter of fresh virus. Serum was considered active if it protected the two treated cattle while the control developed the disease.

A series of tests with Loeffler hyperimmune serum was made by the writers on 12 cattle with results as shown in Table 23.

TABLE 23.—*Results of tests of hyperimmune serum (Loeffler) on cattle*

Cattle test No.	Weight	Date of serum injection	Quantity of serum injected	Time between serum injection and virus exposure	Method of exposure to virus	Observations (1926)							Results [1]
						May 27	May 29	May 30	May 31	June 1	June 2	June 3	June 5	
	Pounds		*Cubic centimeters*											
47	375	May 11, 1926	100	14 days	Local scarification.		Primary inoculation vesicle.	No other lesions developed						Pr.—Sec.
48	350	do	100	do	do									Negative.
61	300	May 17, 1926	75	8 days	do	Primary inoculation vesicle.	Other mouth lesions.	Feet lesions.						Pr.+Sec.
62	225	do	75	do	do	do		No other lesions developed						Pr.—Sec.
75	200	May 25, 1926	60	24 hours	do	do		No other lesions developed						Do.
76	200	do	60	do	do	do	Other mouth lesions.							Pr.+Sec.
49	350	May 11, 1926 May 25, 1926	100	do	Contact infection.									Negative.
50	375	do	100	do	do									Do.
63	400	May 17, 1926	100	5 to 8 days	do									Do.
64	400	do	100	do	do									Do.
59	325	do	100	10 to 12 days	do									Do.
60	325	do	100	do	do				Mouth lesions.				Feet lesions.	Mouth and feet lesions.

[1] The abbreviations "Pr." and "Sec." signify primary and secondary vesicle, respectively.

The serum was injected in several places under the skin of the neck. The cattle were later exposed to foot-and-mouth-disease virus, either by scarification of the upper gum and applying virus, or by contact with animals during the initial stages of the disease. Throughout this experiment the cattle were so handled and stabled that exposure in all instances was limited to the time indicated in the table.

The results of these tests indicate that at 1, 8, and 14 days, respectively, after the administration of the hyperimmune serum, the animals were susceptible to local inoculation of the virus. In animals 61 and 75, exposed 8 days and 1 day, respectively, after the administration of the serum, the disease became generalized. Of two cattle, 47 and 48, exposed to local virus inoculation 14 days after the administration of serum, one showed only a local inoculation vesicle, and the other was unaffected. The failure of one to develop the disease, and the appearance of only local lesions at the site of inoculation in the second animal of this group can hardly be explained by the dose of serum.

In proportion to the weight, there was no great difference in the amount of serum used in the two animals. Both were suffering from papillary stomatitis [34] which involved a considerable portion of the mucous membrane of the gums, lips, and palate. This disease, however, did not necessarily cause the results obtained, since the writers had no difficulty, on several former occasions, in transmitting both types of foot-and-mouth disease and also vesicular stomatitis to animals affected with or recovered from papillary stomatitis. The fact that the serum failed to protect the animals 1 and 8 days after its administration indicates that it alone, could not prevent the development of lesions at the site of inoculation; hence an explanation of the failure in the 14-day animals should be based on other grounds.

In the test of exposure to foot-and-mouth disease by contact, the commission found that one animal, No. 60, exposed from 10 to 12 days after the administration of serum, exhibited lesions in the mouth and in the feet. The others escaped infection. It is evident that the extent and severity of contact exposure can not be so well controlled as the inoculation of virus by scarification. In these tests the writers were unable to control (by means of normal cattle) the infectivity of each animal which acted as a source of the virus for contact exposure. Although exposure in this test appeared to be severe, yet in another contact experiment made at the same time, three out of seven control animals failed to develop symptoms during the period of observation.

These tests indicate that serum in full doses, and even in larger doses than those recommended, fails to protect against local inoculation vesicles and generalization when the virus is administered locally. On the other hand, all but one of the treated animals failed to develop the disease when exposed within 8 to 14 days to ordinary contact infection.

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[34] Papillary or papular stomatitis resembles the disease described by Ostertag and Bugge (*60*). It manifests itself by the formation of circular or oval nodules, varying from 0.3 to 2.5 centimeters in diameter. These are sharply defined by distinct, reddened borders of about 0.5 to 1 millimeter in width. Later they assume a granulating, verrucous growth. They appear usually on the gums, palate, and inner surface of the lips and cheeks, and occasionally and mildly, on the tongue. Changes in other parts of the body were not observed. Complete healing takes place within about two weeks. In France, many of the authorities believe the ailment is caused by an irritating or infectious substance in the feed.

IMMUNITY FOLLOWING INJECTIONS OF IMMUNE SERUM AND VIRUS IN GUINEA PIGS

The writers have already referred to the measure of protective action of immune serum in the guinea-pig test by the appearance in these animals of primary but not secondary lesions if a pad inoculation of the virus follows shortly the injection of serum. On the other hand, failure of protection in guinea pigs is shown by these animals reacting, under similar conditions, with both primary and secondary lesions.

Seventy guinea pigs which were injected with cattle-immune serum and then exposed to virus, were later subjected to pad inoculation of the virus to test their immunity. The results of the later injections are recorded in Table 24.

TABLE 24.—*Immunity following cattle-immune serum plus virus injections in guinea pigs*

Test No.	Number of guinea pigs in test	Immunization with immune serum+virus		Days before second exposure	Immunity test with virus			
		Results[1]			Date	Results[1]		
		Pr.−Sec.	Pr.+Sec.			Pr.+Sec.	Pr.−Sec.	Negative
1	11	5		101–100	Nov. 25, 1925	2	3	
			6	101–106	do		4	2
2	14		14	77–82	do		3	11
3	7		6	29	do			6
		1		29	do	1		
4	12		6	36	do		2	4
		6		36	do	1	5	
5	9	7		41	Nov. 30, 1925	2	5	
			2	41	do		1	1
6	5		5	34	do			5
7	12	1		37	Feb. 12, 1926		1	
			11	37	do			11
Total	70	20				6	14	0
			50			0	10	40

[1] The terms "Pr." and "Sec." indicate primary and secondary vesicles.

It is observed that of the 20 guinea pigs which showed primary but no secondary vesicles following the serum plus virus injections, none resisted completely subsequent pad inoculation. Six developed primary and secondary vesicles and 14 primary vesicles only. Of the 50 which showed both primary and secondary vesicles as a result of serum and virus inoculations, 10 revealed only primary vesicles, none revealed secondary vesicles, and 40 were found to be wholly resistant.

These tests indicate a difference in the degree of immunity in the two groups of guinea pigs and show that when protection is afforded by the cattle serum plus virus injections, the immunity is not so solid as that in guinea pigs which have recovered from infection by the virus itself or in animals in which the serum afforded no protection.

Additional data relating to the effect of this procedure on immunity are available in a similar test on 15 guinea pigs which had recovered from serum plus virus injections and later were given pad inoculations

of virus. The serum injected in these animals was derived from horse, rabbit, and guinea pig. The results follow:

Serum from a horse, supposedly hyperimmunized, was injected into four guinea pigs, which were then exposed to virus by scarification of the pads of the hind legs. All these animals showed primary and secondary lesions. On second injection with virus, 37 days afterwards, all remained normal.

Serum from a hyperimmune rabbit was injected into 8 guinea pigs which were then exposed to virus by the usual scarification method. Four showed primary and secondary vesicles and 4 only primary lesions. All the former remained normal after inoculation with virus 37 days afterwards, but of the 4 of the second group, 2 remained normal and 2 showed primary lesions only.

Pooled serum from hyperimmunized guinea pigs was injected into 3 guinea pigs which were then exposed to virus by scarification of the pads of the hind legs. Two of these animals showed primary lesions only while 1 yielded both primary and secondary vesicles. Of the former, 1 reacted with primary vesicles upon reinoculation of virus 37 days afterwards and the other remained normal, as did the one which showed both primary and secondary vesicles.

To sum up, the resistance shown in these tests was more solid than in those in which cattle-immune serum was used, for in no case were secondary lesions induced by reinoculation with virus. Furthermore, all guinea pigs which had shown primary and secondary vesicles as a result of serum plus virus injections resisted completely subsequent pad inoculations of virus.

The results obtained in these tests are in accord with those reported by Waldmann and Trautwein (*79*). However, their deduction that in the simultaneous inoculation the production of immune bodies is hindered by the use of heterologous, immune serum, whereas when homologous serum is used the effect is the same as obtained when virus alone is used, is not borne out by the findings. In the tests, immune-rabbit serum and virus produced just as solid protection in guinea pigs as did immune guinea-pig serum and virus.

ATTEMPT AT IMMUNIZATION WITH AVIRULENT BLOOD

Active guinea-pig blood was defibrinated and placed in the incubator at 37° C. for 48 hours. Tests on guinea pigs showed this blood to be then avirulent. Of 11 guinea pigs 3 were given three injections of 2 cubic centimeters of this material subcutaneously, 4 received three injections of 0.5 cubic centimeter intradermically in the right thigh, and 4 were given three inoculations in the hairless pads by scarification. These injections were made August 21, 26, and 31. On September 6 all the animals were reinoculated with active virus by scarification of the pads of the hind legs. All animals revealed primary vesicles at the site of inoculation, and later secondary vesicles.

COMPLEMENT-FIXATION TESTS

Complement fixation as a test for specific foot-and-mouth antigen or antibody reactions has been reported hitherto as valueless. The commission's results also were unsuccessful.

Attempts were made by the use of hyperimmune and immune serums to detect antigenic substances in various tissues of animals affected with foot-and-mouth disease. The hyperimmune serums consisted of Loeffler serum and that of guinea pigs and rabbits which had been given repeated injections of virus and which, according to guinea-pig titrations (detailed in the discussion of serum tests in guinea pigs, page 86) showed a high degree of activity. Serum from convalescent cattle was also used.

Virulent guinea-pig blood suspended in physiological salt solution, and virulent blood laked by the addition of distilled water to release any virus contained in the red cells, showed no antigenic value when tested with specific serum. Similarly, negative results were also obtained with fresh, active, vesicular fluid suspended in physiological salt solution or distilled water, with similar material precipitated with acetone, and dried and suspended in saline solution; and also with fluid and coverings of fresh vesicles of guinea pigs, ground in saline or distilled water and shaken for several hours. Most of the material for the preparation of antigen was obtained from guinea pigs, but several tests were made with coverings of vesicles from cattle and from swine.

Because the hyperimmune-rabbit serum showed a high titer as read by guinea-pig test, it was chosen as best for demonstrating antigen. An interesting result was obtained with this serum, the details of which follow.

An antigen was prepared from fluid and coverings of fresh vesicles from guinea pigs. The material was thoroughly ground in sand and distilled water, placed in a shaking machine for two hours, and then in the ice box overnight. The next day this antigen was tested with hyperimmune and normal rabbit serum. The latter was obtained from the same rabbit before hyperimmunization was begun.

Certain rabbit serums when inactivated at 58° C. contain anticomplementary as well as nonspecific, complement-binding properties. However, by carbolizing and heating to 62° for 35 minutes, these objections are eliminated when 0.05 and 0.1 cubic centimeter are used for test.

Therefore, rabbit serums were employed which were inactivated at 62° for 35 minutes, and tested against an antigen prepared from fluid and coverings of vesicles of guinea pigs. A marked complement-fixation reaction was noted; 0.15 cubic centimeter of the antigen produced complete fixation of complement with 0.1 cubic centimeter of the hyperimmune serum, while 2 cubic centimeters of the antigen in the presence of 0.1 cubic centimeter of normal, rabbit serum showed no anticomplementary action. Antigen and serum control tubes showed no evidence of anticomplementary action.

The hyperimmune serum had been prepared by injecting rabbits with fluid and coverings of vesicles from guinea pigs. To check the specificity of the reaction an antigen was prepared in a similar manner with the hairless pads of the hind legs of normal guinea pigs and tested against the hyperimmune and normal rabbit serums. An identical reaction was obtained with this antigen, indicating that the rabbit had developed immune bodies against guinea-pig protein. Such nonspecific reactions should be considered always, since in the absence of a pure culture of virus an antigen containing a mixture of different proteins must of necessity be employed.

Hyperimmune-rabbit serum also gave a negative reaction when tested against an antigen prepared from coverings of vesicles of cattle and hogs.

PERIOD OF INFECTIOUSNESS IN CATTLE AND IN HOGS

A limited study was made of the period of infectiousness of animals with foot-and-mouth disease, by determining the length of time that certain substances removed daily from the animals remained virulent for guinea pigs. These substances consisted of blood, urine, shreds of epithelium from ruptured vesicles, and saliva.

Material from the mouth was obtained by rubbing sterile cotton swabs over all observable lesions. If no lesions were present, the swab was passed over the tongue, gums, and dental pad. In some cases use was made of sterile, wooden spoons whereby lesions on the back of the tongue could be reached. These spoons were valuable as well for collecting considerable saliva. Whenever possible, epithelial shreds from vesicles were used, but as a rule they disappeared quickly after rupture. Later material, therefore, consisted in the main of that collected by the swabs and the spoons.

Material collected was ground in a mortar with physiological saline solution and injected into guinea pigs in the usual manner. The guinea pigs were kept under observation for at least 10 days after inoculation. For the sake of convenience the experimental data are given in Tables 25 to 35. Each animal from which material was collected is considered separately.

TABLE 25.—*Results of inoculations of guinea pigs with material taken daily from infected heifer 1, inoculated intramuscularly September 30, 1925, with Strasbourg guinea-pig virus*

Date	Temperature and symptoms of heifer	Material used for guinea-pig inoculations	Guinea pig No.	Result [1]
1925	° F.			
Oct. 4	101.6 a. m. No evidence of disease			
Oct. 5	102 a. m., 103 p. m. Mouth lesion	Mouth swab	574	Pr.+Sec.
Oct. 6	101.2 a. m., 101.6 p. m.	do	584	Do.
Oct. 7	102 a. m., 101.6 p. m.	do	595	Negative.
Oct. 8	101.4 a. m., 101.6 p. m.	do [2]	600	Pr.+Sec.
			C 1	Do.
Oct. 9	101.6 a. m., 101.8 p. m.	do	C 8	Negative.
			C 9	Do.
Oct. 10	101.6 a. m., 101.4 p. m.	do	C 16	Do.
Oct. 13	101.8 a. m., 101.8 p. m.	do	C 23	Do
			C 24	Do.

[1] The abbreviations "Pr." and "Sec." indicate primary and secondary vesicles of foot-and-mouth disease.
[2] Contained a small piece of tongue epithelium.

Heifer 1 was inoculated intramuscularly September 30, 1925, and showed lesions in the mouth October 5. Swabs of oral cavity collected October 5 and 6 proved to be infectious to guinea pigs. Swab taken October 7 was negative, but that of October 8 was positive. This material contained a small piece of epithelium of a ruptured vesicle from the tongue. Swabs taken October 9, 10, and 13 were negative. Hence, oral swabs contained virus three days after the appearance of the first lesions.

Heifer 2 contracted foot-and-mouth disease September 26, 1925, following natural exposure to heifer 10. Materials taken from the mouth September 26, 27, and 28 were infectious to guinea pigs, but later specimens were negative. Therefore, virus was present two days after lesions were first observed. (See Table 26.)

TABLE 26.—*Results of inoculations of guinea pigs with material taken daily from heifer 2 infected with foot-and-mouth disease September 26, 1925, by natural exposure to heifer 10*

Date	Temperature and symptoms of heifer	Material used for guinea-pig inoculation	Guinea pig No.	Result [1]
1925	°F			
Sept. 26	103.7 a. m., 104.4 p. m. Mouth lesion	Mouth swab	516	Pr.+Sec.
Sept. 27	102 a. m.	do	525	Do.
Sept. 28	100.6 a. m. Feet lesions	do	527	Do.
Sept. 29	101.4 a. m.	do	535	Negative.
Sept. 30	102.6 a. m., 101.6 p. m.	do	545	Do.
Oct. 1	101.2 a. m., 101.4 p. m.	do	548	Do.

[1] The abbreviations "Pr." and "Sec." indicate primary and secondary vesicles.

Heifer 3 was inoculated by scarification of the mouth September 17, 1925, and showed lesions at the site of inoculation September 18. No material was inoculated on that date. On September 19 blood was drawn at 10 a. m., when the temperature of the animal was 102.6° F., and was injected into a guinea pig with negative results. At 5 p. m. the temperature of the heifer had risen to 106°, but there was no opportunity to collect a second sample of blood at that time. Mouth swabs and shreds of epithelium of ruptured foot vesicles taken on September 20, 21, 22, and 23 were capable of infecting guinea pigs. A mouth swab on the 24th was positive and foot material negative.

Guinea pig 503 inoculated with mouth swab September 25 was killed four days after inoculation because it was affected with some intercurrent disease, but up to that time the animal showed no evidence of specific infection. Mouth swab contained virus six days after appearance of first lesion. (See Table 27.)

TABLE 27.—*Results of inoculations of guinea pigs with material taken daily from infected heifer 3, inoculated locally by scarification of the gum and dental pad September 17, 1925, with guinea-pig Strasbourg virus*

Date	Temperature and symptoms of heifer	Material used for guinea-pig inoculations	Guinea pig No.	Result [1]
1925	°F.			
Sept. 18	101 a. m., 103.3 p. m. Mouth lesion			
Sept. 19	102.6 a. m., 106 p. m.	Blood, 10 a. m.	440	Negative.
Sept. 20	103.3 a. m. Foot lesions	Mouth swab	451	Pr.+Sec.
Sept. 21	104.2 a. m., 103.6 p. m.	do	459	Do.
Sept. 22	103.6 a. m., 104 p. m.	do	464	Do.
Sept. 23	102.4 a. m., 103.4 p. m.	Foot material	469	Do.
		Mouth swab	480	Do.
		Foot material	485	Do.
Sept. 24	101.6 a. m., 102.4 p. m.	Mouth swab	491	Do.
		Foot material	496	Negative.
Sept. 25	101.2 a. m., 102 p. m.	Mouth swab	[2] 503	Do.

[1] The abbreviations "Pr." and "Sec." indicate primary and secondary vesicles.
[2] Killed Oct. 26, 1925, on account of intercurrent disease.

Heifer 4 was inoculated intradermically September 30 and showed lesions at site of inoculation at 9 a. m. October 1. The temperature of the animal at this time was 104.8° F. Blood was collected at 10 a. m. and injected into a guinea pig with negative results.

Material collected on mouth swabs October 2 was positive, but on October 3, negative. No further tests were made.

Result: Mouth swabs contained virus on the third day after first appearance of lesions.

TABLE 28.—*Results of inoculations of guinea pigs with material taken daily from infected heifer 4, inoculated September 30, 1925, intradermically on upper gum with guinea-pig Strasbourg virus*

Date	Temperature and symptoms of heifer	Material used for guinea-pig inoculations	Guinea pig No.	Result [1]
1925	°F.			
Sept. 30	102 a. m., 102.2 p. m.			
Oct. 1	104.8 9 a. m. Mouth lesions	Blood collected 10 a. m.	549	Negative.
Oct. 2	104 a. m.	Mouth swab	550	Pr.+Sec.
Oct. 3	102.8 a. m., 104 p. m. Foot lesions	do	566	Negative.
Oct. 4	102.8 a. m.	do	573	Pr.+Sec.
Oct. 5	102.2 a. m., 102.2 p. m.	do	575	Negative.

[1] The abbreviations "Pr." and "Sec." indicate primary and secondary vesicles.

Heifer 6 was inoculated intradermically September 17 and showed lesions of the disease at the site of inoculation September 18. Blood drawn September 19 at 10 a. m., when the temperature of the animal was 104.8° F. yielded a positive result when inoculated into a guinea pig. Urine collected at 3 p. m. on this date was injected into a guinea pig with negative results. Mouth swabs were infectious from September 20 to 24 and negative after that time. Foot material was negative September 22, positive September 23, and negative September 24. Active material was found in mouth swabs six days after first appearance of lesions. (See Table 29.)

TABLE 29.—*Results of inoculations of guinea pigs with material taken daily from infected heifer 6 inoculated intradermically September 17, 1925, in dental pad and lower lip with guinea-pig Strasbourg virus*

Date	Temperature and symptoms of heifer	Material used for guinea-pig inoculations	Guinea pig No.	Result [1]
1925	°F.			
Sept. 18	101 a. m., 103.8 p. m. Lesion at site of inoculation			
Sept. 19	104.8 a. m., 105 p. m.	Blood, 10 a. m.	441	Pr. + Sec.
		Urine, 3 p. m.	445	Negative.
Sept. 20	105 a. m. Foot lesions	Mouth swab	452	Pr. + Sec.
Sept. 21	104 a. m., 104.4 p. m.	do	460	Do.
Sept. 22	103 a. m., 104.2 p. m.	do	465	Do.
		Foot material	470	Negative.
Sept. 23	103 a. m., 103.4 p. m.	Mouth swab	481	Pr. + Sec.
		Foot material	486	Do.
Sept. 24	103.6 a. m., 103.6 p. m.	Mouth swab	492	Pr. + Sec.
		Foot material	497	Negative.
Sept. 25	101 a. m., 101.6 p. m.	Mouth swab	504	Do.
Sept. 26	100.4 a. m.	do	417	Do.
Sept. 29	101 a. m., 101.6 p. m.	do	534	Do.
Sept. 30	102.6 a. m., 102 p. m.	do	544	Do.

[1] The abbreviations "Pr." and "Sec." indicate primary and secondary vesicles.

Heifer 7 was inoculated by scarification of the mouth September 17, 1925, and showed definite lesions at site of inoculation September 19. On this day the temperature of the animal at 9 a. m. was 104°F., and at 5 p. m. 106°. Urine collected from this animal at 3 p. m. was injected into a guinea pig with negative results. Mouth swabs were positive on September 20 and negative after that time. Foot material was positive on September 22 but negative thereafter. Virus was found in the feet three days after first appearance of lesions. (See Table 30.)

TABLE 30.—*Results of inoculations of guinea pigs with material taken daily from infected heifer 7 inoculated by scarification of the gum and dental pad with guinea-pig Strasbourg virus September 17, 1925*

Date	Temperature and symptoms of heifer	Material used for guinea-pig inoculations	Guinea pig No.	Result [1]
1925	°F.			
Sept. 18	102 a. m., 103.4 p. m. No definite lesions.			
Sept. 19	104 a. m., 106 p. m. Mouth lesions	Urine, 3 p. m.	446	Negative.
Sept. 20	104.2 a. m. Foot lesions	Mouth swab	453	Pr.+Sec.
Sept. 21	104.2 a. m.	do	461	Negative.
Sept. 22	102 a. m., 104.4 p. m.	do	460	Do.
		Foot material	471	Pr.+Sec.
Sept. 23	102.6 a. m., 103.4 p. m.	Mouth swab	482	Negative.
		Foot material	487	Do.
Sept. 24	103 a. m.	Mouth swab	493	Do.
		Foot material	498	Do.
Sept. 25	102 a. m., 103 p. m.	Mouth swab	505	Do.
Sept. 26	102 a. m.	do	518	Do.

[1] The abbreviations "Pr." and "Sec." indicate primary and secondary vesicles.

Heifer 8 was inoculated locally September 30, 1925, and showed lesions at the site of inoculation October 1, 1925. Blood collected at 10 a. m. (temperature 102.2° F.) gave negative results when injected into a guinea pig. Mouth swab collected October 2 produced lesions of disease when inoculated into a guinea pig. No further materials were taken from this animal. (See Table 31.)

TABLE 31.—*Results of inoculations of guinea pigs with material taken daily from infected heifer 8 inoculated locally September 30, 1925, with guinea-pig Strasbourg virus*

Date	Temperature and symptoms of heifer	Material used for guinea-pig inoculations	Guinea pig No.	Result [1]
1925	°F.			
Oct. 1	102.2 a. m., 102 p. m. Shows ruptured vesicle	Blood, collected 10 a. m.	550	Negative.
Oct. 2	102.4 a. m., 103 p. m.	Mouth swab	560	Pr.+Sec.

[1] The abbreviations "Pr." and "Sec." indicate primary and secondary vesicles.

Bull 9 was inoculated intramuscularly September 17, 1925. At 4 p. m. September 18 the animal had a temperature of 104.4° F. but no lesions of the disease could be seen. A sterile cotton swab was rubbed over the gum and tongue so that it was well moistened with saliva. Material from this swab injected into a guinea pig gave a positive result, indicating that the virus was in the saliva before any lesions of the disease could be noted. A sample of blood collected at this same time also gave positive results in guinea pigs. Mouth swab was positive September 22; foot material and swab were negative on the 23d. (See Table 32.) On September 24 and 25 material from the mouth and foot were positive, but the results on these days may have been due to probable contamination of material by heifer 10. Heifer 10 had very close contact in the same stall with bull 9. Heifer 10 developed the disease September 24 and from this date the possibility of contamination of bull 9 with virus from heifer 10 can not be excluded. It appears, then, that saliva or mouth secretions removed before the appearance of lesions contain virus. Blood also is infectious at this time.

TABLE 32.—*Results of inoculations of guinea pigs with material taken daily from infected bull 9, inoculated September 17, 1925, intramuscularly with guinea-pig Strasbourg virus*

Date	Temperature and symptoms of heifer	Material used for guinea-pig inoculations	Guinea pig No.	Result [1]
1925	°F			
Sept. 18	101.4 a. m., 104.4 p. m., No indication of lesions.	Mouth swab, collected 4 p. m.	437	Pr.+Sec.
		Blood, collected 4 p. m.	438	Do.
Sept. 19	104.5 a. m., 105.3 p. m. mouth lesion			
Sept. 20	104.6 a. m., Foot lesions	Mouth swab	454	Do.
Sept. 21	103.4 a. m., 104.4 p. m.	do	462	Negative.
Sept. 22	100.8 a. m., 102 p. m.	do	467	Pr.+Sec.
		New tissue [2]	473	Negative.[3]
Sept. 23	101.4 a. m., 101.8 p. m.	Mouth swab	483	Negative.
		Foot material	488	Do.
Sept. 24	101.8 a. m., 101.4 p. m.	Mouth swab	494	Pr.+Sec.
		Foot material	499	Do.
Sept. 25	101.2 a. m., 102.2 p. m.	Mouth swab	502	Do.

[1] The abbreviations "Pr." and "Sec." indicate primary and secondary vesicles.
[2] This refers to the heavy, yellowish well-organized exudate that in many cases covers the erosion in the process of healing.
[3] Positive results later than this date must be attributed to probable contamination of material from heifer 10 which was placed in same stable with bull 9 on Sept. 19, and developed foot-and-mouth disease. Material from heifer 10 proved to be infectious on guinea-pig inoculation Sept. 23. See Table 33.

Heifer 10 was exposed to bull 9 September 19. Mouth swabs taken on the 21st and 22d were negative on guinea-pig inoculation. Mouth swab taken on the 23d was infectious for a guinea pig, although careful examination of the mouth failed to show any evidence of lesions. Mouth swabs were positive on September 25 but negative after that date. Therefore mouth swabs contained virus one day before lesions were seen and for one day after their first appearance. Since animals 9 and 10 were stabled together it is possible that active virus inoculated into guinea pig 484 may have come from bull 9. However, the fact that inoculations from bull 9 were negative three times before the appearance of lesions in heifer 10 reduces this probability. Environmental contamination, however, can not be entirely eliminated in this case. (See Table 33.)

TABLE 33.—*Results of inoculations of guinea pigs with material taken daily from infected heifer 10, exposed to bull 9, September 19, 1925*

Date	Temperature and symptoms of heifer	Material used for guinea-pig inoculations	Guinea pig No.	Result [1]
1925	°F.			
Sept. 19	103 a. m.			
Sept. 20	102. No indication of lesions.			
Sept. 21	101.8 a. m., 103 p. m.	Mouth swab	463	Negative.
Sept. 22	101.7 a. m., 103.2 p. m. No indication of lesions.	do	468	Do.
Sept. 23	101.6 a. m., 102.6 p. m. No indication of lesions.	do	484	Pr.+Sec.
Sept. 24	104.4 a. m., 104 p. m. First evidence of disease. Mouth lesion.	do	495	Do.
Sept. 25	103.4 a. m., 105 p. m.	do	505	Do.
Sept. 26	102.6 a. m.	do	519	Negative.
Sept. 27	102.4 a. m.	do	524	Do.
Sept. 28	101.6 a. m., 102 p. m.	do	526	Do.

[1] The abbreviations "Pr." and "Sec." indicate primary and secondary vesicles.

Hog 1 was inoculated by scarification of the mouth September 30 and showed lesions at the site October 1, 1925. Shreds of epitholium of vesicles from the feet were infectious for guinea pigs until October

4. After that time guinea-pig inoculations were negative. Hence, epithelium from coverings of vesicles in the feet were infectious three days after vesicles were first observed, but not later. (See Table 34.)

TABLE 34.—*Results of inoculations of guinea pigs with material taken daily from infected hog 1, inoculated September 30, 1925, by scarification of the snout with guinea-pig Strasbourg virus*

Date	Temperature and symptoms of hog	Material used for guinea-pig inoculation	Guinea pig No.	Result [1]
1925	°F.			
Oct. 1	103.6 a. m., 105 p. m. Mouth lesion.			
Oct. 2	105.4 a. m., 106.6 p. m. Feet lesions.			
Oct. 3	106 a. m., 105 p. m.	Covering from unruptured vesicle of foot.	568	Pr. and Sec.
Oct. 4	103.6 a. m.	Covering from ruptured foot vesicle.	570	Do.
Oct. 5	103.4 a. m., 104 p. m.	Foot material, ruptured vesicle	576	Negative.
Oct. 6	104.6 a. m., 100.2 p. m.	do	582	Do.
Oct. 7	104 a. m., 105 p. m.	Foot material apparently unruptured vesicle.	592	Do.
Oct. 8	105 a. m., 105 p. m.	Loose epithelium and covering of apparently unruptured vesicle.	C-2	Do.
			C-3	Do.
Oct. 9	104.8 a. m., 104.2 p. m.	Foot material, ruptured vesicle	C-10	Do.
			C-11	Do.

[1] The abbreviations "Pr." and "Sec." indicate primary and secondary vesicles.

Hog 2 was inoculated intravenously September 30, 1925, and foot lesions were noted October 1. Pieces of epithelium of ruptured vesicles on the feet October 5, 6, and 7 were negative on guinea-pig inoculation. In this case vesicle coverings collected four days after first lesions were observed were not infectious for guinea pigs. (See Table 35.)

TABLE 35.—*Results of inoculation of guinea pigs with material taken daily from infected hog 2, injected intravenously September 30, 1925, with guinea-pig Strasbourg virus*

Date	Temperature and symptoms of hog	Material used for guinea-pig inoculation	Guinea pig No.	Result [1]
1925	°F.			
Oct. 1	105.2 a. m., 106 p. m. Foot lesion.	None collected		
Oct. 2	104.8 a. m., 105.4 p. m.	do		
Oct. 3	104 a. m., 105 p. m.	do		
Oct. 4	104.8 a. m.	do		
Oct. 5	103.8 a. m., 104.4 p. m.	Covering of ruptured vesicle	577	Negative.
Oct. 6	104.8 a. m., 105.4 p. m.	Coverings from ruptured and apparently unruptured vesicles.	583	Do.
Oct. 7	103.6 a. m., 104.2 p. m.	Coverings from ruptured vesicle	593	Do.

In conclusion, it should be stated that the results of the series of tests are fragmentary because of limitations of time and also of animals. Negative results are hedged in by doubt since a larger number, fully four or five, of guinea pigs should be used when dealing with small quantities of virus, for under such conditions it sometimes happens that only one of the test animals may show positive results.

These tests confirm the findings of others, that blood and saliva may harbor the active virus of foot-and-mouth disease before the lesions of the disease are present. The activity of the virus in the vesicular fluid and in the coverings of both ruptured and unruptured

vesicles decreases rather rapidly. Its presence was not demonstrated by the writers later than six days after the appearance of lesions; and in the majority of tests with one guinea-pig inoculation active virus was not demonstrated later than three days after the appearance of the lesions. The important practical significance of these findings is that in animal inoculations for the purpose of establishing diagnosis only material from fresh, vesicles not more than 2 days old should be depended on.

That the virus was present even before the rise of temperature is strongly indicated, but not proved, by the tests with mouth swabs of heifer 10. (Table 33.) Circumstances surrounding these tests are set forth in the protocols.

SURVIVAL OF THE VIRUS OUTSIDE THE ANIMAL

Experiments on the survival of the virus outside the animal were limited by the shortness of time at the disposal of the commission. An attempt, however, was made to study this problem and in a previous section were described some tests on viability of the virus in earthworms and soil. In addition, the following data concern the survival of the active agent in tissue fragments in hay.

Coverings of unruptured, lingual vesicles from two cows with foot-and-mouth disease were collected and cut into fragments approximately 10 by 7 by 3 millimeters. Each piece was loosely wrapped in a small bundle of hay and placed in a cheesecloth sack (12 by 25 centimeters). The sacks were fastened with long strings and distributed in a stack of hay in one of the stables. At definite intervals a sack was withdrawn. The tissues when removed were found to be considerably shrunken and brittle, having a refractile appearance. They were ground in a mortar with sand and physiological saline solution and inoculated into guinea pigs in the usual manner. The results of the inoculations are given in Table 36.

TABLE 36.—*Results of guinea-pig inoculations with virus kept in hay for varying periods*

Sample No.	Time kept in hay	Guinea pig inoculated	Result [1]	Sample No.	Time kept in hay	Guinea pig inoculated	Result [1]
	Days	*Number*			*Days*	*Number*	
1	4	C6	Pr.+Sec.	5	24	197	Pr.+Sec.
		C7	Do.			198	Pr.—Sec.
2	7	C20	Do.			205	Do.
		C21	Do.			206	Do.
3	12	C39	Pr.—Sec.	6	30	207	Negative.
		C40	Pr. Died after 4 days.			208	Do.
4	20	174	Pr.—Sec.	7	50	251	Do.
		175	Do.			252	Do.
		176	Do.			253	Do.
5	24	196	M. P.—Sec.				

[1] The abbreviations "Pr.," "M. P.," and "Sec." indicate primary, mild primary, and secondary vesicles, respectively.

Table 36 shows that the virus of foot-and-mouth disease as it exists in the epithelial coverings of unruptured tongue vesicles of cattle and kept in hay at the temperature of the stable was active for guinea pigs for 30 days but not for 50 days.

It is pointed out elsewhere in this report that in the living animal the virus contained in the epithelial coverings of vesicles rapidly loses its ability to induce the disease in guinea pigs. It may so happen, however, that coverings of a freshly ruptured vesicle rich in virus may become detached from the animal and fall to the ground. That under favorable conditions such virus may retain its activity for a long period is indicated by the results of the foregoing experiment.

CARRIERS OF FOOT-AND-MOUTH-DISEASE VIRUS

It has been generally accepted by workers on foot-and-mouth disease that a very small proportion of animals recovered from foot-and-mouth disease retain active virus and later eliminate it from their bodies. (Loeffler, Nevermann, Bartolucci, Bang, Bürgi, Hess, Guillebeau, and others.) Thus are explained otherwise inexplicable origins of foot-and-mouth-disease outbreaks. Experimental work on this subject is, however, meager and the preponderance of the evidence on which the existence of carriers is based consists of field observations. This field evidence is usually derived from one of two premises: (1) Animals develop the disease after having been placed in contact with recovered cases on previously infected farms; (2) recovered animals introduced among previously noninfected herds cause infection among the latter.

The first can not be accepted as good ground on which to base the presence of carriers, since the possibility of infection from contaminated materials on the premises can not be eliminated. In spite of the conclusions reached by Lebailly (*44*), Vallée and Carré (*71*), that environs of animals soon free themselves from active virus, experimental and field evidence definitely indicate that virus may remain active and capable of producing the disease for weeks and even for months after the last animal of a herd has passed through the active stage of the disease.

The second presents more convincing evidence for the existence of carriers, for in many of the reported cases no other source of virus could be found. In Switzerland the question of the carrier has received special attention and data covering a period of years have been collected, an interpretation of which strongly favors the probability of carriers.

For controlling epizootics of foot-and-mouth disease in Switzerland special regulations are in force with reference to the danger of spread by carriers. Animals recovered from the disease are prohibited from contact with susceptible animals until eight months after recovery. The Swiss have collected numerous instances in which there appears no other explanation for infection than that of contact with recovered cases. Thus, the cantonal (*1*) statistics for the years 1919 to 1923 show 408 outbreaks in which association with previously infected animals were found. (Fig. 4.) These statistics name and include the two classes of evidence mentioned above, but unfortunately do not give separately the number of cases for each one. The records of the federal veterinarian's office in Berne for 1925 and 1926 show infection occurring in instances when recovered animals were brought into direct and indirect contact with previously noninfected cattle, for summering in Graubünden and Waadt Cantons,

in the Alps. The records also contain reports of cases in which foot-and-mouth disease occurred in previously noninfected places after the introduction of animals recovered from the disease. The following offers an example:

On premises in the vicinity of Chateau d'Œx, foot-and-mouth disease appeared December 23, 1925, eight days after the introduction of cattle which recovered from the disease in September of the same year.

The commission's discussions with the federal and cantonal veterinarians of Switzerland indicate that may outbreaks attributed to carriers belong to the group in which infection followed the placing of recovered animals on previously noninfected premises. Hofstetter's (*38*) study on the outbreak in Canton Zurich, Switzerland, contains practically the same kind of information for this canton as found

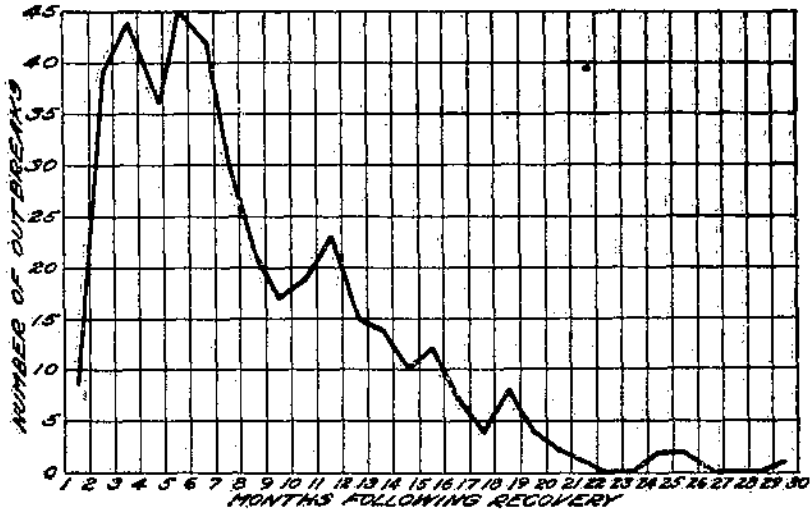

FIG. 4.—Outbreaks of foot-and-mouth disease in Switzerland caused by carriers, 1919 to 1923, inclusive. Of the total 408 outbreaks, 237 were caused by animals between the second and eighth months after recovery, and one outbreak was caused by an animal as late as 20 months after recovery from the disease

in the cantonal statistics. The same comment applies to the value of his reported cases as evidence on the carrier in foot-and-mouth disease. The governmental report of Canton Zurich for the outbreak of 1920–21 contains similar reports on the carriers in foot-and-mouth disease. It gives, however, the following general and specific incidences of infection in previously noninfected herds which followed the introduction among them of recovered animals. In Stäfa, Rafz, and Hirzel, cattle on noninfected premises became infected in 16 to 17 days and even up to 4 months after introduction of cattle which had been affected with foot-and-mouth disease from 3 to 4 months previously. An ox which recovered from foot-and-mouth disease in the early part of November, 1920, was brought to a noninfected farm in March, 1921. Seventeen days afterwards the previously noninfected cattle on this farm came down with the disease.

The British Ministry of Agriculture and Fisheries (*33*, *36*) reports two cases as follows:

Case (17) In Lancashire. At Maghull (near Liverpool) on the 11th August: In this outbreak, two animals had been purchased from premises where foot-and-mouth disease had occurred about eight months previously and in which the animals were isolated. It is possible, therefore, that in this instance the disease was introduced by one of these animals, which though making a normal recovery nevertheless was a "carrier" of infection. The great majority of recovered animals are not infective, but a very small proportion of them is believed to be capable of infecting others. It is believed that this occurs through the release of infective material held in the horn structures of the hoof which, in course of time, allows the escape of infection.

Case (8) Yorks (W. R.) (Wadsley Bridge, Sheffield) confirmed 26th March: Seven cattle were found affected out of a total of 15 cattle and 14 pigs. A bull and a heifer were purchased on July 9, 1924, from certain premises in Cheshire where an outbreak was confirmed in November, 1923, and in which isolation was adopted. During that outbreak both the bull and heifer had passed through an attack of foot-and-mouth disease, the bull recovering early in January, 1924, and the heifer about the middle of January, 1924. It would appear possible, therefore, that one of these animals was a "carrier" of infection. No further cases occurred in this area.

Mohler (*54*) reports the following observations during the 1914 and 1915 outbreak of foot-and-mouth disease in the United States:

In the early part of November, 1914, foot-and-mouth disease appeared among 747 cattle on exhibition at the National Dairy Show in Chicago. Special permission was granted by the Secretary of Agriculture to retain these cattle under absolute quarantine until they had fully recovered, and by test proved not to be disseminators of the virus. December 26, 1914, arrangements had been made to remove all but seven cattle to Cicero, Ill. These seven cows were slaughtered. Five had persistent mastitis and two reacted positively to tuberculin. The other 740 cattle were sprayed and scrubbed with 3 per cent cresol solution, were taken through a foot bath and placed overnight in cleaned and disinfected quarters, and were then placed in box cars and transported to Cicero, Ill. On March 25, 1915, 50 head of young cattle were placed in contact with the recovered cases. In addition to this contact exposure, injections of saliva, feces, urine, vaginal discharges, and scrapings from the interdigital spaces were made into the susceptible animals. On April 8, 50 hogs were added to extend the test. They were fed on milk from the recovered cows and were also allowed to consume the leavings and droppings from the cattle. Until May 30 no cases of foot-and-mouth disease had developed and the animals were released from quarantine.

The Prussian veterinary service reports many outbreaks of foot-and-mouth disease which have apparently followed the release from quarantine of recovered animals. One such outbreak followed the contact with an animal recovered from the disease seven months previously.

A resolution by the Bavarian ministry, December 27, 1912, contained the following:

There is evidence that here (in these outbreaks) are cases in which the virus (almost without exception) in the deeper cracks or in otherwise difficulty reached areas of the hoof horn of recovered animals, is finally eliminated with the worn-off horn portions of the hoof, and then taken up by susceptible animals.

The literature contains many similar references and conclusions. Although many of the reported cases are apparently free from other means of infection, the possibility of infection from unrecognized sources can not be entirely overlooked, because the disease is enzootic and at times widespread in Europe. The fact that England is freer from foot-and-mouth disease than the Continent adds weight to their observations as evidence of the existence of carriers. Nevertheless, it should be borne in mind that in England, as elsewhere,

outbreaks of the disease have occurred in which the source of infection could not be definitely determined, although carriers as the cause could be absolutely eliminated.

Assel (*4*), in 1913, in attempting to explain the origin of certain outbreaks, studied the infectivity of saliva, urine, feces, and horn tissue from animals 251 days after recovery from foot-and-mouth disease. He succeeded in producing in 1, and possibly in 2 of 3 animals, foot-and-mouth disease by exposing their injured buccal mucosa to horn tissue. He could not, however, demonstrate active foot-and-mouth disease virus in the saliva, urine, or feces from the recovered cases. De Blieck (*30*) demonstrated experimentally the active virus of foot-and-mouth disease in the hoofs of cattle 30 days but not 3 months after recovery.

Zschokke (*84*), in his gross and microscopical, anatomical studies of foot lesions in cattle, showed that the anterior portions of the sole and other areas of the hoof may contain incased lesions which can not be detected by physical examination. He suggests that in such cases the virus is protected and does not reach the exterior until the old hoof is worn down or cut out artificially. In a similar study, Böhm (*9*) shows that hogs may have hidden lesions of foot-and-mouth disease in their feet and virus contained therein is not eliminated until the hoof is worn down. He also suggests that the source of certain outbreaks that can not otherwise be traced may be due in some instances to virus which had been preserved in this manner. Böhm reports that he has induced foot-and-mouth disease experimentally with portions of horn from cattle eight months after recovery. Details are not given in his report. Lebailly (*46*) distributed 62 recovered cattle among 450 animals without the occurrence of any infection among the latter. The exposed cattle were presumably susceptible, but no definite statement to that effect was given by Lebailly nor did he test these animals for their sensitiveness to the virus.

The possibility that the virus may be retained in other portions of the body has been suggested. However, no data other than that reported by Horváth (*39*) have been submitted in support of this possibility. The experimental disease was induced with material from a cardiac abscess from an animal slaughtered from three to four months after recovery from foot-and-mouth disease.

The disease was introduced by two cows with abscesses in the valves of the left side of the heart one year after they had malignant foot-and-mouth disease. Fortner (*20*) explains the deaths in affected guinea pigs, which occur weeks after the inoculation of the virus, by the prolonged retention of the virus in bodies of the animals. He demonstrated the presence of virus in one case in the blood and salivary glands 19 days after infection. In another guinea pig he found the virus in the blood and urine 22 days, and in a third, in the blood, 34 days after inoculation. In still another guinea pig, remarkable as it may seem, the virus was found in the blood, urine, and unruptured tongue vesicles 198 days after infection.

EXPERIMENTS IN CARRIERS AT ALFORT, FRANCE

The French Ministry of Agriculture, through Professor Vallée, placed at the writers' disposal its excellent experiment station, the Laboratoire National de Recherches, at Alfort. There the American commission found an opportunity to study the carrier problem in

foot-and-mouth disease. The Swiss veterinary service, through Prof. M. Bürgi and his staff, cooperated with the writers in obtaining cattle which the former believed might be carriers. All these animals were specially selected and were of the type of recovered animals that are usually associated with outbreaks of the disease in Switzerland. Professor Vallée and Doctor Rinjard aided whole-heartedly and made the experiment possible.

On February 25, 1926, there arrived at Alfort the recovered cases of foot-and-mouth disease recorded in Table 37, which Professor Bürgi purchased for the experiment.

TABLE 37.—*List of cattle recovered from foot-and-mouth disease and used in carrier experiment*

LOT 1

Cattle No.	Age	Date foot-and-mouth disease was contracted	Cattle No.	Age	Date foot-and-mouth disease was contracted
Bull 421	15 months	Dec. 17, 1925	Cow 426	8 years	Oct. 16, 1925
Cow 422	12 years	Jan. 4, 1926	Cow 427	4 years	Sept. 6, 1925
Steer 423	2 years	Jan. 7, 1926	Heifer 428	15 months	Aug. 28, 1925
Heifer 424	2½ years	Nov. 4, 1925	Cow 429	6 years	Aug. 8, 1925
Heifer 425	2 years	Sept. 24, 1925	Heifer 430	2 years	Nov. 26, 1925

These cattle were placed for observation in the isolated foot-and-mouth-disease stalls of the laboratory. Even though special care had been taken to prevent contamination with foot-and-mouth-disease virus in transit, the bodies of the animals were freed from gross contamination of litter and manure and their feet were thoroughly washed with tap water on arrival at Alfort.

Sixteen Bretonne cattle were purchased from a locality from which the laboratory for many years obtained its susceptible cattle for experimental work on foot-and-mouth disease. These cattle, numbered 402 to 417, inclusive, arrived at Alfort February 25, 1926, and were isolated at the horse pavilion at the veterinary college and cared for by a special attendant until March 9, 1926. They were then brought to the research laboratories and placed in contact with the recovered cattle from Switzerland. The animals were moved about so that every normal, susceptible animal was continuously in contact with one or two recovered ones. During this period the stables were not cleaned for periods varying from four to seven days.

On March 12, 1926, four hogs, shipped in individual crates from Molsheim, Alsace, arrived at Alfort. They were quarantined and kept under observation for 11 days. They were then placed in the stables occupied by the cattle, in which the litter still remained. The hogs were kept in these stables for periods of from three to five days and their feed was placed on the litter.

At least two daily temperatures of all cattle and hogs were taken, and frequent examinations of the animals were made. No foot-and-mouth disease developed in the hogs or in the Bretonne cattle.

Early in April it was decided to extend the experiment by adding a second lot of recovered animals from Switzerland. These cattle arrived in Alfort April 9, 1926, and were placed in one large stable on the grounds of the laboratory. The feet of these animals were not

cleaned or washed, as was done with the first lot. Table 38 describes the second group of recovered cases:

TABLE 38.—*List of cattle recovered from foot-and-mouth disease and used in carrier experiment*

LOT 2

Cattle No.	Age	Date foot-and-mouth disease was contracted	Cattle No.	Age	Date foot-and-mouth disease was contracted
Cow 443	13 years	Oct. 30, 1925	Heifer 449	2 years	Feb. 10, 1926
Bull 448	13 months	Oct. 8, 1925	Cow 451	4 years	Feb. 5, 1926
Heifer 446	22 months	Sept. 10, 1925	Cow 444	do	Aug. 18, 1925
Cow 445	4 years	Jan. 25, 1926	Bull 450	14 months	Aug. 25, 1925
Cow 447	9 years	Jan. 20, 1926	Heifer 452	15 months	Nov. 4, 1925

Another group of 12 Bretonne cattle (431 to 442) arrived in Alfort March 30, 1926, and were placed in the horse pavilion. After 10 days' observation, on April 9, 1926, they were brought into contact with the second group of Swiss recovered cattle in one large stable. The normal animals were at all times in close contact with the recovered Swiss cattle.

The experiment was in progress several weeks but no infection occurred. It was then suggested that perhaps the small Bretonne cattle were not so susceptible as the heavier Swiss Simmenthal cattle; therefore, seven supposedly susceptible Swiss cattle (453 to 459, inclusive) were purchased. They arrived at Alfort April 15, 1926, and were kept in the horse pavilion of the veterinary college until April 24, 1926. They were then brought into contact with the second group of recovered cattle, in the large stable at the laboratory.

Until the latter part of May no disease occurred. Thereafter more rigid tests were made. The hoofs of the recovered cattle were trimmed and the removed horn allowed to remain in the stalls. The susceptible animals were exposed to this material and were also kept in contact with recovered cases for 10 days before the latter were finally slaughtered. At that time none of the exposed animals had developed foot-and-mouth disease. It was decided, however, to test the infectivity of the bile and the hoof materials from the recovered cases by injecting these materials into the Bretonne and Simmenthal susceptible animals.

On June 2 the 19 (heifer 452 died of dystocia) recovered animals were slaughtered. Samples of the bile from all the recovered cattle except Nos. 422, 424, 426, 429, and 430 were collected separately and 20 cubic centimeters of each injected subcutaneously into the following cattle June 4, 1926:

Bile from animal No.	Inoculated into animal No.	Bile from animal No.	Inoculated into animal No.
421	402	446	413
423	408	447	432
425	405	448	433
428	411	449	410
443	439	450	407
444	435	451	438
445	412		

Twenty cubic centimeters of a mixture of equal parts of the samples of bile were inoculated into each of three Simmenthal cattle (453, 455, and 457).

The feet from animals 421, 422, 423, 425, 429, 443, 447, 449, 450, and 451 showed evidence of previous foot-and-mouth-disease infection. The individual feet of these animals were kept separate and material from the lesions was cut into very small fragments, ground with salt solution, and placed under vacuum in refrigerator at −5° C. for 18 hours. The hoof lesions in cattle 421, 423, and 443 were particularly interesting because they were less dry and more hemorrhagic than the others. The material from these was treated as others, except that to each was added sufficient physiologic salt solution to cover the material. The inoculations of these suspensions were made intradermically into the mucous membrane of the lips of the following animals, each of which received 1.5 cubic centimeters. Each of the injected animals also swallowed about 2 cubic centimeters of the suspension.

Hoof material from No. 421 was inoculated into Simmenthal 456.
Hoof material from 423 was inoculated into Simmenthal 458.
Hoof material from 443 was inoculated into Simmenthal 459.

Bretonne cattle 431, 436, and 441 were inoculated with a mixture of suspensions of materials from cattle 422, 425, 429, 447, 449, 450, and 451.

None of the inoculated cattle developed foot-and-mouth disease. In view of the negative results, the next step was to test the susceptibility of the exposed animals to foot-and-mouth-disease virus. On June 14, 33 of the animals were injected intradermically in the mucous membrane of the upper lip with type O guinea-pig virus, and 32 developed typical and severe experimental foot-and-mouth disease. The period of incubation varied from 32 hours to 4 days. One animal (410), however, showed no evidence of the disease.

On July 14, 13 of the cattle were available for test for susceptibility to type A virus. The test was made by intradermic injection into the mucous membrane of the upper lip with fresh vesicle coverings from a heifer. Eleven of these animals developed severe foot-and-mouth disease. Two (402 and 417) proved to be resistant to this injection.

The four hogs developed foot-and-mouth disease as a result of inoculation with type O virus, June 14. One month later they proved to be susceptible to type A virus. Each inoculation induced a severe form of the disease. None succumbed.

Following is a summary of the results of these experiments. Twenty specially selected recovered cattle, from 58 to 234 days after infection with foot-and-mouth disease, did not transmit the disease when placed in contact with 35 cattle and 4 hogs. The periods of contact in these cases were from 56 to 87 days.

The bile from 13 of the recovered cases was injected subcutaneously into 16 cattle. Material from the foot lesions of 10 recovered cases was injected into the mucous membrane of the lips of 6 cattle. All these injections failed to infect.

Thirty-two of 33 cattle and the 4 hogs were proved to be susceptible to type O virus, and 11 of 13 of the same cattle and the 4 hogs were infected with type A virus one month after exposure to type O virus.

In addition to these experiments at Alfort, another series of tests was undertaken at Strasbourg. This comprised the injection of

guinea pigs with material from hoof lesions of the animals given in Table 39 which had recovered from experimental foot-and-mouth disease.

TABLE 39.—*Result of guinea-pig inoculations with hoof material from cattle and a hog recovered from experimental foot-and-mouth disease*

Number of animal used as hoof material source	Date of infection	Type of virus	Date of slaughter	Number of guinea pig inoculated [1]	Result
			1926		
3	Sept. 17, 1925	O	Mar. 22	839	Negative.
				840	Do.
				841	Do.
				842	Do.
4	Sept. 30, 1925	O	do	855	Do.
				856	Do.
	Feb. 2, 1926	A	do	857	Do.
				858	Do.
5	Oct. 26, 1925	A	do	835	Do.
				836	Do.
	Nov. 26, 1925	O	do	837	Do.
				838	Do.
7	Sept. 17, 1925	O	Apr. 13	840	Do.
				B75	Do.
	Jan. 8, 1926	A	do	C94	Do.
				C98	Do.
8	Sept. 20, 1925	O	do	835	Do.
				836	Do.
				848	Do.
				855	Do.
10	Sept. 25, 1925	O	Mar. 22	843	Do.
				844	Do.
	Feb. 8, 1926	A	do	845	Do.
				846	Do.
11	Nov. 26, 1925	O	Apr. 13	C95	Do.
				C83	Do.
	Jan. 4, 1926	A	do	851	Do.
				847	Do.
12	Nov. 26, 1925	O	Apr. 14	C94	Do.
				851	Do.
	Jan. 4, 1926	A	do	B74	Do.
				838	Do.
13	Dec. 9, 1925	O	Mar. 22	847	Do.
				848	Do.
	Jan. 7, 1926	A	do	849	Do.
				850	Do.
14	Jan. 15, 1926	A	May 4	----------	Do.
				967	Do.
	Apr. 14, 1926	O	do	968	Do.
15	Feb. 15, 1926	A	Apr. 14	842	Do.
				837	Do.
	Feb. 2, 1926	O	do	849	Do.
				B44	Do.
16	Mar. 8, 1926	A	Mar. 23	851	Do.
				852	Do.
				853	Do.
				854	Do.
17	Apr. 14, 1926	O	May 13	960	Do.
				961	Do.
18	May 14, 1926	O	May 4	969	Do.
				970	Do.
19	do	O	do	965	Do.
				966	Do.
20	do	O	do	976	Do.
				974	Do.
				971	Do.
				972	Do.
23	Apr. 14, 1926	O	May 18	54	Do.
				55	Do.
26	do	O	do	56	Do.
				57	Do.
30	do	O	do	62	Do.
				63	Positive.
32	do	O	do	52	Negative.
				53	Do.
33	do	O	do	58	Do.
				59	Do.
Hog 9	Mar. 2, 1926	A	May 15	42	Do.
	Apr. 14, 1926	O	do	43	Do.

[1] Some tag numbers were duplicated.

Table 39 shows that material was used from the foot lesions of 21 recovered cattle and 1 recovered hog slaughtered from 20 to 186 days after experimental infection with foot-and-mouth disease. On inoculation intradermically into the posterior pads of 68 guinea pigs, experimental foot-and-mouth disease was induced in only 1 (*63*). This guinea pig was inoculated with material from heifer 30, 34 days after infection, and was the last guinea pig of the series inoculated with hoof material. From the pad lesions of this pig, typical foot-and-mouth-disease lesions were produced in other guinea pigs. The question arises in this instance whether there is a remote possibility that virus may have contaminated foreign material which entered the space between the old and new horn where it was retained and was later included in the inoculum. The hoofs, however, were washed, scrubbed, and were otherwise carefully controlled against contamination with outside virus, but since the latter may be active in a dilution of 1:10,000,000, the question as to whether such contamination occurred in this single instance must remain unanswered at present.

FOOT-AND-MOUTH DISEASE IN THE HORSE

The similarity between foot-and-mouth disease and vesicular stomatitis, as shown elsewhere in this report, and the use of the horse as the chief means of differentiating the two diseases, make the question of the horse's susceptibility to foot-and-mouth disease important.

The writers have been unable to find in the literature any account of the definite transference of foot-and-mouth disease experimentally to horses, although a few unsuccessful attempts are recorded. Hutyra and Marek (*40*) state that experimental foot-and-mouth disease in horses was shown by Brauer and Woestendieck, but Gins and Krause (*30*) state that the latter investigators noted the disease as occurring only spontaneously and not as a result of experimental inoculation, as incorrectly stated by Hutyra and Marek. In the widespread epizootic of 1919–1921 it was reported that foot-and-mouth disease occurred in the horse in a number of instances. In these cases the animals were in direct contact with infected cattle.

The clinical picture of the disease as reported in equines is practically the same as that in cattle. A short description of four cases of foot-and-mouth disease, as recorded by Wildsfeuer (*83*), follows:

Case 1.—In a stable of six cattle infected with foot-and-mouth disease of the malignant type stood two horses from 18 to 20 years old. The horse closest to the cattle ate the same feed. On the fifth day after the outbreak of the disease in the cattle this horse began to drool profusely and did not eat. The next day its temperature was 40.2° C., pulse 60, respiration 28. There was some swelling of the throat lymphatics, drooling, the saliva was ropy and tenacious in character, almost like gum arabic, and the mucous membrane of the mouth and tongue was markedly swollen. On the buccal mucosa numerous small vesicles were also observed. At the next observation, after five days, the temperature was 39.6° C., pulse 56, respiration 28, and drooling was still pronounced. Erosions were present in the mouth at the site of the vesicles. The tongue was still swollen and showed a large area denuded of epithelium. The fever gradually disappeared, although the drooling lasted more than three weeks. The normal appetite returned after four weeks. The second animal remained healthy.

Case 2.—On a farm of 14 cattle in which a malignant form of foot-and-mouth disease appeared, a yard of 20 meters separated the horse from the cattle stable. Different attendants cared for each species. In the horse stable were 10 horses,

of which one was a yearling and the remainder from 8 to 20 years old. On the sixteenth day after the outbreak of foot-and-mouth disease in the cattle, one horse, 15 years old, became sick. His temperature was 39.8° C., pulse 55, respiration 25, he had a poor appetite, and drooling was marked. Vesicles appeared on the mucous membrane of the mouth. The course of the disease was milder than in case 1. Recovery occurred after 10 days.

Cases 3 and 4.—On a farm in which the mild type of foot-and-mouth disease broke out in cattle, the horse barn was situated 40 meters from the cow barn, a shed intervening. The animals in each inclosure were cared for by different attendants. The horse stock consisted of five animals. One animal, 3 years old, was found affected. The floor in front of this animal was found wet with saliva. When moved the horse was somewhat unsteady on his hind legs. His temperature was 40.8° C., respiration 30, and pulse 68. The throat lymphatics were slightly swollen. The buccal mucosa and tongue were swollen and vesicles were observed in the mouth. Drooling occurred. The animal was observed four days later and was found to be somewhat improved. The owner reported that the animal stabled next to the sick horse had shown symptoms of the disease the previous day. On examination this animal showed the same conditions as the first, but not in so marked a form. Six days later both horses were recovered. Wildsfeuer concludes, therefore, that horses can be infected with foot-and-mouth disease either directly or indirectly; that this condition is more common than heretofore supposed, and that owners, perhaps, may hide the fact for fear of quarantine restrictions of horse stock.

As a result of a questionnaire of the Reichsgesundheitsamt (government health office) sent to official and practicing veterinarians throughout Germany following the epizootic of 1919–1921, these reports were received on foot-and-mouth disease in the horse (*82, p. 519*):

Cases were observed in which individual horses contracted the disease after close contact with affected cattle or contaminated feed and in which the exposure was heavy. The symptoms were loss of appetite, fever, vesicles on lips and mucous membrane of mouth, erosions on the tongue, and ulcers on the gums, and more or less drooling. The symptoms were similar to those seen in cattle. General disturbances, as colic and abortions, were also noted. In the main the disease was nonlethal and mild, and recovery took place in from 3 to 14 days. In foals, however, deaths occured when milk from infected cows was consumed. Changes in the heart muscles were noted, similar to those found in fatal bovine infections.

Giovine (*31*) observed foot-and-mouth disease in a mule which was in contact with two affected cattle and ate of the same feed moistened with saliva of the cattle. It reacted with typical vesicles on the lips and tongue and recovered in 20 days.

In comparative studies which the writers made with the virus of foot-and-mouth disease and vesicular stomatitis, the susceptibility of horses to both viruses was put to test. Six horses injected with foot-and-mouth-disease virus (two of which were inoculated with type A) were unaffected. Four of the horses were inoculated with the virus of vesicular stomatitis and all contracted the disease. The detailed results of inoculations follow:

Horse 1.—On November 26, 1925, the horse was inoculated by scarification of the mucous membrane of the lower lip and tongue with guinea-pig type O foot-and-mouth-disease virus. The animal remained normal. Two cattle inoculated by scarification of the mucous membrane of the mouth, one intramuscularly, and three hogs intravenously with the same material, developed the disease.

On December 12 hyperimmunization of horse 1 was begun. This animal received 17 injections of active foot-and-mouth-disease virus subcutaneously, intravenously, and intradermically as shown in Table 21. At no time was any evidence of foot-and-mouth disease seen as a result of these injections. Furthermore, its serum failed to protect against foot-and-mouth-disease virus in guinea-pig tests.

On January 25, 1926, the animal was exposed by scarification of the tongue and mucous membrane of the lower lip to active type A virus. It remained normal. Control guinea pigs inoculated with this same material contracted the disease in the usual manner. On February 15 the horse was exposed to guinea-pig, vesicular-stomatitis virus by scarification of the tongue and the mucous membrane of the lower lip. On February 17 it showed well-marked lesions of vesicular stomatitis. On March 4 it was again exposed to vesicular-stomatitis virus by scarification of the mucous membrane of the lower lip. The animal remained normal.

Horse 1 was therefore resistant to the effects of both O and A types of foot-and-mouth-disease virus, but was susceptible to later injections of vesicular-stomatitis virus.

Horse 2.—On January 25 horse 2 was inoculated by scarification of the tongue and mucous membrane of the lower lip with active type A foot-and-mouth-disease virus. The animal remained normal. Control guinea pigs inoculated with the same material became infected.

Horse 3.—On April 21 horse 3 was inoculated by scarification of the tongue and mucous membrane of the upper lip with type O virus. The animal failed to show any evidence of disease. Control guinea pigs inoculated with the same material contracted the disease. On March 26 horse 3 was exposed to vesicular-stomatitis virus by scarification of the tongue and the mucous membrane of the lower lip. On March 29 the tongue showed well-marked lesions of vesicular stomatitis.

Horse 4.—On April 21 horse 4 was similarly inoculated with type O virus. The animal failed to show any evidence of disease. Control guinea pigs injected with the same material contracted the disease in the usual manner. On March 29 the horse contracted vesicular stomatitis as a result of exposure to this virus by scarification of the tongue and mucous membrane of the upper lip.

Horse 5.—On April 21 horse 5 was injected intramuscularly with type O virus. It remained normal. Control guinea pigs injected with this material were positive. On April 5 the horse contracted vesicular stomatitis as a result of inoculation by scarification of the tongue and mucous membrane of the lower lip.

Horse 6.—Horse 6 on May 28 was injected intramuscularly with type O virus. The animal remained normal, although control guinea pigs inoculated with the same material became infected.

These experiments show the marked resistance of the horse to types A and O of the virus of foot-and-mouth disease. Its insusceptibility is further borne out by field experience in the United States. With reference to the few prior reports on the infrequent and irregular occurrence of the disease in horses, the experimental evidence presented indicates that there is a possibility of confusion of foot-and-mouth disease with vesicular stomatitis in the horse, especially since the clinical picture of both diseases in cattle may at times be very much alike. Until direct experimental proof is presented to the contrary, the horse should be considered as practically insusceptible to the virus of foot-and-mouth disease.

COMPARATIVE STUDIES ON VESICULAR STOMATITIS AND FOOT-AND-MOUTH DISEASE

The similarity in the clinical picture of vesicular stomatitis and of foot-and-mouth disease in cattle is at times strikingly close. The determination of a distinction between the two should be important from the points of view of epizoology and control. Hence, during the course of the commission's studies on the virus of foot-and-mouth disease, an investigation was made of the comparison of one disease with the other.

This section deals with comparative studies on the two viruses; their action in guinea pigs, cattle, swine, and horses; the immunity induced, the results of cross-immunity tests with the different animals, and the filtrability of the viruses.

EXPERIMENTAL VESICULAR STOMATITIS IN GUINEA PIGS

Waldmann and Pape (*77*) succeeded in producing experimental foot-and-mouth disease quite regularly in guinea pigs. Their results were amply confirmed by others, including the British and American commissions. The transmissibility of this virus to guinea pigs is now regarded as an established fact.

The writers' first experiment in a comparative study of vesicular stomatitis and foot-and-mouth disease began, therefore, with an attempt to transfer the virus of the former to guinea pigs. Portions of the tongue of a horse showing lesions were received November 18, 1925, from Washington, D. C. This was the only strain of vesicular-stomatitis virus available for the work. A salt-solution suspension of this virus, which was sent in 50 per cent glycerol, was applied to the scarified hind pads of three guinea pigs November 21, 1925. On November 22 all three showed inoculation, or primary, vesicles.[35] Generalization of the disease, as evidenced by secondary-vesicle formations on the pads of the front legs of one guinea pig, appeared November 29. The other two failed to exhibit secondary lesions. Cotton (*14*) recently has also reported the transmission of vesicular stomatitis to guinea pigs.

In general, the virus of vesicular stomatitis induced in guinea pigs lesions similar to those yielded by that of foot-and-mouth disease. In the writers' experience with these viruses it was found that as a rule vesicular-stomatitis virus was slower in producing primary and secondary vesicles. Primary vesicles appeared from the thirtieth to the forty-eighth hour, and secondary usually from the seventy-second to the ninety-sixth hour after inoculation. But this is not a definite distinction between the viruses, for infrequently there may be a delay in the appearance of the lesions of foot-and-mouth disease. Indeed, with the strain of the type A virus used by the writers, primary vesicles did not usually appear until between the thirtieth and forty-eighth hour, though occasionally, as after the first inoculation with vesicular-stomatitis virus, the primary vesicles may appear within the first 24 hours.

In 250 guinea pigs inoculated with vesicular-stomatitis virus, one-half the number developed secondary vesicles, while the remainder showed only primary lesions on the inoculated pads. With foot-and-mouth-disease virus, on the other hand, the development of the secondary vesicles is the rule. Nevertheless, there are some strains of foot-and-mouth-disease virus which may also fail to produce secondary vesicles, especially in early guinea-pig passages, in a certain, though small, number of cases. Although there is a difference between the time of the appearance of the lesions and the number of cases in which secondary vesicles develop, this difference offers no definite basis for differentiating the viruses. The results of the test are shown in Table 40.

Furthermore, Table 40 indicates that the number of guinea-pig passages had no appreciable effect on the development of secondary lesions.

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[35] The term "primary" vesicle as used in this report refers to the vesicle at the site of inoculation and the term "secondary" vesicle refers to generalization of the disease as evidenced by the formation of vesicles at points other than the site of inoculation.

TABLE 40.—*Effect of guinea-pig passage on the development of secondary vesicles in vesicular stomatitis*

Vesicle development [1]	First to tenth passage	Eleventh to twentieth passage	Twenty-first to thirtieth passage
Guinea pigs developing primary but no secondary vesicles (Pr.—Sec.)	87	27	11
Guinea pigs developing both primary and secondary vesicles (Pr.+Sec.)	02	25	8

[1] The abbreviations "Pr." and "Sec." refer to primary and secondary vesicles.

Of all the guinea pigs inoculated by intradermic injection or by local application of virus to the hind pads (injured by scarification or puncture with a fine knife or needle), only one animal exhibited a tongue lesion. This, as well, is not a difference between the viruses, since a number of guinea pigs inoculated with some strains of foot-and-mouth-disease virus also fail to develop vesicles on the tongue. However, when larger doses of vesicular-stomatitis virus were employed, and these were injected either intramuscularly or intradermically in hairy areas, no vesicles developed at the site of inoculation, but in a considerable number of cases, lesions were noted on the tongue.

Thus of 15 guinea pigs inoculated intradermically in the abdominal region, 6, or 40 per cent, and of 15 inoculated intramuscularly, 3, or 20 per cent, exhibited lesions on the tongue. The erosions following the rupture of the vesicle in these cases were more severe and healed much more slowly than those which occurred after inoculation of the foot-and-mouth-disease virus in the pads of the feet. One animal of these series, as a result of intramuscular inoculation, yielded vesicles on two feet as well as on the tongue, while after the intradermic inoculation a single animal developed lesions on the feet only; the other 19 failed to show any evidence of disease.

From the foregoing it will be observed that so far as the effects of injecting the virus of foot-and-mouth disease or of vesicular stomatitis into guinea pigs are concerned, there is little, if any, difference between them.

CROSS-IMMUNITY TESTS OF VESICULAR STOMATITIS AND FOOT-AND-MOUTH-DISEASE IN GUINEA PIGS

The writers then attempted to discover any differential factor in the immunity reactions of these two viruses.

Table 41, test 1, shows that 18 guinea pigs which had recovered from vesicular stomatitis failed to yield lesions when again inoculated with vesicular-stomatitis virus, while of 31 guinea pigs, recovered from one or more types of foot-and-mouth disease, 30 were susceptible to vesicular stomatitis.

Test 2 shows that when 14 guinea pigs recovered from vesicular stomatitis, were inoculated with type A foot-and-mouth-disease virus, all exhibited primary and secondary vesicles. Moreover, 6 control animals, recovered from the type A virus, after reinoculation with the same type A virus, revealed no secondary lesions, but in 3 only mild, primary vesicles.

Test 3 reveals that of 11 vesicular-stomatitis convalescents, inoculated with type O foot-and-mouth-disease virus, all showed primary and secondary vesicles. Of the 12 recovered from type O foot-and-

mouth-disease virus, 10 resisted infection with this strain completely, and 2 developed mild primary but no secondary lesions.[36]

TABLE 41.—*Results of cross-immunity tests with vesicular stomatitis and foot-and-mouth-disease viruses in guinea pigs*

Test	Number of guinea pigs in test	First exposure: Virus used [1]	First exposure: Result [2]	First exposure: Period before second exposure	Second exposure: Virus used	Second exposure: Date	Second exposure: Result [2]
				Days		1926	
1	18	Vesicular stomatitis.	11 Pr.+Sec., 7 Pr.—Sec.	22 to 25	Vesicular stomatitis.	Jan. 15	18 negative.
	12	Foot-and-mouth O-1.	12 Pr.+Sec.	21 to 54			8 Pr.+Sec., 4 Pr.—Sec.
	11	Foot-and-mouth A[3].	6 Pr.+Sec., 5 Pr.—Sec.	35 to 54			8 Pr.+Sec., 3 Pr.—Sec.
	3	Foot-and-mouth O-1	3 Pr.+Sec.	92 to 94			2 Pr.+Sec., 1 Pr.—Sec.
		Foot-and-mouth A	do	36			
		do	2 Pr.+Sec., 1 Pr.—Sec.	76 to 81			1 Pr.+Sec.
	3	Foot-and-mouth O-1.	3 Pr.+Sec.	51			2 Pr.—Sec.
		Foot-and-mouth A.	2 Pr.+Sec.	74 to 76			1 Pr.+Sec. 1 Negative.
	2	Foot-and-mouth O-2.	do	46			
2	14	Vesicular stomatitis.	9 Pr.+Sec., 5 Pr.—Sec.	31 to 61	Foot-and-mouth A	Jan. 21	14 Pr.+Sec.
	6	Foot-and-mouth A.	4 Pr.+Sec., 2 Pr.—Sec.	43 to 56			3 M. P.—Sec. 3 negative.
3	11	Vesicular stomatitis.	9 Pr.+Sec., 2 Pr.—Sec.	39 to 66	Foot-and-mouth O[4]	Jan. 25	11 Pr+Sec.
	12	Foot-and-mouth O-1.	12 PR.+Sec.	20 to 84			10 negative. 2 M. P.—Sec.

[1] In these tests one vesicular-stomatitis virus, two type O and one type A foot-and-mouth-disease viruses were used.

[2] "Pr.—Sec." indicates development of primary vesicles but no secondary lesions. "Pr.+Sec." indicates development of both primary and secondary vesicles. "M. P." (mild primary) indicates small, primary vesicles.

[3] In the early guinea-pig passages with foot-and-mouth-disease virus type A secondary lesions were not produced regularly.

These series of tests, the results of which are recorded in Table 41, demonstrate definitely that vesicular-stomatitis virus can readily and regularly be distinguished from the two types of foot-and-mouth-disease virus by guinea-pig cross-immunity tests, and these therefore should be aids in differentiating the two diseases.

As discussed elsewhere in this report, the serum of an animal collected a short time after recovery from foot-and-mouth disease contains antibodies which are specific for the type of virus inducing the disease. The serum of animals recovered from vesicular stomatitis also contains protective substances against the virus of vesicular stomatitis.

Cross-immunity tests were made with the serum of animals recovered from vesicular-stomatitis and from foot-and-mouth-disease types A and O. Guinea pigs were injected subcutaneously with serum and then exposed to virus by scarification of the pads of the hind legs in the usual manner. (See Table 42.)

[36] Immunity in foot-and-mouth disease may be local and manifest itself by the prevention of primary-vesicle formation at the site of inoculation, or it may be general and prevent the development of secondary lesions. The local immunity is the first to disappear. Thus guinea pigs may still possess immunity against a strain of foot-and-mouth-disease virus even though primary inoculation vesicles do occur, provided such guinea pigs are protected from secondary vesicles against a virus which constantly produces secondary lesions. The guinea pigs recorded "Pr."—"Sec." (primary vesicles only) with the viruses used are considered immune to that virus.

TABLE 42.—*Serum cross-immunity tests with guinea pigs*

Guinea pig No.	Virus	Serum used		Result [1]	Guinea pig No.	Virus	Serum used		Result [1]	Guinea pig No.	Virus	Serum used		Result [1]
		Kind	Quantity				Kind	Quantity				Kind	Quantity	
			C. c.					C. c.					C. c.	
1	Vesicular stomatitis.	Vesicular stomatitis.	0.1	Pr.—Sec.	1	Foot-and-mouth type O.	Vesicular stomatitis.	0.1	Pr.+Sec.	1	Foot-and-mouth type A.	Vesicular stomatitis.	0.1	Pr.+Sec.
2	do	do	.1	Do.	2	do	do	.1	Do.	2	do	do	.1	Do.
3	do	do	.5	Do.	3	do	do	.5	Do.	3	do	do	.5	Do.
4	do	do	.5	Do.	4	do	do	.5	Do.	4	do	do	.5	?Pr.—Sec.
5	do	do	1.0	Do.	5	do	do	1.0	Do.	5	do	do	1.0	Pr.+Sec.
6	do	do	1.0	Do.	6	do	do	1.0	Do.	6	do	do	1.0	Do.
7	do	do	3.0	Do.	7	do	do	3.0	Do.	7	do	do	3.0	Do.
8	do	do	3.0	Do.	8	do	do	3.0	Do.	8	do	do	3.0	Pr.—Sec.
9	do	Foot-and-mouth type O.	.1	Do.	9	do	Foot-and-mouth type O.	.1	Do.	9	do	Foot-and-mouth type O.	.1	Pr.+Sec.
10	do	do	.1	Do.	10	do	do	.1	Do.	10	do	do	.1	Do.
11	do	do	.5	Pr.+Sec.	11	do	do	.5	Pr.—Sec.	11	do	do	.5	Do.
12	do	do	.5	Do.	12	do	do	.5	Do.	12	do	do	.5	Do.
13	do	do	1.0	Do.	13	do	do	1.0	Do.	13	do	do	1.0	Do.
14	do	do	1.0	Pr.—Sec.	14	do	do	1.0	Do.	14	do	do	1.0	Do.
15	do	do	3.0	Pr.+Sec.	15	do	do	3.0	Do.	15	do	do	3.0	Do.
16	do	do	3.0	Do.	16	do	do	3.0	Do.	16	do	do	3.0	Do.
17	do	Foot-and-mouth type A.	.1	Do.	17	do	Foot-and-mouth type A.	.1	Pr.+Sec.	17	do	Foot-and-mouth type A.	.1	Do.
18	do	do	.1	Pr.—Sec.	18	do	do	.1	Do.	18	do	do	.1	Do.
19	do	do	.5	Do.	19	do	do	.5	Do.	19	do	do	.5	Pr.—Sec.
20	do	do	.5	Pr.+Sec.	20	do	do	.5	Do.	20	do	do	.5	Do.
21	do	do	1.0	Do.	21	do	do	1.0	Do.	21	do	do	1.0	Do.
22	do	do	1.0	Do.	22	do	do	1.0	Do.	22	do	do	1.0	Do.
23	do	do	3.0	Pr.? Sec.	23	do	do	3.0	Do.	23	do	do	3.0	Do.
24	do	do	3.0	Pr.—Sec.	24	do	do	3.0	Do.	24	do	do	3.0	Do.
25	do	Control		Pr.+Sec.	25	do	Control		Do.	25	do	Control		Pr.+Sec.
26	do	do		Do.	26	do	do		Do.	26	do	do		Do.
27	do	do		Do.	27	do	do		Do.	27	do	do		Do.

[1] "Pr.+Sec." indicates primary and secondary vesicles. "Pr.—Sec." indicates primary vesicles only. "?Pr." indicates questionable primary vesicles. "?Sec." indicates questionable secondary vesicles.

As will be seen in Table 42, 0.1 cubic centimeter of vesicular-stomatitis-immune serum prevented generalization of vesicular-stomatitis virus, while 3 cubic centimeters failed to protect against generalization of foot-and-mouth-disease-virus types O and A. Five-tenths of 1 cubic centimeter of convalescent serum from type O foot-and-mouth disease prevented generalization of the type O virus, while 3 cubic centimeters of this serum failed to prevent generalization of type A foot-and-mouth-disease virus and of the vesicular-stomatitis virus. Five-tenths of 1 cubic centimeter of convalescent serum from type A foot-and-mouth disease prevented generalization of the type A virus, while 3 cubic centimeters failed to prevent generalization against type O virus. One cubic centimeter of this serum failed to protect against generalization of the vesicular-stomatitis virus. Of two guinea pigs inoculated with 3 cubic centimeters of serum, one showed questionable secondary lesions, while the second exhibited only primary lesions. These results, therefore, indicate that serum from recovered animals which were previously infected with one type of virus fails to neutralize the effects of another type.

EXPERIMENTAL VESICULAR STOMATITIS IN CATTLE

The commission then directed its attention to a study of the lesions produced by these viruses in large animals.

Vesicular stomatitis, like foot-and-mouth disease, is characterized by the formation of vesicles on the mucous membrane of the mouth which are followed by erosions. Before and during the appearance of the vesicles there is fever. In foot-and-mouth disease, as a rule, the lesions are more extensive and more severe. But in vesicular stomatitis the injury may be so extensive, and in foot-and-mouth disease so mild that a differential diagnosis becomes extremely hazardous. In the inoculated animals this condition was oftentimes strongly manifest.

Table 43 shows that 40 cattle were exposed to the virus of vesicular stomatitis by one of several modes of injection. The 12 cattle inoculated locally exhibited the disease. The injection in each case was made by applying the virus to scarified areas on the upper gum and tongue. Foot lesions were not found in any of the cases so inoculated, nor did the writers observe definite, secondary vesicles in the mouth, although extension from the point of injection was often seen.

Of the 14 cattle inoculated intramuscularly with 1 to 3 cubic centimeters of heavy suspensions of virus, none developed definite lesions of vesicular stomatitis. One animal so treated showed a series of exfoliations of epithelium on the tongue, but two susceptible guinea pigs injected with material obtained on the day of the appearance of the lesions failed to develop the disease. On the other hand, the commission's experience, confirming that of others, with intramuscular inoculations with foot-and-mouth-disease virus indicates that this procedure is very efficient in producing the disease in cattle.

Eight cattle were inoculated intravenously with a heavy suspension of vesicular-stomatitis virus. Six remained unaffected while two exhibited the disease. Of these two, one showed a mild lesion on one foot only, material from which induced vesicular stomatitis in

guinea pigs. The other positive case showed lesions around the nostrils and muzzle. These two animals owing to unavoidable circumstances, were handled by the same attendant in the same stable in adjoining stalls. The location of the lesions on the nostrils and muzzle of the one suggests that the virus may have been transferred by the attendant while holding this animal for examination.

TABLE 43.—*Results of cattle inoculations with vesicular-stomatitis virus*

Source of virus	Date of inoculation	Method of exposure							
		Local injection	Result	Intramuscular injection	Result	Intravenous injection	Result	Contact	Result
Horse 1 (vesicle coverings).	Feb. 17	4	4 positive	4	4 negative			6	3 positive, 3 negative.[1]
Guinea pigs 834, 835, 837, 839.	Mar. 24	2	2 positive						
Guinea pigs 821–23, B–75.	Mar. 10					1	1 negative		
Horse 4	Mar. 29	2	2 positive						
Guinea pigs 849 and 850.	Mar. 25	1	1 positive						
Horse 4	Mar. 29	2	2 positive						
Heifer 28	Mar. 30	1	1 positive	2	2 negative	7	2 positive, 5 negative.		
Guinea pigs 976–981.	May 2			8	8 negative				
Total		12	12 positive	14	14 negative	8	6 negative, 2 positive.	6	3 positive, 3 negative.

[1] Record of exposure of three negative cases: Heifer 8 was placed in same stall with heifer 3 Feb. 17, 1926. On this day heifer 3 was inoculated by scarification with vesicular-stomatitis virus and showed lesions of the disease Feb. 19, 1926; heifer 15 was placed in same stall with heifer 5 Feb. 17, 1926. On this day heifer 5 was inoculated by scarification with vesicular-stomatitis virus and showed lesions of the disease Feb. 19, 1926; bull 18 was placed in same stall with heifer 7 Feb. 19, 1926. Heifer 7 showed first symptoms of vesicular stomatitis on that day.

Six animals were exposed by contact to others suffering from the disease. Three contracted the malady and three remained well. As the footnote of Table 43 indicates, the animals were brought together at the time when the lesions first appeared, or even before that time. Contact at such time has been found necessary in foot-and-mouth disease, to insure exposure during the infective stage, and this probably applies also to vesicular stomatitis. Hence, it may be concluded that the virus of vesicular stomatitis induces lesions indistinguishable from those of foot-and-mouth disease. However, a difference was noted in the results of various methods of inoculations: Intramuscular or intravenous injection of vesicular-stomatitis virus, even with massive doses, failed in the commission's experiments to induce obvious lesions regularly, but the same methods with foot-and-mouth-disease virus yielded, as a rule, the typical vesicles.

IMMUNITY IN CATTLE FOLLOWING EXPOSURE TO VESICULAR-STOMATITIS VIRUS

Owing to the limited time for its studies, the commission had no opportunity to observe the duration of immunity in cattle which follows recovery from the natural or artificially induced disease. From the data presented in the preceding pages it is evident that immunity to vesicular-stomatitis infection should be based on the

resistance to inoculations by scarification, since the intramuscular or the intravenous methods of inoculation usually fail to provoke visible lesions. The following is a summary of the results of immunity tests in animals which were exposed, by different methods, to the active agent of vesicular stomatitis.

Three cattle which recovered from the disease after local inoculation (by scarification) were proved to be resistant when given, subsequently, similar injection.

Six cattle received the first inoculation intramuscularly, with negative results. Later, 4 were injected locally; of these, 3 were immune and 1 developed the disease. The second inoculation in the positive case was made only five days after the first. The remaining 2 cattle in this group were later injected intravenously, with negative results. Hence no conclusions could be drawn as to their immunity, for this method may fail to reveal demonstrable lesions.

Six cattle inoculated intravenously with negative results were injected later locally. All proved to be resistant.

Six cattle were first exposed to vesicular stomatitis by being brought into contact with infected cattle. Three animals developed the disease. The three unaffected ones were later injected intravenously and therefore yielded no evidence on which to draw conclusions as to their immunity.

These results indicate that immunity in vesicular stomatitis in cattle is easily induced. It is important to note that resistance follows intramuscular or intravenous injection of virus, although no visible lesions are produced, at least by intramuscular injection.

CROSS-IMMUNITY TESTS OF VESICULAR STOMATITIS AND FOOT-AND-MOUTH DISEASE IN CATTLE

The immunity in cattle following exposure to the virus of vesicular stomatitis has been demonstrated; similarly, animals recovered from foot-and-mouth disease are resistant to reinfection with the same type of foot-and-mouth-disease virus for varying periods of time.

The close similarity between foot-and-mouth disease and vesicular stomatitis gave rise to the idea of the possible resistance to foot-and-mouth disease of cattle recovered from or immune to vesicular stomatitis. The ability to produce immunity to vesicular stomatitis in cattle without producing visible lesions as a result of intramuscular injections lent encouragement to this idea. Additional support to it is found in the case of cowpox and smallpox. The two viruses are closely related but not identical, yet the cowpox virus produces an immunity against smallpox. The results of cross-immunity tests between foot-and-mouth-disease virus and vesicular stomatitis in guinea pigs failed to indicate such protection. However, guinea pigs, while furnishing an ideal means for the study of foot-and-mouth-disease virus, are not naturally susceptible to the disease, but cattle are. Hence, cross-immunity tests in cattle were considered with especial interest to determine this point.

Table 44 shows the results of inoculation with foot-and-mouth-disease virus of cattle recovered from or immune to vesicular stomatitis. The animals were exposed by means of scarification of the mucous membrane of the gum, dental pad, or tongue, or by intramuscular injection, and it will be seen that all the animals thus inoculated developed foot-and-mouth disease.

TABLE 44.—*Results of immunity tests of cattle immune to or recovered from vesicular stomatitis when exposed to foot-and-mouth-disease virus by scarification or intramuscular injection*

Animal No.	Previous history	Date of exposure to foot-and-mouth disease	Method of exposure and type of virus used	Result [1]
		1926		
14	Immune to vesicular stomatitis, Mar. 10, 1926.[2]	Apr. 14	Intramuscular, O virus	Positive, Apr. 16, 1926.
17	Recovered from vesicular stomatitis, Feb. 17, 1926.	do	Scarification, O virus	Do.
18	Immune to vesicular stomatitis, Mar. 4, 1926.	do	do	Do.
19	Immune to vesicular stomatitis, Feb. 17, 1926.	do	Intramuscular, O virus	Do.
20	Recovered from vesicular stomatitis, Mar. 24, 1926.	do	Scarification, O virus	Do.
23	Immune to vesicular stomatitis, Mar. 30, 1926.	do	Intramuscular, O virus	Positive, Apr. 17, 1926.
25	Recovered from vesicular stomatitis, Mar. 30, 1926.	do	do	Positive, Apr. 18, 1926.
26	Immune to vesicular stomatitis, Mar. 30, 1926.	do	Scarification, O virus	Positive, Apr. 17, 1926.
27	do	do	Intramuscular, O virus	Positive, Apr. 16, 1926.
30	do	do	Scarification, O virus	Positive, Apr. 17, 1926.
31	do	do	Intramuscular, O virus	Positive, Apr. 16, 1926.
32	do	do	Scarification, O virus	Do.
33	do	do	do	Do.
34	Recovered from vesicular stomatitis, Mar. 30, 1926.	do	Intramuscular, O virus	Do.

[1] All animals contracted foot-and-mouth disease in typical form.
[2] Immune as a result of intramuscular or intravenous injection. These animals showed no visible lesions of vesicular stomatitis.

Exposure was next made by contact. Table 45 gives the results of the first experiment. On April 27, four cattle (Nos. 21, 22, 28, and 29), that had recovered from vesicular stomatitis, were placed in a shed with two normal cattle (Nos. 37 and 38). On that day the two normals were inoculated with foot-and-mouth-disease virus by scarification of the mucous membranes of the gum and dental pad of the upper jaw. In 48 hours both animals showed primary vesicles at the site of inoculation, followed in a few days by vesicle formation on other parts of the mouth and on the feet. The four test animals were in very close contact with the affected animals and all were fed and watered by the same attendant. On May 7 animal 28 developed foot-and-mouth disease; the remaining three test animals were were again placed in contact with No. 28 but failed to contract the disease. The experiment was terminated May 20. Control animals for this experiment were not available and for that reason the experiment was not conclusive. However, three out of four animals recovered from vesicular stomatitis, placed in contact with cattle suffering from acute foot-and-mouth disease, and thus actively exposed to the virus, resisted infection.

As a result of the apparent resistance to foot-and-mouth disease, by contact, of three out of four cattle recovered from vesicular stomatitis, it was desirable to obtain further information on this subject, particularly as to the resistance of animals that have become immune to vesicular stomatitis as a result of intramuscular injection.[37]

[37] Through limitation of time, immunological studies on foot-and-mouth disease and on vesicular stomatitis in large animals were begun only toward the end of the commission's stay in Europe.

TABLE 45.—*Results of immunity tests of cattle recovered from vesicular stomatitis when exposed to foot-and-mouth disease by contact*

Animal No.	Previous history	Date of exposure to foot-and-mouth disease	Method of exposure and type of virus used	Result[1]
		1926		
21	Recovered from vesicular stomatitis Mar. 24, 1926.	Apr. 27	Natural exposure to Nos. 37 and 38.	Negative.
		May 7	Natural exposure to No. 28.	
22	Recovered from vesicular stomatitis Mar. 28, 1926.	Apr. 27	do	Do.
		May 7		
29	Recovered from vesicular stomatitis Mar. 30, 1926.	Apr. 27	do	Do.
		May 7		
28	Recovered from vesicular stomatitis Mar. 29, 1926.	Apr. 27	Exposure to Nos. 37 and 38	Positive, May 7, 1926.
37	Normal animal	do	Local scarification guinea pig O virus.	Positive, Apr. 29, 1926.
38	do	do	do	Do.

[1] Experiment terminated May 20, 1926.

TABLE 46.—*Results of immunity tests of cattle recovered from or immune to vesicular stomatitis when exposed to foot-and-mouth disease by contact*

Animal No.	History of vesicular stomatitis	Date of exposure to foot-and-mouth disease	Method of exposure and type of virus	Result
		1926		
51	Intramuscular injection, May 11, 1926.	May 25	Natural exposure to virus, animals 39, 40, 41, 42, 43, 44, 45, 46, 65, 66, 74.[1]	Positive, June 9, 1926.
52	do	do	do	Positive, May 30, 1926.
53	Intramuscular injection, May 11, 1926, May 15.	do	do	Positive, June 1, 1926.
54	do	do	do	Positive, June 4, 1926.
55	Intramuscular injection, May 11, 1926.	do	do	Positive, May 31, 1926.
57	Intramuscular injection, May 11, 1926, May 15.	do	do	Negative.
58	do	do	do	Do.
21	See Table 44	do	do	Do.
23	do	do	do	Positive, May 31, 1926.
29	do	do	do	Negative.
67	Normal animal (control)	do	do	Positive, June 10, 1926.
68	do	do	do	Negative.
69	do	do	do	Positive, June 1, 1926.
70	do	do	do	Positive, May 31, 1926.
71	do	do	do	Negative.
72	do	do	do	Do.
73	do	do	do	Positive, June 3, 1926.
39	Normal animal (virus animals)	do	Local scarification, guinea-pig virus O.[2]	Positive, May 27, 1926.
40	do	do	do	Do.
41	do	do	do	Do.
42	do	do	do	Positive, May 29, 1926.
43	do	do	do	Do.
44	do	do	do	Positive, May 27, 1926.
45	do	do	do	Do.
46	do	do	do	Do.
65	do	do	do	Positive, May 30, 1926.
66	do	do	do	Positive, May 27, 1926.
74	do	do	do	Positive, May 29, 1926.

[1] The virus animals were so distributed among the test animals as to cause uniform exposure. The animals in this experiment were housed in two sheds. Animals were chained to a headboard for restraint. There were no partitions between individual animals. Consequently contact with large amounts of virus was thus obtained.

[2] These animals were inoculated by scarification of the mucous membrane of the gum and pad with foot-and-mouth-disease virus type O derived from guinea pigs.

Therefore, on May 11, 1926, seven cattle (Nos. 51, 52, 53, 54, 55, 57, and 58) were injected intramuscularly with 2 cubic centimeters of a heavy suspension of active, vesicular-stomatitis virus obtained from guinea pigs. On May 13 heifer 55 showed a series of exfoliations of epithelium on the tongue, but two susceptible guinea pigs injected with material obtained on the day of the appearance of these lesions failed to exhibit any indication of disease. On May 15 heifers 53, 54, 57, and 58 received a second intramuscular injection of 2 cubic centimeters of a heavy suspension of active vesicular-stomatitis virus derived from guinea pigs. All the animals remained normal. On May 25 these 7 animals, together with Nos. 21, 22, and 29, which had resisted the effects of foot-and-mouth-disease virus before, and 7 normal, control animals, were exposed, by contact, to 11 cattle inoculated by scarification of the mucous membranes of the dental pad and gum with foot-and-mouth-disease virus (guinea-pig type O). All the virus animals showed primary vesicles in from 48 to 105 hours, followed by generalization of the disease. The affected animals were placed so that the test animals (contacts) were in constant close contiguity with animals in the virus-spreading stage of the disease. The commission's impending departure for the United States compelled the termination of the experiment June 10. Table 46 records the results of the experiment.

As will be seen in Table 46, the results were as follows: Of 7 animals injected intramuscularly with vesicular-stomatitis virus, 5 (Nos. 51, 52, 53, 54, and 55) became infected with foot-and-mouth disease and 2 remained well. Of the 3 animals which had previously resisted foot-and-mouth-disease virus, 1 (No. 22) became infected with foot-and-mouth disease and 2 remained normal. Of 7 normal animals used as controls in this experiment, 4 (Nos. 67, 69, 70, and 73) developed the disease, while 3 remained well.

From this experiment it appears, therefore, that cattle immune to vesicular stomatitis, either as a result of recovery from the disease or following intramuscular injections of the virus, were not resistant to any appreciable extent to foot-and-mouth-disease virus when placed in contact with animals affected with the latter disease.

Table 47 shows the results of infection with the virus of vesicular stomatitis of cattle recovered from either or both types of foot-and-mouth disease.

It will be noted in Table 47 that 10 cattle recovered from one or both types of foot-and-mouth disease were exposed to vesicular-stomatitis virus. Five of them were inoculated locally and all revealed the disease. The other 5 were placed in contact with vesicular-stomatitis-infected cattle. Three developed the disease while the other 2 remained well.

From the foregoing tests it may be conculded that cattle recovered from vesicular stomatitis are susceptible to foot-and-mouth disease, and, conversely, animals recovered from foot-and-mouth disease can readily be infected with vesicular stomatitis.

EXPERIMENTAL VESICULAR STOMATITIS IN SWINE

Mohler (*53*) reported the transference of vesicular stomatitis to three hogs by local inoculation. The commission's experience confirms this finding, as shown by the fact that of eight hogs exposed to the virus of vesicular stomatitis, by local or intravenous inoculations,

TABLE 47.—*Results of immunity tests of cattle recovered from types of A and O foot-and-mouth disease when exposed to vesicular-stomatitis virus*

Animal No.	History	Date of exposure to vesicular stomatitis	Method of exposure	Result
		1926		
3	Recovered from foot-and-mouth disease O virus, Sept. 17, 1925.	Feb. 17	Scarification	Positive, Feb. 18, 1926.
5	Recovered from foot-and-mouth-disease A virus, Oct. 26, 1925, and O virus, Nov. 26, 1925.	do	do	Positive, Feb. 19, 1926.
7	Recovered from foot-and-mouth-disease O virus, Sept. 17, 1925, and A virus, Jan. 8, 1926.	do	do	Do.
8	Recovered from foot-and-mouth-disease O virus, Sept. 30, 1925.	do	Natural	Negative.
		Mar. 4	Intravenous	Do.
		Mar. 10	do	Do.
10	Recovered from foot-and-mouth-disease O virus, Sept. 25, 1925, and A virus, Jan. 8, 1926.	Feb. 17	Natural	Positive, Feb. 27, 1926.
4	Recovered from foot-and-mouth-disease O virus, Sept. 30, 1925, and A virus, Feb. 3, 1926.	Feb. 22	do	Do.
15	Recovered from foot-and-mouth-disease A virus, Jan. 15, 1926, and O virus, Feb. 2, 1926.	Feb. 17	Natural	Negative.
		Mar. 11	Intravenous	Do.
		do	do	Do.
		Mar. 30	Scarification	Do.
16	Recovered from foot-and-mouth-disease A virus, Feb. 3, 1926.	Feb. 22	Natural	Positive, Feb. 26, 1926.
35	Recovered from foot-and-mouth-disease O virus, Apr. 14, 1926.	May 7	Scarification	Positive, May 9, 1926.
36	do	do	do	Do.

six revealed the disease. The disease produced by intravenous inoculations was clinically indistinguishable from foot-and-mouth disease in swine.

The injected animal shows, after from 24 to 48 hours, a marked rise in temperature. Usually, in 48 hours, distinct vesicles made their appearance on the feet, involving the interdigital space, the coronary band, and the heels, extending in some instances to the plantar surfaces. In some cases the dewclaws also are involved. The animals become very lame and move about with difficulty, often walking on their knees, as is seen in foot-and-mouth disease. Vesicle formation may also occur on the snout. The animals are rather sick and as a rule do not eat for several days. Within a few days the temperature drops to normal, the animals begin to eat, the feet become less sensitive, and healing gradually takes place. After a few weeks a distinct line of separation occurs between the new and old horn. These processes are identical with those of foot-and-mouth disease.

As a result of local inoculations, swine show a vesicle at the site of inoculation and, following its rupture, an erosion occurs with a raw, red base. The vesicular-stomatitis lesions at the site of inoculation appear to be more pronounced than those of foot-and-mouth disease, following the same method of inoculation. A marked rise in temperature is coincident with the appearance of the local lesion, or it may take place later. In the three animals inoculated in this way no secondary lesions could be seen. In foot-and-mouth disease, as a rule, secondary lesions are noted following local injection. Only three hogs, however, were inoculated locally with vesicular-stomatitis virus. This number is, of course, too small to be of any value upon which to establish points of difference between the two diseases.

Two hogs (Nos. 4 and 7, Table 48) were exposed to natural infection by placing them in contact, in the same pens, with hogs artificially infected. The animals were together from the time the latter were injected. Neither of the two exposed animals exhibited the disease. Hog 4 was resistant to two later inoculations, one intravenous and one by scarification, while hog 7 was proved to be susceptible by reacting to a later inoculation of active virus by scarification.

Thus it appears that swine are susceptible to vesicular stomatitis and react with lesions similar to those of foot-and-mouth disease.

CROSS-IMMUNITY TESTS WITH VESICULAR STOMATITIS AND FOOT-AND-MOUTH DISEASE IN SWINE

Cross-immunity tests in swine demonstrated, as in similar tests in guinea pigs and cattle, that animals recovered from foot-and-mouth disease were susceptible in general to infection with vesicular stomatitis and that hogs, recovered from vesicular stomatitis, were readily infected with type O of foot-and-mouth-disease virus. Type A virus was not used.

Table 48 shows that of nine hogs recovered from one or both types of foot-and-mouth disease and then inoculated locally or intravenously with vesicular-stomatitis virus, all except hogs 5 and 6 revealed clear-cut evidence of the latter disease. Hog 5, as a result of an intravenous inoculation, showed a questionable lesion of the disease on one foot only. This animal was, however, resistant to a subsequent, intravenous injection. Hog 6 failed to show any evidence of disease following an intravenous inoculation. It was later found to be resistant to one intravenous and one local injection. Hog 4, recovered from foot-and-mouth disease, was exposed, by contact, to vesicular-stomatitis virus, but failed to show any evidence of disease. It was resistant to later injections, two intravenous and one local, of active vesicular-stomatitis virus.

Two of the hogs (Nos. 10 and 11), recovered from vesicular stomatitis, revealed foot-and-mouth disease following an intravenous inoculation of the latter virus. It is to be noted, therefore, that cross immunity between the two viruses does not exist in this species.

TABLE 48.—*Results of cross-immunity tests in swine with foot-and-mouth disease and vesicular stomatitis*

Hog No.	Virus to which immune	Virus to which exposed	Method of exposure	Result
1	Foot-and-mouth O and A	Vesicular stomatitis	Scarification	Positive.
2	do	do	Intravenous	Do.
3	do	do	do	Do.
4	do	do	Natural	Negative.
			Intravenous	Do.
			Scarification	Do.
5	do	do	Intravenous	Doubtful.
			do	Negative.
6	Foot-and-mouth A	do	do	Do.
			do	Do.
			Local	Do.
7	do	do	Natural	Do.
			Scarification	Positive.
8	Foot-and-mouth O and A	do	do	Do.
9	Foot-and-mouth A	do	Intravenous	Do.
10	Vesicular stomatitis	Foot-and-mouth O	do	Do.
11	do	do	do	Do.

EXPERIMENTAL VESICULAR STOMATITIS IN HORSES

Five horses were inoculated locally with vesicular-stomatitis virus as follows: A small portion of the dorsal surface of the tongue and a portion of either the upper gum or inner surface of the lips were scarified. Vesicular fluid and epithelial coverings from either guinea-pig or cattle lesions were then applied to these scarified areas. Four of these horses developed severe lesions at the site of inoculation on the tongue. The fifth failed to show evidence of disease at the points of inoculation. It did, however, reveal a temperature of 104.8° F. on the fourth day. Five days after inoculation, this horse was killed while in the agonal stage of meningitis. The cause of the meningitis was not determined.

In general, the appearance of the experimental disease in the horse is as follows: From 36 to 72 hours after injection the tongue shows, at the site of inoculation, areas of blanched, easily detachable epithelium, which soon fill with clear, straw-colored fluid. These areas coalesce, forming a large vesicle containing from 5 to 10 cubic centimeters of fluid. Within the next 24 hours the vesicle ruptures, leaving a deep, red, raw erosion, which heals very slowly. Definite, secondary lesions have not been observed, although extension of lesions have been seen beyond the points of inoculation.

The lesions at the other points of inoculation, such as on the gum and on the lip, are mild, and limited to small vesicle formations, followed by erosions which heal rapidly. In one of the four cases no definite lesions developed in any other site than on the tongue. In the horse the tongue appears to be a more suitable place for inoculation of vesicular-stomatitis virus than other parts of the buccal mucosa. Such was not the writers' experience in cattle. In cattle there seemed to be no marked difference between the severity of the lesions produced on the tongue and those on other portions of the mucous membrane of the mouth.

Six horses were inoculated, locally or intramuscularly, with types O or A of foot-and-mouth-disease virus. These inoculations failed in every case to produce any evidence of disease. The literature, however, contains references to natural cases of foot-and-mouth disease in horses which are discussed in another section of this report under "Foot-and-mouth disease in the horse." The commission concludes, from its tests, that the horse is very resistant to infection with foot-and-mouth disease, but is highly susceptible to the virus of vesicular stomatitis.

FILTRATION EXPERIMENTS

It is generally accepted that the virus of foot-and-mouth disease is filtrable. The members of the commission, in more extensive observations, found that the virus ordinarily carries an electropositive charge—its isoelectric range centering at P_H = about 8—and that this condition influences filtration through the commonly employed electronegative filters. To summarize the results of the commission's experiments: The foot-and-mouth-disease virus, in its electropositive condition, is filtrable through the Seitz asbestos disk, through Berkefeld V and N candles, and through the various sizes of Chamberland bougies up to L 11. With respect to the L 11 filter, electropositive virus failed to traverse this type of Chamber-

land bougie, but when the charge of the virus was shifted to electronegative it then passed freely through this same filter. These experiments are described in detail elsewhere in this report.

On the other hand, the virus of vesicular stomatitis was regarded, at the inception of the commission's studies on its filtration in November, 1925, as nonfiltrable. Mohler (*53*), in 1918, and Cotton (*14*), in 1926, reported that the virus failed to pass filters which retain bacteria of the ordinary species such as *Bacillus prodigiosus*.

Following are the results of 18 different filtration tests from 13 of which an unequivocal conclusion can be drawn that the virus of vesicular stomatitis is also filtrable and that a distinction between this virus and that of foot-and-mouth disease on the basis of filtrability does not exist.

The first test was made, November 30, 1925, with a Berkefeld V filter.

PROTOCOL 27

A new Berkefeld filter, type V, was employed with a negative pressure of 20 centimeters of mercury. A vigorously growing culture of *Bacillus prodigiosus* was first filtered, with the result that the material before filtration yielded a profuse growth but the filtrate remained sterile. Then the aspirated lymph from six guinea pigs with experimental vesicular stomatitis was diluted 1 : 8 in physiological saline at $P_H=7.6$ and filtered through the same candle. Two guinea pigs were injected intradermically in the posterior pads with the filtrate and four with the unfiltrated materials. All six exhibited the typical lesions of vesicular stomatitis within 48 hours.

Thus, in a carefully controlled filtration experiment, it was found that the virus of vesicular stomatitis was filtrable through a Berkefeld V candle. This experiment was repeated under exactly similar conditions except that the virus material was diluted 1:15 in physiological saline at $P_H=7.8$. In this case 4 guinea pigs were injected, 2 with the unfiltered and 2 with the filtered substance. All showed typical vesicular-stomatitis lesions within 48 hours. Another repetition was made with the virus diluted 1:25 in the saline. Here again the 2 guinea pigs injected with the unfiltered and the two with the filtered material yielded typical experimental vesicular stomatitis within 48 hours. A fourth test was made with the aspirated lymph diluted 1:20 in phosphate buffer at $P_H=7.5$. The other conditions of the experiment remained the same. Three guinea pigs injected with the unfiltered and 3 with the filtered virus showed the typical vesicles of vesicular stomatitis within 48 hours.

Hence, in four different tests, in which active virus was used from different sources, and diluted from 1:8 to 1:25, either in physiological saline or phosphate buffer at $P_H=7.5$ to 7.8, filtration was effected through an equal number of new Berkefeld V candles, impervious to a culture of *B. prodigiosus*.

The next experiments concerned the filtrability of the virus through Berkefeld N candles, which are less porous than the V type.

PROTOCOL 28

In all, 10 different filtration tests were made, employing a new Berkefeld N filter in each instance. All these filters were tested, for absence of leakage, with *Bacillus prodigiosus* cultures and in every case the unfiltered culture grew profusely but the filtered material remained sterile. A negative pressure of 20 centimeters of mercury was used throughout these tests. The sources of the virus were the vesicular fluid or finely ground tissue covering, the vesicles from

the tongue of a heifer, and of four different horses. In addition there was employed the vesicular fluid from the foot lesions of a hog and those of three guinea pigs.

The diluents were either physiological saline solution or phosphate buffer at P_H=7.5 to 7.6 and the dilutions of the virus materials in them were from 1:10 to 1:150. In each experiment, as a control, the unfiltered, diluted virus was injected into two guinea pigs which showed, within 48 hours, as a rule, the typical vesicles of vesicular stomatitis. The filtrate was injected intradermically in the posterior pads of from two to three guinea pigs for each test. The results of the inoculation of filtrates are given in Table 49.

TABLE 49.—*Results of inoculation of filtrates of vesicular-stomatitis virus through Berkefeld N filter*

Test	Source of material	Dilution	Injection of filtrate in guinea pigs
1	Tongue vesicle, horse	1:25	Positive.
2	Foot vesicle, guinea pig	1:10	Do.
3	Tongue vesicle, horse	1:10	Negative.
4	Foot vesicle, hog	1:15	Do.
5	Foot vesicle, guinea pig	1:40	Do.
6	....do	1:40	Do.
7	Tongue vesicle, horse	1:20	Positive.
8	....do	1:150	Do.
9	Tongue vesicle, heifer	1:20	Do.
10	....do	1:20	Do.

This series of 10 separate filtration tests, made with active virus diluted 1:10 to 1:150 and obtained from different sources, shows that the active agent was also filtrable through Berkefeld N candles which were impermeable to *Bacillus prodigiosus*. In 4 of the 10 experiments, the virus, however, failed to traverse the filters.

Filtration of the virus through a Seitz filter was then attempted. The Manteufel, E. K. model, with one asbestos disk, was used at a negative pressure of 20 centimeters of mercury. The virus comprised the fluid and ground coverings of a vesicle on the tongue of a horse, diluted 1:150 in phosphate buffer at P_H=7.5. Both the unfiltered and filtered material could induce experimental vesicular stomatitis in guinea pigs.

Finally, filtrations were made through Chamberland bougies, sizes L 3, L 7, and L 11,[38] all of which were proved impermeable to living *B. prodigiosus* cultures.

PROTOCOL 20

Single experiments were made with each of the L 3, L 7, and L 11 types of Chamberland filters. No negative or positive pressure was applied, but the virus materials were allowed to drip through the walls for about one hour. The virus consisted of the fluid or ground coverings of vesicles on the tongues of two horses with vesicular stomatitis, diluted about 1:20 in phosphate buffer at P_H=7.5 to 7.6.

The unfiltered material from each horse was injected into three guinea pigs, or six in all. These six showed the typical vesicles of vesicular stomatitis within 48 hours after injection.

The filtrate of the L 3 type bougie was inoculated into three guinea pigs. One showed primary and secondary vesicles; the other two were apparently unaffected.

[38] An L 5 type filter was also used but it was found to contain Javelle water (a chlorine compound) with which the filter was cleaned. The negative results in this case, therefore, could not be considered as conclusive.

But a reinoculation of 1 of the negative guinea pigs with active virus 15 days later again revealed no symptoms. Hence, lesions may have been present after the first injection but they were probably so slight as to be masked or overlooked. The other negative pig died.

The filtrate of the L 7 type bougie was inoculated into three guinea pigs. One exhibited the typical disease which could be transferred to a normal animal, but the second and third were negative. On reinoculation with active virus 14 days later one of the latter two were positive, the other negative. It is apparent that here again the symptoms after the first injection may have been overlooked in one of these animals.

The filtrate of the L 11 type bougie was injected into three guinea pigs. All remained well and a reinoculation of active virus after 14 days induced in these three animals the typical primary and secondary vesicles of vesicular stomatitis.

This experiment demonstrates that the virus can traverse the L 3 and L 7 types of Chamberland filters but not under ordinary conditions the L 11. In this respect an analogue is to be found in the similar behavior of the foot-and-mouth-disease virus—a condition to be discussed later. It is noteworthy that no pressure, positive or negative, was employed in the experiment and that the filters were capable of retaining *Bacillus prodigiosus*.

Table 50 summarizes the results of the separate filtration tests.

TABLE 50.—*Results of filtration of the virus of vesicular stomatitis through various filters*

Type of filter used	Number of separate filtrations	Infectiousness of filtrate in guinea pigs	Type of filter used	Number of separate filtrations	Infectiousness of filtrate in guinea pigs
Berkefeld V. S.	4	4 positive.	Chamberland L 3	1	1 positive.
Berkefeld N.	10	6 positive; 4 negative.	Chamberland L 7	1	Do.
Seitz	1	1 positive.	Chamberland L 11	1	1 negative.

To prove the specificity of the virus contained in the vesicular-stomatitis filtrates, a cross-immunity test was made in which a Berkefeld N filtrate of the virus was injected into three guinea pigs recovered from vesicular stomatitis; into three having had a type A foot-and-mouth disease, and into a like number convalescent from type O foot-and-mouth disease. The results of this test showed that the filtered vesicular-stomatitis virus induced the typical experimental disease in recovered type A and type O foot-and-mouth-disease guinea pigs, as well as in the normal controls, but was without effect in the animals which had been through an attack of vesicular stomatitis.[39]

DISCUSSION

In the comparative studies of foot-and-mouth disease and vesicular stomatitis, the commission's efforts were directed to find criteria by which one could be differentiated from the other.

That the vesicular-stomatis virus produces lesions in the guinea pig has been shown. In general these lesions are indistinguishable from those of experimental foot-and-mouth disease. It has been pointed out that as a rule the vesicular-stomatitis virus requires a longer time to exhibit vesicles than does the foot-and-mouth virus. Furthermore,

[39] The conditions under which these filtrations were carried on are similar to those described under "Physical and chemical properties of the virus," p. 33.

secondary vesicles were not so constantly present in experimental vesicular stomatitis in guinea pigs. These findings were not constant for the virus of either disease and can not be relied upon as a criterion for differentiating the two diseases by guinea-pig inoculations.

The cross-immunity tests in guinea pigs, however, indicate definitely that one disease does not immunize against the other. Hence, this can be used in supplementing other means of differentiating vesicular stomatitis from foot-and-mouth disease. For example, a supply of guinea pigs recovered from or immunized against vesicular stomatitis could be kept constantly on hand by the Bureau of Animal Industry. Suspected material is then inoculated into a series of 5 or more vesicular-stomatitis-immune guinea pigs and also into 2 or 3 normal guinea pigs. If the suspected material induces lesions in the normal guinea pigs and not in the vesicular-stomatitis immunes, it would be proof that the inoculated material contained vesicular-stomatitis virus. On the other hand, if both the control normal guinea pigs and the vesicular-stomatitis immunes became infected, it would indicate that the suspected material contained foot-and-mouth-disease virus.[40] It is not possible under existing conditions in the United States to have a supply of foot-and-mouth-disease-immune guinea pigs to complete these cross-immunity tests.

The use of foot-and-mouth-disease and vesicular-stomatitis immune serums in guinea-pig tests for differentiating the two diseases also suggests itself.

Cross-immunity experiments with cattle yielded practically the same results as those in guinea pigs. It is apparent, however, that unless cattle immune to vesicular stomatitis are by chance available, the constant, regular supply of immunized guinea pigs is more economical and quite as effective.

The cattle inoculations, however, yield differences, which, if carefully considered, may be of some aid in differentiating the two diseases. It has been pointed out that definite lesions of vesicular stomatitis were not observed in cattle inoculated intramuscularly with as much as from 1 to 3 cubic centimeters of heavy suspensions of vesicular-stomatitis virus. On the other hand, with foot-and-mouth-disease virus, this mode of inoculation induced typical, pronounced lesions in all 11 cattle injected. Other investigators also report positive results with intramuscular injection of foot-and-mouth-disease virus.

The intravenous inoculation of vesicular-stomatitis virus into seven cattle resulted in one animal's showing a definite but mild affection. The writers did not inoculate foot-and-mouth-disease virus into cattle by the intravenous route. Others, however, report positive results by that method.

Even with local inoculation there is almost always a difference in the behavior of the two viruses. When cattle were inoculated on the tongue or gums with vesicular-stomatitis virus, no foot or other secondary lesions were observed. With foot-and-mouth-disease virus, inoculation by scarification, as a rule, causes foot and other secondary lesions.

[40] W. E. Cotton (*15*) has recently found a plurality of vesicular-stomatitis virus to exist, and this fact must therefore be considered when interpreting results where vesicular-stomatitis-immune animals show lesions.

For proper interpretation of the inoculation results it is essential that the injected material, either from foot-and-mouth disease or from vesicular-stomatitis suspects, should contain fluid or coverings from fresh, or recently ruptured, vesicles. Under certain conditions the virus loses its virulence rapidly.

Hogs appear to be at least as susceptible to vesicular stomatitis as to foot-and-mouth-disease virus, when inoculated locally or intravenously, and offer no means of differentiating the two diseases. Cross-immunity tests, however, reveal a sharp difference, but here there is no advantage over guinea pigs for differential tests.

The horse is the most reliable animal for distinguishing between the two diseases. While this animal is readily infected with vesicular-stomatitis virus, the writers were unable to infect it with either types A or O of foot-and-mouth-disease virus. The results of the writers' experiments with horses and the field experiences in foot-and-mouth disease in the United States and other countries may be accepted as evidence of the resistance of this animal to that disease.

In differentiating between foot-and-mouth disease and vesicular stomatitis by the use of the horse as a test animal, however, the possibility exists, although it may be very rare, of the two diseases being present in the same herd or in the same animal simultaneously. Vallée [41] has shown that an animal can be infected with both the A and O types of foot-and-mouth disease simultaneously and that both types of the disease can be produced with virus from such an animal. While lack of time prevented experimental study of the action of mixtures of the viruses of vesicular stomatitis and foot-and-mouth disease, it is thought that the following procedure might be an aid in eliminating a possible mixed infection.

With the suspected material one or more horses and two or more cattle are inoculated by scarification of the mucous membrane of the tongue of the horse and the mucous membrane of the gum or dental pad of cattle. It is of course essential that susceptible animals be used.

The development of the disease in the horse and the cattle would indicate that the inoculated material contains at least the virus of vesicular stomatitis. Two or more normal cattle are now inoculated with the material collected from the horse. This should contain only vesicular-stomatitis virus, assuming that the foot-and-mouth-disease virus (if present in the original material) had been lost because of the insusceptibility of the horse to infection with foot-and-mouth disease.

Thus we have two groups of cattle immune to subsequent exposure to the virus with which they were inoculated: Group A, inoculated with the original material, and Group B, inoculated with that derived from the horse. After an interval of one week both groups are injected with the original substance which should be kept in a cold chamber in phosphate buffered 50 per cent glycerol, the preparation of which is described elsewhere in this report. Then,

1. If animals in Group A are unaffected, and those in Group B are infected, it would indicate that the original material contains both vesicular-stomatitis and foot-and-mouth-disease viruses.
2. If animals in Groups A and B are unaffected, it would indicate that the original material contained only the virus of vesicular stomatitis.

[41] Personal communication.

Guinea pigs may be used as test animals in conjunction with cattle or alone. In each group check animals should control the activity of the virus.

It was demonstrated that cattle are readily immunized against vesicular stomatitis by local, intramuscular, or intravenous inoculation of the virus, even though no evidence of the disease is observed. By analogy to the immunity produced in certain other diseases, it was hoped that this strong, easily produced immunity in vesicular stomatitis would also protect against foot-and-mouth-disease infection. A large series of cross-immunity tests proved, however, that cattle, recovered from vesicular stomatitis, become infected when exposed to foot-and-mouth-disease virus by contact, by scarification, or by intramuscular inoculations.

In view of the fact that the intramuscular injection of cattle with vesicular-stomatitis virus is followed by no demonstrable lesions (at least in the limited number of animals used by the writers), and that this procedure renders the animal solidly immune, it is suggested that this may be a means of immunization against vesicular stomatitis in field work. For such a purpose, virus propagated in guinea pigs could be used.

In respect to filtration, there is no distinction between the virus of vesicular stomatitis and that of foot-and-mouth disease. Both respond to the test in a fairly parallel manner. It appears that the virus of vesicular stomatitis under ordinary conditions has a tendency to become adsorbed in the walls of the denser types of filters, as in the case of the Berkefeld N and the L 11 type of the Chamberland. With the foot-and-mouth-disease virus this condition also prevails and has been found to be a consequence of its electropositive charge. No cataphoresis tests were made, however, with the active agent of vesicular stomatitis, so that a definite interpretation of this phenomenon is not possible. The results of the experiments are suggestive, however, if such adsorption occurs as a consequence of electropositive charge, that the virus of vesicular stomatitis may be of the order of magnitude of that of foot-and-mouth disease.[42]

CONCLUSIONS

From the foregoing work the following conclusions may be drawn:

1. Vesicular-stomatitis virus is readily transferred to guinea pigs. There is little to differentiate this disease from foot-and-mouth disease by a study of the lesions induced. Cross-immunity tests in these animals, however, offer an aid in differentiating the two diseases.

2. Vesicular-stomatitis virus induces lesions in cattle, which are at times indistinguishable from those of foot-and-mouth disease. Differences were noted, however, in the response to various methods of inoculation of the two viruses. Divergence in the course of the two diseases was also observed. Immunity produced in cattle by vesicular stomatitis and foot-and-mouth disease is solid for its respective virus, but in the considerable number of cattle inoculated with, or exposed by contact to, the two viruses, no evidence of cross immunity was found.

[42] Since this was written, one of the writers has shown that both viruses are identical in several other physical, chemical, and biological characters. (Olitsky, P. K., in Exp. Med., 1927, XIV, 969.)

3. Swine are susceptible to the active agent of vesicular stomatitis; the disease induced is then indistinguishable from foot-and-mouth disease. Here again no evidence of cross immunity between the two was discovered.

4. Horses are very sensitive to infection with vesicular-stomatitis virus by inoculation on the tongue. On the other hand, these animals are resistant to foot-and-mouth disease when inoculated on the tongue, mucous membrane of the lips, or intramuscularly. The horse can be regarded as the best of test animals to differentiate vesicular stomatitis from foot-and-mouth disease.

5. In 13 of 18 carefully controlled filtration experiments it was found that the virus of vesicular stomatitis was filtrable through Berkefeld V and N candles, through Seitz asbestos disks, and through Chamberland bougies, sizes L 3 and L 7. The virus, similar to that of foot-and-mouth disease, is not filtrable under ordinary conditions through Chamberland L 11 type bougies and shows the same tendency to adsorption in the walls of denser electronegative filters.

REGULATIONS FOR THE CONTROL OF FOOT-AND-MOUTH DISEASE IN EUROPEAN COUNTRIES

[Compiled by the Swedish Foot-and-Mouth-Disease Commission]

These rules and regulations for the control of foot-and-mouth disease in certain European countries were compiled, in 1925, by the Swedish foot-and-mouth-disease commission, which has given permission for their publication in English.

The American commission takes this opportunity of expressing to the Swedish commission appreciation of its courtesy. The writers also thank Charles H. Heisler, American consul at Malmö, Sweden, and Verner Carlsson, of the American consulate at Malmö, for the translation of these regulations into English.

Regulations for the control of infectious diseases are based on the nature of the disease and are in accordance with conditions existing in a country. Generally speaking, there is no marked difference between the United and European countries in the rules and regulations governing the control of most animal diseases. Foot-and-mouth disease, however, is one distinct exception. The methods employed in the United States in handling this disease differ from those of European countries, except the United Kingdom, and the rules and regulations of these countries, therefore, differ from those of the United States.

In the United States foot-and-mouth disease is combated by the slaughter or "stamping out" method, while in continental Europe, as a general thing, it is handled by means of isolation and quarantine.

A knowledge of the rules and regulations for the control of foot-and-mouth disease in Europe is of value to the United States Department of Agriculture and others charged with the suppression of this disease. The knowledge is valuable not only as a source of exact information as to what is being done in the various countries, but also because this information is at hand in the event of any necessity to change existing methods of dealing with foot-and-mouth disease in the United States. Furthermore, a report of an epizoological study of foot-and-mouth disease in Europe must of necessity include methods of con-

trol. The rules and regulations for the control of the disease in certain European countries are, therefore, submitted by the United States Department of Agriculture foot-and-mouth-disease commission as a section of its report.

The rules and regulations and methods of controlling foot-and-mouth disease in the various countries [43] are presented for each country in the following order: Belgium, Denmark, France, Germany, Sweden, Switzerland, The Netherlands, and United Kingdom.

BELGIUM

METHOD OF REPORTING

Report shall be made to the mayor of the municipality in which the animals are located at the time of infection. Owners and possessors of animals, veterinarians, and meat inspectors are under obligation to report.

In fixing the infected area consideration is paid to three points, namely, whether there are on the farms in the area animals that are (1) infected with the disease; (2) suspected of being infected, or (3) suspected of being exposed to the disease. Owners of such farms shall report the matter to the mayor and thereafter keep their animals separated in such manner that they can not come in contact with animals that can become infected with the disease.

Animals that are suspected of being contaminated with foot-and-mouth disease are all ruminants and pigs, (1) which have been at a place suspected of being infected or in a herd comprising animals infected or suspected of being infected with the disease; (2) which have been on a pasture separated from other pastures only by a fence that permits contact with the neighboring animals, or only by means of a body of water or watercourse that is a common watering place for the animals; (3) which have been on the first adjacent meadow downstream, that receives water from the infected pasture; (4) which have followed a road which might have been infected during the previous four days by the passing of animals infected with the disease; (5) which have been touched or tended by a person who the same day has been in contact with an infected animal; and (6) which are or have been subject to other possible source of infection, as established by an official veterinarian.

Distinction is made between the following kinds of infections areas: Infected premises, infected zone, and safety zone.

The area of infected premises includes the farm, pasture, or other place where infected animal or animals suspected of being infected or of being contaminated are confined or inclosed.

By "infected zone" is understood the territory, outside of the infected premises, within a radius of 300 meters (about one-fifth of a mile) or in such larger or smaller radius as the mayor may decide.

The measures taken for combating foot-and-mouth disease are adopted according to the spread of the disease, so that a distinction is made between two periods, viz, the first period, when the disease is raging in only a restricted area and there is hope of eradicating or checking the disease; and the second period, when an economical disposition of the cattle would be seriously impeded should the regulation be applied in all their rigorousness.

[43] A general discussion of the foot-and-mouth-disease situation in Italy, Austria, and Hungary is given under "Epizoology and control of epizootics, p. 3."

SLAUGHTER AND APPRAISAL OF ANIMALS

In cases prescribed by the minister of agriculture the mayor orders slaughter of infected animals or animals suspected of being infected. Compensation in such cases is granted to the owner of the animals. It is fixed by appraisal of one expert, who definitely settles the matter. The expert is appointed by and sworn into office by the governor of the Province. The president of "la Fédération provinciale d'élevage" (about the same as an agricultural association) nominates the expert.

QUARANTINE OF PERSONS

A person who has entered an infected place or has been in contact with infected animals, when he leaves the place, shall be disinfected according to the instructions of the veterinarian.

Admission to an infected place shall be prohibited to all persons except veterinarians, supervising disinfectors, policemen, and attendants.

Public roads leading to the infected place may be closed by the mayor on the recommendation of the veterinary inspector. Such roads may be traveled only by persons holding pass bills issued by the mayor.

Persons who do not belong to the farm must not stroll on its premises. This regulation applies to the whole safety zone.

QUARANTINE OF ANIMALS

INFECTED PREMISES

During the first period animals declared by a veterinarian to be infected or suspected of being infected or contaminated shall be kept in stables.

If the veterinarian finds it impossible or inadvisable to keep the animals in the stables and their slaughter is deemed inopportune, they may be placed, at the direction of the veterinarian, in an inclosure either together or in groups.

Animals for slaughter that show no symptoms of the disease and have no fever may, under police surveillance and according to the direction of the veterinarian, be transported in carts to a public slaughterhouse or other slaughterhouse which has been assigned by the veterinary authorities.

During the second period infected and suspected animals are isolated either in stables or on pastures, according to the direction of the authorities.

INFECTED ZONE

During the first period, ruminants, pigs, dogs, and poultry may not enter public roads or premises other than those to which they belong, except however, that they may cross public roads in order to reach pasture, and that draft cattle may be used for such purposes outside the farm. As a condition for these exceptions it is stipulated that the animals before entering the pasture and prior to being brought back into the stable shall pass through a tank of lime solution to a height of 22 centimeters, that they shall when leaving and returning to the stable pass through the lime solution as above, and that in

both cases the animals do not belong to a farm which is an infected place. Animals also may leave the infected zone to be brought to a public or other recognized abattoir where they shall be slaughtered within 24 hours. The transportation may not take place unless the veterinarian has issued a pass the same day or the day before. The animals may also be exhibited at market places reserved for animals intended for slaughter.

During the second period the movement of animals is prohibited but the veterinary inspector may grant general modifications in this respect under such conditions as he may find it reasonable to prescribe; this shall not apply, however, to animals belonging to an infected place.

SAFETY ZONE

Cloven-footed animals may not leave the zone so long as this prohibition is not revoked by the veterinary inspector in charge.

The holding of fairs, the assembling of young cattle and pigs for sale, the transportation of boars, and trespassing of strangers and animals from other farms on the pastures are likewise prohibited.

The use of common pastures and the bringing together of animals are regulated by the veterinary inspector.

When the disease has reached the second period the veterinary inspector may issue modified regulations regarding the sale of cloven-footed animals.

RESTRICTIONS ON GOODS

The removal, from the infected place, of manure, muck, hay, straw, or other products that might spread the infection to other animals is prohibited until the isolation regulations have been canceled. In emergency cases the transportation of muck may be permitted by the veterinary inspector. Special regulations are in force with regard to milk.

MILK

Milk from an infected farm may be brought to creameries if the management of the creamery is willing to receive it, but not until all work with milk from uninfected farms has been completed.

The milk may not be sold unless heated to 80° C.

The containers shall be disinfected both inside and outside by being immersed in boiling 3 per cent solution of soda.

This disinfection takes place at the creamery.

Newly slaked lime shall be spread every day on the roads leading to the creamery and on its yard, as well as on the bottom of the bodies of the vehicles used for the transportation.

A vehicle used for the transportation of the milk may not be brought from an infected to an uninfected farm. The same cart may not be used for infected and clean farms.

No compensation for losses caused by milk restrictions is provided for in the laws.

HORSES, DOGS, CATS, AND POULTRY

Horses, other single-hoofed animals, and draft dogs may leave an infected place during the first period in order to perform work, provided the hoofs and paws are disinfected prior to the departure. No restrictions are prescribed pertaining to the use of these animals during the second period. It is provided, however, that a veteri-

narian has a right to give instructions to that effect so far as the infected place is concerned.

Poultry, except pigeons, and dogs on infected farms shall be locked up or tied in such manner that they can not reach a public road or enter the property of other persons.

In the entire safety zone it is prohibited to allow dogs to run at large.

DURATION OF QUARANTINE

INFECTED PLACE

If the veterinary inspector does not oppose a measure of the kind the mayor cancels the regulations which isolate a stable or inclosure provided disinfection was performed within three days after the veterinarian established the recovery of the animals and a general disinfection of the animals and of all infected places and articles has taken place. Certificate of the recovery may not be issued, however, until at least 10 days have elapsed since the declaration of infection.

Access to infected stables and pastures is prohibited to men and animals for a period of not less than 14 days after establishment of the recovery. During the same period the animals remain under public surveillance and may not be sold or come in contact with other animals susceptible to the disease; and finally they may not enter roads used by other cloven-footed animals.

INFECTED ZONE

The regulations are canceled 14 days after establishing the recovery of the last case of infection. Animals that have been quarantined in a stable and have recovered for more than two weeks may, however, be taken on public roads before such prohibition is revoked.

SAFETY ZONE

The veterinary inspector in the district where the disease has broken out fixes the limits of the safety zone and informs the mayor both of this fact and of a suitable time for the abolishing of the regulations.

GENERAL PRECAUTIONARY MEASURES

Special regulations apply, with regard to markets for cloven-footed animals, in which among other things veterinary examination of each animal is prescribed.

The Minister of Agriculture may prohibit the holding of such markets when a great spreading of foot-and-mouth disease may be be expected.

The Minister of Agriculture may also issue special instructions for such markets which are considered particularly infectious. When the market is closed animals may be brought to public abattoirs where they shall be slaughtered within five days. If the animals are brought to a slaughterhouse at some other place they are marked in a specified manner. The transportation may take place in a cart or by railway.

NOTES AND COMMENTS

The most characteristic feature of the Belgian laws is that they recognize the need of special methods of combat as the disease spreads. Special regulations apply to the period when the disease is new and of less prevalence, "the first period," and to the period thereafter, "the second period." Furthermore, the system which recognizes three kinds of infected areas is provided for in the laws; and the laws are well and clearly arranged.

DENMARK

METHOD OF REPORTING

In cases in which foot-and-mouth disease is suspected the owner or the individual in charge of the animals shall either call in a veterinarian or report the matter to the respective chief of police or his representatives.

EXTENT OF INFECTED AREA

According to regulations now in force, included in the provisional regulations of February 13, 1925, regarding measures for combating foot-and-mouth disease, the infected area comprises partly the farm on which the disease has broken out and partly other farms in the immediate vicinity. Different regulations are, however, in force for an infected farm and adjacent farms.

Prior to the regulations of 1925 those of November 28, 1924, were in force, which mention two kinds of infected areas.

"Lokelafspaerring" (local isolation) comprised the infected farm and an area surrounding it. If the infected farm was located in a village, the local isolation should comprise the whole of the village. Otherwise the isolation should include, besides the infected farm, those adjacent farms the buildings of which were located at a distance of about 400 meters from the buildings of the infected farm.

"Icktagelseedigtrikt" (observation district), which included an area with a radius of not less than 2 kilometers, provided, however, that a village falling partly within the limits thereof should in its entirety belong to the observation district.

Contiguous local isolation areas should be considered as a unit, and likewise contiguous observation districts.

If the Minister of Agriculture prescribed quarantine measures referring to a larger area, the establishing of observation districts could be omitted.

QUARANTINE OF PERSONS

INFECTED FARM

The owner shall see to it that strangers are not admitted to stables, manure piles, or any part of the yard. This applies particularly to persons such as butchers and cattle dealers who, on account of their trade, constantly come in contact with cloven-footed animals. If such persons have been on the farm after the outbreak of the disease the police shall order disinfection of their clothing.

Everybody living on the premises is forbidden to leave except when absolutely necessary, and then only after a complete change of clothing and footwear. Persons working on the infected farm but living elsewhere shall, so far as possible, remain on the farm

until the disease is over. If this is not possible, they shall, before they leave the farm, carefully wash their hands and faces with soap and water and change their clothing and footwear. They shall go directly to their homes and may not visit farms or houses where there are cloven-footed animals.

If any person on the farm moves from there, his working clothes shall be disinfected, which if possible shall be done by boiling all wearing apparel that will withstand such treatment. The chief of police shall keep himself informed as to where the person in question moves and see that the new employer is informed of the fact that he comes from an infected farm. If such person has been engaged in the care and treatment of cloven-footed animals, the chief of police shall prohibit such person from entering a stable or coming into contact with cloven-footed animals for eight days.

Attendance at school and church and taking part in meetings is prohibited for every person residing on an infected farm and also for persons who work on the farm but live outside the farm area.

Those who milk or attend infected cows shall change their clothes or put on overall clothing and change their shoes before they go into the stable, and every milker shall in addition use a hood or head cloth during the milking. The change shall take place in the stable. When the clothing used in the stable has been taken off, the hands shall be thoroughly washed with water and soap.

Farm in the immediate vicinity of the infected farm which is placed under "public surveillance," having been declared infected or suspected of being infected. For such farms the same regulations apply in regard to personal intercourse as for infected farms.

According to the regulations of November 28, 1924, there were in force for the local isolation the same regulations as have been given above for infected farms. Relative to attendance at schools it should be stated, however, that under special circumstances and in the event of a greater spreading of the disease, the Minister of Agriculture may, on the request of chief of police, grant exceptions to the regulations pertaining thereto.

In observation districts there is no restriction on personal intercourse.

QUARANTINE OF ANIMALS

INFECTED FARM

Infected animals as well as animals that have been exposed to infection shall be kept in stables.

It may, on the other hand, be permitted that animals which have mingled with livestock which have not been exposed to infection (for instance, because they have been on special pastures) may remain there if they are kept together and watched, so that they can not enter premises belonging to a neighbor or escape to a highway. If the disease should appear among such animals, all the stock shall immediately be stabled. In exceptional cases the chief of police may, on permission from the veterinarian allow the animals to remain on the pasture, if they are kept within inclosures at a distance from a neighbor's animals, or kept tethered and under constant watch day and night. Such permission may not be granted except in cases where only part of the livestock is on the pasture and the disease has appeared only among that part; or, when the police, after consultation with

the agricultural representative, consider it impossible to procure stable fodder; or when on account of local conditions they are of the opinion that it would be more dangerous to take the animals home than to let them remain in the open. The chief of police may also under the circumstances mentioned above permit animals from infected livestock to be placed on pasture.

When the disease on the farm is over, so that the disinfection of the stables can be performed, the chief of police may grant permission to place the animals on pasture before the disinfection is performed. Prior thereto the hoofs of the animals shall be carefully cleaned and hoofs and clefts of the hoofs smeared with tar or tar alcohol (3 parts tar and 1 part alcohol), in addition to which the animals shall be scrubbed over the entire body with a disinfectant. The animals if possible, shall not be allowed to enter the public highways and shall not be placed in the vicinity of the neighboring farm, and may not, without special permission, be brought home if they have to pass over a highway.

Cattle, sheep, goats, and pigs must not be moved from an infected farm to other parts of the country or to another country except as follows.

When the disease has been found on a farm but is not prevalent among the pigs and if the pigs are kept in a building which is not in direct communication with or in the immediate vicinity of the cattle stable, the veterinary police authorities may grant permission to have the pigs transported under satisfactory control to the nearest slaughterhouse under veterinary supervision for immediate slaughter, provided the animals have been found sound at a veterinary examination performed immediately prior to the removal. The transportation to the abattoir shall take place direct in cart or truck and the animals shall be accompanied by a bill of health.

Permission may furthermore be granted, eight days after the completion of the disinfection, to ship in cart or truck to the nearest slaughterhouse for immediate slaughter, under veterinary control, cloven-footed animals that have gone through the disease and also those that may be suffering from results of the disease, such as vesicles of the feet, inflammation of the udder, or the like. Before the animals are shipped from the farm they shall be washed and the hoofs cleaned and tarred. A bill of health shall accompany the animals.

ADJACENT FARMS UNDER PUBLIC OBSERVATION

The same regulations as are in force for infected farms apply to adjacent farms. However, the owner (though not a buyer) is permitted to ship animals for slaughter to a near-by slaughterhouse under the following conditions:

(1) The animals shall be examined by a veterinarian who, if the animal is found sound, puts on a visible mark (earmark, clipping, or painting) and issues a bill of health, in which are stated the number of animals, kinds and marks, the slaughterhouse to which they shall be brought, and a statement to the effect that the owner has declared that the animals have belonged to him during the last two weeks. The veterinarian also issues a certificate which, when the bill of health is returned, must contain an indorsement regarding the receipt of the animals.

(2) The slaughter shall take place at a public slaughterhouse or at an export abattoir authorized by the minister of agriculture, or at a private slaughterhouse under public control, and the slaughter shall take place within 24 hours after the issuance of the bill of health. If there is no such slaughterhouse in the vicinity, the chief of police may permit slaughter at a private slaughterhouse and instruct the veterinarian to exercise the necessary control. At the slaughterhouse the animals shall be examined both prior to and after the slaughter.

(3) The bill of health shall be delivered to the veterinarian of the slaughterhouse, who makes a notation thereon to the effect that slaughter has been performed and returns it within two days to the veterinarian who issued it. If the latter veterinarian has not received the bill of health within four days he shall inform the chief of police who investigates the matter as soon as possible and prosecutes for any offense committed.

LOCAL QUARANTINE

Prior to the law of February 13, 1925, certain regulations were in force with regard to local isolation and the transportation of animals for slaughter. If the transportation was performed with truck, it should be performed direct and without stops.

Trucks should not proceed, in isolated districts, to collect animals from different herds, and should not, at the same time, transport animals from isolated areas and from areas not isolated. The cart or truck used for the transportation should be cleaned and disinfected, which should be arranged by the slaughterhouse. Manure and straw remaining on the vehicle should be either buried or burned. If the transportation is performed by railway, it should take place in a locked car, into which no other animals should be brought than those intended for slaughter at the same abattoir. If a vessel is used, other animals than those intended for slaughter at the same abattoir should not be transported on the same voyage. Railway cars and the ship's hold where the cattle were stabled should be cleaned and disinfected after the transportation is performed. The space at railroad stations and the quays used for such transportation should immediately after each shipment be cleaned and disinfected, which should be arranged by the respective stations. If the slaughterhouse had no connection by rail with the station or vessel docks, the transportation of the animals from the railway or from the vessel should take place by carts which should be disinfected at the slaughterhouse, or under the supervision of the proper chief or police. The transportation of pigs to a slaughterhouse for pigs should be so performed that the animals from isolated areas are received after pigs from other districts. The place where they were discharged should be cleaned and covered with lime after the pigs have been received.

Within the area of local isolation cloven-footed, domestic animals are not allowed to be driven loose, and animals should not be taken from one herd to another for breeding or for any other purpose.

OBSERVATION DISTRICT

The regulations for an observation district apply only to transportation of cloven-footed animals. Thus, such animals must not be brought to other parts of the country or to any other country. On

the other hand, the owners (not buyers) are entitled to bring their animals to a near-by slaughterhouse for slaughter on the conditions set forth for transportation of animals from an area of local isolation, provided, however, that instead of the system with bill of health and the marking of the animals the following should apply: Before the removal, and not earlier than the day before, the owner should fill out and sign a pass bill and file it with the authorities designated by the chief of police. This bill should contain information corresponding to the information furnished in a bill of health, and the owner, in attaching his signature, should make a sworn statement that the animals had belonged to him for not less than 14 days and that there were no symptoms of foot-and-mouth disease in his herd. Authorities designated by the police should certify to the correctness of the pass bill and fill out the corresponding certificate. In case of transportation by railway, the railway administration should receive the pass bill which should accompany the shipment to the slaughterhouse, the veterinarian of which should make a notation of the arrival of the animals and return it to the representatives of the police, who should note on the corresponding certificate the return of the pass bill.

RESTRICTIONS ON GOODS

INFECTED FARM

Hay and straw may not be removed from an infected farm; the chief of police, however, may permit hay and straw from ricks on the ground to be removed if the persons of the farm do not assist in the removal. Hay, straw, and turnips that have been particularly exposed to the contagion may not be removed even after the public control of the farm has ceased. Bags and other packing used for forage products may not be removed from the farm until the disinfection has taken place.

The chief of police is authorized to give permission to remove grain from the farm when necessary, and then on the condition that such bags are used for its transportation as have not been in contact with cloven-footed animals since the outbreak of the disease on the farm or have had any communication with cattle stables or pigpens on the farm.

Fertilizer kept on the farm at the outbreak of the disease may not be carried away without the permission of the veterinarian, but shall be covered with a thick layer of straw, chaff, or the like.

If the manure pile is in the immediate vicinity of a road, the owner shall provide wire netting or similar fencing of such a height that poultry can not have access to the manure pile.

Under special circumstances, for example, the veterinary police authorities may make special arrangements as to treatment and removal of manure on farms in cities. The infectious manure shall be kept covered until removal, which shall take place in carts with tight bottoms.

If a container of urine is full at the outbreak of the disease, the contents shall immediately be removed. Where there is a urine pit, its contents shall be mixed daily with chloride of lime.

Slaughter of animals under public surveillance may take place only under special permit from the Department of Agriculture and on premises under the control of a veterinarian. The meat from infected

animals may not be removed from the farm without special permission from the department. The hoofs shall be buried, the hides disinfected and then, if possible, taken direct to a tannery. The head and the tongue shall be either buried or boiled on the spot.

Carcasses of cattle, sheep, and goats, which have been found sound prior to slaughter may be freely removed, but hides and hoofs shall first be disinfected. Carcasses of pigs which were sound when slaughtered may, when scalded and cleaned, likewise be removed from the farm.

Poultry, including pigeons, may be removed if slaughtered.

ADJACENT FARMS UNDER PUBLIC SURVEILLANCE

What has been stipulated for infected farms with regard to meat, carcasses, etc., of slaughtered animals applies to adjacent farms under public surveillance, but not what has otherwise been stipulated for infected farms.

LOCAL ISOLATION

In each area cloven-footed animals, either alive or slaughtered, may not be removed except by special permit. The restrictions extend also to cloven-footed game, and to hay, straw, and manure. Hides, horn, hoofs, hair, and wool of cloven-footed animals may not be exported unless previously disinfected and salted. It is also prohibited to remove, from the farm, implements and articles that had been in the stables or that had in some other manner been in contact with a cloven-footed beast. The chief of police is authorized to grant exemption from the prohibition to remove hay and straw.

OBSERVATION DISTRICT

There is no provision for restricting the removal of commodities from such areas.

MILK

According to the law of April 14, 1920, regarding infectious diseases in domestic animals, milk may not be removed from an infected farm unless the Minister of Agriculture has granted permission. It is prescribed in a special ordinance that the chief of police may prohibit the exportation of milk from an infected farm, if the disease has occurred sporadically in a dairy district and provided that none of the infected herds, either alone or together, have more than 50 milk cows. In case of a wider spread of the disease within the dairy districts or in case it occurs in herds with more than 50 milk cows the chief of police may not issue prohibition against the exportation of milk without the consent of the veterinarian. According to the instructions of the veterinary police, the Minister of Agriculture may authorize the chief of police to permit the exportation of raw milk to a creamery under certain precautionary measures. Such authorization has also been granted. At least as early as in November, 1920, before 1,000 cases of the disease had occurred in the country, such transportation of milk was general.

If prohibition has been issued against the exportation of milk from an infected farm the owner receives compensation therefor to an amount which is the difference between the market value of the milk at the time in question and its feeding value. The owner shall make

out a sworn statement as to the quantity of milk produced during the period of infection.

The restrictions on the delivery of milk from infected herds are as follows:

(1) The milk containers may not be brought into the stable and before they are removed from the farm they shall be carefully limed either by being wholly immersed in newly prepared lime white (1 part slaked lime and 8 parts water), or at any rate by dipping the bottom while the other part of the container and the cover are washed with lime. It is, on the other hand, not satisfactory to lime the containers with a broom. The liming of the containers shall be done, so far as possible, by a person who does not come in contact with infected animals. The cover shall fit tightly. If necessary, parchment or a rubber ring should be placed between the container and the cover.

(2) Milk from infected herds may not be brought to the creamery together with milk from sound herds, but shall be brought either in a collection cart intended for such milk or directly from the infected farm.

(3) The driving to the creamery shall be done by a person who has nothing to do with the care and grooming of infected animals, and shall be performed as carefully as possible to prevent the spilling of milk.

(4) Milk from infected farms may not be received at the creamery until all milk from noninfected herds has been treated and the milk carts have left. The drivers may not enter the creamery or come in contact with its personnel, with other persons at the creamery, or with cloven-footed animals.

(5) When emptied, the containers from infected farms shall be carefully scalded, both inside and outside. If raw milk is spilled anywhere, boiling water or lime white shall be poured on the spilled milk. The receiving platform at the creamery and the place outside of it shall be limed daily. All the milk carts shall be scalded and limed every day before they leave the creamery and they may not, so long as engaged in such traffic, be used for the transportation of living animals, or forage products, hay, straw, or forage roots outside of the infected farm. They may not be placed or used on farms where foot-and-mouth disease is not prevalent.

(6) Those of the personnel of the creamery who handle the infectious milk shall wear during this work special overall trousers, blouse, and footwear, and afterwards wash themselves carefully. Every day immediately after having been used the overall clothing shall be boiled and then dried at the boiler. The footwear shall be cleaned immediately after having been used.

(7) The milk shall be heated to not less than 90° C. either prior to being brought into the creamery proper, which in general is preferable, or prior to the centrifugalization, if regenerator is used, or else both the cream and the skimmed milk shall be warmed to the required temperature. If the raw milk has passed through a weighing vat, preheater, and centrifuge, these implements shall be cleaned and scalded very carefully after being used. Finally the floor in the creamery shall be scalded.

(8) At a conspicuous place in the creamery and at a man's height there shall be placed a printed poster of the measures prescribed. No other persons than those employed in the creamery may enter it.

(9) Milk, cream, and whey delivered to the contractors of every creamery receiving milk from herds infected with foot-and-mouth disease shall be heated to 90° C. before they leave the creamery.

(10) If cattle or pig raising is conducted on the creamery premises or near by or if any person employed in the creamery is the owner of cattle, sheep, goats, or pigs, this fact shall immediately be reported to the police or to the veterinarian so that necessary precautions can be taken.

Transportation of milk from infected herds by railway or vessel is prohibited.

HORSES, DOGS, CATS, AND POULTRY

No animal may be removed from an infected farm without special permit from the veterinary police, but the owner is permitted to use his horses outside the limits of the farm if they are separated from cloven-footed animals on the farm. If the animals have been exposed to the contagion through the manure from cloven-footed animals, the soiled parts must be carefully cleaned and disinfected prior to the animals being used outside the farm. The horses may not be placed in a stable outside of the farm.

Horses from infected farms may not be lent for use anywhere else; neither may they be used for the transportation of milk from sound livestock. Eight days after the completion of the disinfection the veterinary police may give permission to remove the horses to other farms after the horses have been carefully washed with a disinfectant and their hoofs cleaned and tarred. Stallions stabled on infected farms may not be used to serve mares from other farms, and mares from such farms may not be brought to infected farms for service.

Within a radius of 5 kilometers (about 3 miles) from an infected farm all dogs shall be tied or led by leash. Loose dogs shall be taken in charge by the police and killed if not claimed within four days. Hunting with dogs is prohibited. If a dog running loose can not be caught the chief of police shall have it shot. For cities and like communities the chief of police may issue special regulations.

The cats of an infected farm shall be killed or kept locked up; the same applies to cats on adjacent farms.

Poultry and pigeons on infected and adjacent farms shall be penned.

If there are rats on an infected farm, the chief of police may at the suggestion of the veterinarian prescribe the use of an effective rat poison at public expense. This poisoning shall be performed as soon as possible, and not later than the beginning of the final disinfection.

GENERAL PRECAUTIONARY MEASURES

The holding of cattle markets (not horse markets) and auctions for the sale of cloven-footed animals, the bringing together of animals for purchase and sale, or any other gathering of cloven-footed animals for sale or for any other purpose is prohibited. It is permissible, however, to hold export animals in certain specified cities for the sale of animals for export from the country.

The admission of strangers, particularly tradesmen, to stables is prohibited.

Tradesmen's stables where pregnant cows are stabled, and the assembling stables of export associations are under veterinary supervision.

If considered necessary and not accompanied by considerable inconveniences to communication, the chief of police is authorized to prohibit all communications on public roads with the exception of highways leading through or immediately adjacent to an infected farm; from this prohibition he may exempt specific individuals residing near by, but not their domestic animals.

Owners of trucks or other vehicles used for the conveyance of cattle, pigs, fertilizer, or raw hides shall report to the proper chief of police. All such vehicles shall be disinfected after each transportation, and the disinfection shall be arranged by the respective slaughterhouse or cattle market immediately after the discharge, provided the transportation takes place to an export abattoir or cattle market approved by the Department of Agriculture. With regard to other transportation the chief of police shall see that disinfection is performed under control of authorities designated by him.

FRANCE

METHOD OF REPORTING

Cases or suspected cases of disease shall be reported to the mayor of the community. Under obligation to report are the owner, assistants, shepherds, and veterinarian consulted. Veterinarians supervising fairs or markets shall report any observed cases of infectious animal disease to the local police authorities.

EXTENT OF INFECTED AREA

The prefect declares an area infected which includes the farm with the stables, fences, meadows, and pastures where infected animals are kept, and in addition surrounding territory, according to circumstances. There appears to be nothing to prevent the inclusion of other territory than that belonging to the farm. Notices regarding the infection shall be posted on the door of the office of the mayor, on the boundary posts and on the ways out of the village or municipality.

SLAUGHTER AND APPRAISAL OF ANIMALS

The statutes contain no stipulation regarding slaughter of animals on account of foot-and-mouth disease, with the exception of emergency slaughter.

QUARANTINE OF PERSONS

In the declaration of infection which is issued by the prefect, prohibition is given against entering the infected area, the limits of which are established according to circumstances. No prohibition to depart from the area appears to be given.

QUARANTINE OF ANIMALS

The declaration of infection may include isolation of the cloven-footed animals, separation of sound and infected animals, examination, listing, and marking. Infected animals may not be sold for other purposes than slaughter. In such case they shall be slaughtered on the spot. The same applies to animals suspected of being infected, with the exception that they may be taken to an official slaughterhouse. In the latter case they shall be marked with a

brander and transported by cart or by railway, in addition to which the veterinarian shall issue a pass bill which shall be visaed by the mayor. The pass bill shall be sent to the mayor within five days accompanied with a certificate from the manager of the slaughterhouse to the effect that slaughter has been performed.

RESTRICTIONS ON GOODS

There is nothing stipulated in the laws regarding the delivery of goods from infected farms. Where local regulations are issued by the prefect they are believed to be based partly on the regulation that when foot-and-mouth disease has been found, a certain area may be placed under "prohibition." From the circular communication from the Department to the prefects it appears that foodstuffs, forage, manure, etc., may not be exported unless a guaranty is furnished that these articles are absolutely free from contagion.

MILK

Milk from infected livestock is included among the commodities for which export prohibition is stipulated, unless guaranty can be furnished to the effect that such commodities do not transfer the contagion.

In the regulations available there is no provision for compensation for milk which can not be utilized on account of the quarantine prescribed.

HORSES, DOGS, CATS, AND POULTRY

No regulations regarding these animals exist except that the Minister of Agriculture has pointed out in a communication to the prefects the importance of preventing the animals from coming in contact with animals infected or suspected of being infected.

DURATION OF QUARANTINE

The declaration of infection is not revoked until 14 days after the recovery of the animal last infected and after the completion of the disinfection.

GENERAL PRECAUTIONARY MEASURES

When foot-and-mouth disease assumes a more serious character, the prefect prohibits the holding of markets of all kinds for cloven-footed animals and the exhibition or marketing of such animals on public roads or in the yard of an inn. Exception is made for markets in cities which have slaughterhouses under veterinary control; but all animals brought to such market shall be slaughtered at the slaughterhouse. The prefect also prohibits the bringing of pigs within the Department or part thereof except those transported on vehicles.

In addition, the prefect may prescribe that a cattle dealer who brings cloven-footed animals into his stables shall report such fact and the animals may not leave the place until the expiration of five days and until a veterinarian has certified that they are free from foot-and-mouth disease.

The prefect's instructions above referred to can not be revoked until the conditions causing them have ceased to exist.

If an infectious animal disease is found at a market, the infected or suspected animals belonging to the same owner shall be placed in

in an inclosure until they have recovered. The owner may demand slaughter, when the infected animals are slaughtered on the spot and those suspected of being infected may be brought to a public abattoir under observation and prescribed precautionary measures. Animals of other owners which have been in contact with the infected animals shall be marked with clips and may not leave the market without the permission of the veterinary inspector. Such permit, or pass bill, shall be returned to him within five days accompanied with a certificate of the mayor in the community to which the animals have been sent.

NOTES AND COMMENT

There is no hindrance to the consumption of meat animals infected with foot-and-mouth disease which have been slaughtered.

In France there is an advisory committee for epizootics. It consists of eight members: One cabinet minister appointed by certain Departments, the director, and the assistant director of agriculture, the director general of the customs, the inspector general of the veterinary colleges, of the public health, and of the veterinary departments, and the assistant secretary of the veterinary division of the Department of Agriculture.

GERMANY

METHOD OF REPORTING

Cases or suspected cases of disease shall be reported to the police authorities or to other authority, as fixed by the government of each State. Under obligation to report are the possessor of the animals or his agent, attendants, watchmen, transportation officials, veterinarians, and others who are engaged in the curing of animal diseases; professional castrators, meat inspectors, slaughterers, butchers, and others professionally engaged in the preparation, use, and destruction of slaughtered animals and their parts.

EXTENT OF INFECTED AREA

In the law in force in the whole of the German Republic with regard to infectious animal diseases, as well as in administrative regulations pertaining thereto, four kinds of infected areas are mentioned, a larger one including the smaller one, viz:

(1) Farm where infected animals are kept, (2) closed area, (3) observation area, and (4) a larger area where certain regulations relative to trade in cloven-footed animals are in force.

SLAUGHTER AND APPRAISAL OF ANIMALS

The regulations for the control of infectious animal diseases contain the stipulation that, if foot-and-mouth disease appears sporadically in a district otherwise free from the disease, authorization may be given for the slaughter of animals, infected or suspected of being infected, provided it can be assumed that the contagion will thereby be exterminated. In the explanation of this stipulation it is stated:

> If the infection has already gained ground or if there is reason to presume, after a sporadic outbreak, that the contagion has already spread to a great extent, for instance, by means of the propagation of the infection from a large cattle market, slaughter must be omitted as being useless from the start.

Compensation for animals thus slaughtered is paid out of public funds.

The basis for compensation is the market value of the animals, regardless of such lower value as may have arisen through the infection. In case of foot-and-mouth disease the total value shall be compensated, provided, however, that deduction shall be made partly for what may be paid for slaughtered animals on account of insurance agreements and partly for the value of such parts of the animal as the owner may dispose of under the regulations of the police authorities. Compensation is not given for illegally imported animals or for animals which have been found infected with foot-and-mouth disease within 14 days after their importation, unless it can be proved that they were not infected until after their importation. The right to compensation is forfeited in certain instances, as when the owner of the animals has neglected to report the outbreak of foot-and-mouth disease or violated resulting regulations.

Furthermore, every individual State of the confederation issues special instructions with regard to the payment of compensation, for instance, as to how the compensation shall be determined. In Prussia the following regulations apply with regard to this matter: The appraisal is made (1) either by a veterinary official or (2) by him and two arbitrators. The veterinarian appraises alone (*a*) when the owner consents thereto, or (*b*) when it is permitted in the regulations issued by provincial or municipal confederations of the same standing, provided the total amount appraised does not exceed in case (1) 1,500 marks and in case (2) 500 marks. The regulation in question was issued in 1911. Otherwise the appraisal shall be performed by a veterinarian and two arbitrators.

For each kreis (an administrative district comparable to a county) the managing board of the district appoints a number of persons to serve as arbitrators for a period of three years. A kreis may also be divided into several arbitration districts. The arbitrators shall be sworn. The same applies to veterinarians acting in behalf of the official veterinarian. The police authority calls in arbitrators in case appraisal is to take place. Relationship with the owner and other circumstances of the kind are cause for being barred as arbitrators. In case of difference of opinion among the appraisers the average of the various values appraised shall as a rule be considered as the value actually appraised. If, however, the values determined by two of the appraisers agreeing with each other be lower than the average amount, the lowest value shall apply. The appraisers make a record of the appraisal. The decision may not be appealed from. The appraisal shall, if possible, take place prior to the slaughter and at the place where the animals are kept.

Money for the payment of compensation for animals slaughtered on account of foot-and-mouth disease is paid in Prussia partly from the funds of the provincial or municipal confederation of the same standing and partly from the public treasury, and in equal shares. The confederation may decide to assess the owners of animals for contributions for the payment of compensation and for administrative expenses, to such extent as they are not borne by the public treasury. With regard to the foot-and-mouth disease, only owners of animals that can be infected with the disease may be assessed, and the number of livestock is used as a basis for the contribution.

Assessment can also be made, however, for the forming of a fund for the purpose.

QUARANTINE OF PERSONS

A quarantined stable on an infected farm may be entered only by the owner or his representative or by those who have to look after or attend the animals. Persons having remained in a quarantined stable are subject to disinfection if they desire to leave the farm. The disinfection consists of the cleaning and disinfection of such shoes and clothing as are soiled and not changed, as well as of the hands and other parts of the body that have been in contact with the animals. Other prohibition to leave or enter an infected farm is not expressly stipulated in the general laws, but restrictions in the personal intercourse may be prescribed by the police authorities for certain places within the infected district, such as farm, stable, pasture, or market place. Such restrictions may serve to prevent certain tradesmen, such as cattle dealers and butchers, from entering a designated area, but may also include instructions for the disinfection of persons visiting infected places.

QUARANTINE OF ANIMALS

INFECTED FARM

Cloven-footed animals may be moved from an infected farm only for slaughter with the permission of the police authorities. If the animals are grazing, they shall as a rule be placed in stables.

Cloven-footed beasts may not be brought to the farm.

The infected animals shall be separated from the sound ones.

ISOLATED AREA

Animals suspected of being infected shall be separated, but, on permission of the police authorities may be removed for slaughter. Importation of cloven-footed animals into the area may not take place except for immediate slaughter.

OBSERVATION AREA

Cloven-footed animals may not be driven through the district. Exportation for feeding and breeding purposes may be granted by the police authorities. Cloven-footed animals may be brought to a near-by slaughterhouse or railway station for transporation to a slaughterhouse if on veterinary examination not earlier than 48 hours prior to the departure the herd has been found sound.

For the whole district common grazing may be prohibited as well as the use of common watering places for cloven-footed animals. In this manner some kind of compulsory insurance is obtained. The decisions reached by the confederations in regard to matters of the kind must be ratified by the minister of agriculture in order to become effective.

RESTRICTIONS ON GOODS

INFECTED FARM

Manure may be transported from infected stables only under the observation of certain disinfection procedure. Like conditions apply to the removal from the farm of manure and urine of cloven-footed animals. Without the permit of the police authorities forage and

litter may not be removed, and then only if, with consideration paid to their storage and manner of transporation, they can not be considered as bearers of infection. Implements, vehicles, containers, and other articles, if they have been in contact with animals infected or suspected of being infected or with waste from such animals, must be disinfected prior to being removed from the farm.

ISOLATED AREA

Manure and urine from cloven-footed beasts as well as implements and other articles may be removed from the area only with the permit of the police authorities and under observance of such precautionary measures as those authorities may prescribe.

With regard to the other two kinds of isolated areas there are no regulations pertaining to the exportation of goods.

MILK

As a general rule it is prescribed that milk from an infected farm may not be removed unless it has been heated to a certain temperature. Exemption, however, can be granted for the transportation of milk to an establishment where such heating can be performed. As a condition for the granting of exemption it is stipulated that all milk shall be warmed to a certain degree of heat. This is determined in the following manner: (1) Heating over open fire until boiling takes place; (2) heating to not less than 85° C. through steam acting directly or indirectly; (3) heating in a water bath, at not less than 85° C. for 1 minute or not less than 75° C. for 30 minutes, the last method carried out being under conditions prescribed by the proper authority.

In Germany, exemption has become the rule. The milk is sent to the creamery, or other dairy establishment, immediately after the outbreak of the disease, without any interruption.

The German statutes contain no provisions for compensation for milk which it has not been possible to utilize in the usual manner on account of the regulations in force.

HORSES, DOGS, CATS, AND POULTRY

Horses and other single-hoofed animals may be used outside of the infected farm, provided, if they are placed in quarantined stable, that their hoofs are disinfected before they leave the farm.

Within the isolation area all dogs shall be tied. A dog which is led by a leash or put to a vehicle is to be considered as tied. Exemption may be given for watchdogs when they accompany watchmen, and for hounds during hunting.

On an infected farm the poultry shall be penned. The same applies with regard to pigeons where circumstances permit.

DURATION OF QUARANTINE

It is prescribed with regard to an infected farm that it shall be declared free (1) if all the cloven-footed animals have died or have been slaughtered or removed; (2) if no recurrence has occurred within three weeks after the removal of animals infected or suspected of being infected or after recovery is established by veterinary examination; and (3) if, in both cases, disinfection has been performed and been approved.

Relative to other isolated areas it is stipulated that the regulations issued with regard to them be revoked as soon as the risk of the disease being transmitted to noninfected farms within the areas does not exist.

INOCULATION

For the professional production of serums as a protection against or for the cure of infectious animal disease a license is required which is issued in Prussia by the Minister of Agriculture when the applicant is a public institution and otherwise by the president of the local government. License is granted only for specific serums and only to experts having suitable facilities. The institution is placed under public veterinary inspection.

GENERAL PRECAUTIONARY MEASURES

The regulations for the control of infectious animal diseases and laws relating thereto contain a number of measures pertaining to protection against the permanent danger of infectious animal diseases. They refer, for example, to the supervision of cattle, markets, examination of animals for railway and sea transportation, limitation of the right to drive animals on public roads, certificate of origin and bills of health for animals being brought to markets and exhibitions, compulsory Pasteurization for creameries, and the establishment and management of slaughterhouses and markets.

NOTES AND COMMENTS

The German laws are characterized by great detail. Germany's form of government is responsible for a lack of conciseness. First come the regulations concerning animal diseases, valid for the whole country (82 paragraphs), with the addition of export regulations comprising 15 paragraphs and supplements. Each State has its export laws pertaining to the law regarding animal diseases, to which belong special regulations, and finally come in each State police regulations regarding animal diseases (315 paragraphs and various supplements). There are also a few special regulations. As a result the same subject and the same wording occur several times.

SWEDEN

PERSONS ENTERING STABLES, ETC.

According to royal proclamations in 1924 (1) owners of susceptible animals must see to it that cattle buyers, butchers, or other persons not caretakers of the animals do not enter buildings where cattle are kept unless they have a permit from the medical department. (2) Persons coming from a foreign country infected by foot-and-mouth disease are prohibited for six months from association with cloven-footed animals unless they pass a medical examination and are duly disinfected.

MILK

A law of June 6, 1925, prohibits, within a certain area, any persons from selling or delivering milk, buttermilk, or whey intended for use as cattle feed unless such product has been heated to at least 80° C.

TRANSPORTATION OF ANIMALS AND GOODS

Horses and cloven-footed animals must not be moved to infected counties except by permission from the medical board. Exports of animals and certain described offal, bones, hides, and skins, and hay and straw may not take place from infected territories without a permit. It is provided, however, that horses may be moved to and from an infected county when harnessed to a cart.

GENERAL REGULATIONS

Regulations formulated in 1898 to suppress foot-and-mouth disease and other diseases describe in detail the functions of the municipal and health boards, also län (county) government boards, and the rights and duties of the police and owners of the domestic animals.

Where foot-and-mouth disease has broken out a veterinarian appointed by the län government board makes a thorough investigation and furnishes the board and the owner of the animals with written instructions concerning measures necessary to stop the spread of the infection. The functions of the local police are set forth, also the duties of owners regarding segregation of infected animals, attendants for same, disposal of dead animals, and of manure, milk, etc. Dogs must be kept in a shed or tied up until the premises are declared free. Provisions concerning disinfection of premises, compensation to owners, and penalties for violations are detailed.

When foot-and-mouth disease was prevalent, especially in the early stage of the epizootic, the län government boards suspended all church services and closed the public schools and amusement places. The report states, however, that when the disease made greater progress it became necessary to relax some of the prohibitory measures.

SWITZERLAND

METHOD OF REPORTING

Report shall be made to the competent cantonal authority. Persons under obligation to report are the owner, watchman, and attendants of animals, all departments of the veterinary police, veterinarians, meat inspectors, slaughterers, customs officials, cantonal and municipal police departments, proprietors of inns, and cattle dealers with regard to animals stabled with them; all institutions, both those of the confederation as well as those of the cantons, and finally everybody who can assume that otherwise a report would not be made.

EXTENT OF INFECTED AREA

The law specifies two kinds of closed areas, viz, local quarantine and extended quarantine.

The latter contains two areas with different regulations, viz, the infected area and safety area. The area of local quarantine includes inclosed territory, such as stable, premises within fences, etc. The area of extended quarantine includes, for example, a village or a whole district. It seems, however, that in the combat of foot-and-mouth disease extended quarantine is resorted to immediately when a case of the disease has been found.

In fixing the limits of the safety area, consideration is taken, if possible, of the geographic conditions, but the limits may be fixed

without consideration to the borders of municipalities of cantons. If it is a question of including in a zone more than one canton, the national veterinary authorities fix the zone limits.

SLAUGHTER AND APPRAISAL OF ANIMALS

Inasmuch as slaughter appears to be a means of successfully combating the infection, the veterinary authorities, or the cantonal board of health shall prescribe that infected animals or animals suspected of being infected shall be slaughtered. Slaughter shall particularly be resorted to if in a district only sporadic cases of the disease appear or if the disease appears at an especially dangerous place.

The extent of the slaughter is determined by the veterinary authorities in concurrence with the cantonal boards of health. When differences in opinion arise between them the matter is decided by the department of agriculture.

In this connection it should be especially noted that animals not fully recovered may be slaughtered when the quarantine ceases. This refers to animals that are considered to be carriers of the infection.

Before the slaughter, official appraisal shall be made on the basis of the price the owner would have to pay for a like animal.

The authorities ordering the slaughter shall prescribe whether and in what manner the carcasses of slaughtered animals may be utilized.

If many animals are slaughtered the veterinarian shall endeavor to facilitate the utilization of the slaughtered animals.

Slaughter shall take place if possible where the animals are kept. The necessary arrangements for slaughter shall be made by the cantonal veterinarian. In order to render the wastes harmless they shall be buried on the spot. Heads, tongues, and viscera may be used only in boiled condition. If they can not be thus treated they shall be immediately burned or buried. The feet shall be cut off and be burned or buried. The buried parts shall be covered with lime before the grave is filled. The hides shall be disinfected.

If the slaughter can not take place on the spot, the animals shall be brought to a slaughtering place with the greatest precautions. The cantonal veterinarian shall personally supervise the transportation. The following precautionary measures shall particularly be taken:

(1) The hoofs of the animals shall be cleaned and disinfected; they shall then be painted with iodine and covered with a thick layer of tar. In addition a bag soaked with a disinfectant shall be folded around the feet.

(2) The cavities of the mouth and of the nose shall be protected with a bag of impervious material to prevent mucus from falling to the ground.

(3) Transportation shall take place in a cart. If this is not possible, only such roads may be used as are neither open to passenger traffic nor used by animals from other farms.

(4) The parts of the roads used shall be thoroughly disinfected, and the excrement shall be removed and burned.

In exceptional cases the veterinary authorities may permit the transportation of animals by railway to the place of slaughter.

QUARANTINE OF PERSONS

SIMPLE QUARANTINE

Admission to quarantined animals is prohibited unless an official veterinarian has given permission.

INFECTED ZONE

Persons living in buildings in which there are quarantined animals must not leave the buildings without permission. If they have no contact with the animals the cantonal veterinarian may grant them permission to live at some other place during the period of isolation. Before such transfer they shall be disinfected.

The personnel used for the attendance of the animals must not leave the premises assigned to them by the veterinarian. On instructions of the cantonal veterinarian specified persons may be granted permission to leave the quarantined area for the purpose of performing agricultural work of an urgent nature.

SAFETY ZONE

No special regulations are in force for this zone with regard to personal intercourse.

QUARANTINE OF ANIMALS

Animals infected or suspected of being infected are isolated within a fixed space, stable, inclosure, or the like. The animals may not leave the area and healthy animals may not be placed there. With the permission of the veterinarian, animals in the area may be transported to slaughter.

EXTENDED QUARANTINE

INFECTED ZONE

No animals of any kind may leave the area where they are isolated, and if the animals are grazing (on the Alps), where there are no stables, they shall be assembled in herds and watched day and night. Animals not susceptible to foot-and-mouth disease may be removed, however, with the permission of the cantonal veterinarian after they have been disinfected.

Importation of animals susceptible to the disease is prohibited.

SAFETY ZONE

Animals within this zone (outside of infected zone) may not be removed or offered for sale. With the permission of the cantonal veterinarian animals may be used, however, for agricultural work and also may be taken to near-by pastures. Transportation of animals for slaughter may also be permitted by the veterinarian.

RESTRICTIONS ON GOODS

The removal from the infected zone of commodities that might transfer the disease is prohibited.

MILK

Milk may not be sent from a farm where foot-and-mouth disease has been found or is suspected of being present. It may be used only at the place of production. Butter produced from such milk shall be melted.

A creamery that has received milk from a farm that is infected or suspected of being infected shall be isolated as well as the farms that have delivered milk to the creamery during the last 14 days. Milk from these farms may not be placed on the market and may be delivered to the creamery only after having been boiled. It must be transported by persons who do not come in contact with the animals infected or suspected of being infected.

In exceptional cases and upon permission of the cantonal veterinarian raw milk may be gathered in carts from the various owners of animals and brought to the creamery.

The containers shall be returned in a cleaned and disinfected condition to the owners of the animals. So long as such arrangements are applied, only such persons may be admitted to the creamery as are employed in it.

According to the statutes no compensation is paid for damage on account of milk restrictions.

HORSES, DOGS, CATS, AND POULTRY

Horses, dogs, cats, and poultry are not specifically mentioned in the laws. Reference should be made, however, to the general regulations in force regarding animals within the area of extended modification, according to which no animals may leave the area except by permit from a veterinarian.

DURATION OF QUARANTINE

As to the duration of the isolation regulations it is in general stipulated that they shall remain in force until expressly changed or revoked. As a basis for the duration of quarantine the final examination of the official veterinarian is accepted. The following regulations, however, should be noted. If slaughter of the animals has taken place, the farm remains quarantined for four weeks thereafter. If the animals have not been slaughtered, they shall be marked, after recovery, with the year and month when infected. The marked animals may be used within the isolated area, but until eight months have elapsed they may not be removed for any other purpose than slaughter or in order to be brought together with animals belonging to a herd that has had the disease. Through a special decision after the passing of the law this period of eight months was reduced to three months, but later was set again at eight months.

At places where slaughter has been resorted to, animals may not be taken until four weeks after the final disinfection, and at places where the animals have had the disease, new animals may not be placed until eight months after the quarantine has ceased to exist.

INOCULATION

The veterinary authorities consider the scientific and practical value of biological products and are authorized to supervise the production of such articles. Enterprises engaged in the production

of, or trade in, biological products are under the supervision of the veterinary authorities.

GENERAL PRECAUTIONARY MEASURES

The trade in horses, cattle, sheep, goats, and pigs is regulated. This is made possible by dividing the cantons into cattle-inspection districts in each of which there is a cattle inspector, and if possible a veterinarian employed by the canton. This official makes a record of all domestic animals, of the kinds mentioned, which are imported into or exported from the district. Exportation can not take place unless the inspector has issued a bill of health which may include either one animal or several animals. Cattle markets and exhibitions are under veterinary and police supervision. Animals may not be imported into such district without bills of health, and veterinary examination takes place prior to the importation. The trade in animals by taking them from farm to farm is prohibited, but the cantons may grant exemption for such trade in poultry.

NOTES AND COMMENTS

The Swiss regulations pertaining to the combat of infectious diseases among domestic animals consist of a law and an executive order, the former of June 13, 1917, and the latter of the August 30, 1920. They are detailed but clearly arranged. The laws contain 50 paragraphs and the executive order 286 paragraphs.

THE NETHERLANDS

METHOD OF REPORTING

Report shall be made to the mayor of the community owner, possessor, or attendant of animals.

EXTENT OF INFECTED AREA

The extent of infected area is fixed by the managing official of the municipality upon recommendation of the official veterinarian. In case of divergent opinions the matter is referred to the Minister of Agriculture. The area is made known by means of posters of which there are two kinds—white and blue. The white ones are posted at farms infected or suspected of being infected; the blue ones, at other farms in order to warn against danger of infection. The latter cause no infringement in the liberty of action of the owner, while, on the other hand, infringement is the case with the white posters.

SLAUGHTER AND APPRAISAL OF ANIMALS

The owner of an animal is subject to regulations prescribing slaughter of infected animals or animals suspected of being infected. Compensation is paid at the rate of 90 per cent of the value of the sound animal.

The appraisal is performed by an expert, appointed by the mayor. If the owner, the chief of the veterinary district, or the mayor is dissatisfied with the appraisal, the mayor informs the judge of this fact, who appoints two experts to perform the appraisal together with the one first appointed. For decision the majority rule applies,

but if no majority is obtained the value that is neither the highest nor the lowest is taken. As soon as the appraisal has taken place the mayor informs the owner of the animals that the compensation due him will be paid by the public treasury.

QUARANTINE OF PERSONS

A farm, including the grounds, placarded with a white poster, is quarantined in such manner that nobody may leave it without being disinfected. Disinfection regulations require that, if necessary after previous disinfection, hands and other soiled parts of the body be well washed with warm water and soap; and that clothing and shoes, if not changed, be scrubbed with soap lye to free them from adherent dirt.

The law does not prohibit entrance to an infected area.

QUARANTINE OF ANIMALS

The regulations regarding restrictions on animals provide for the separation of animals infected or suspected of being infected, and, if they are grazing, that they be placed in stables, that they be marked, and that they be not brought from the farm without permission. Prohibition against the removal of sound animals has not been enacted, and the mayor may grant permission to export infected animals or animals suspected of being infected under such conditions as the chief of the veterinary district may prescribe.

RESTRICTIONS ON GOODS

There are restrictions against the exportation of milk, meat, hides (unless disinfected), horns, and hoofs of ruminants, wool, manure, and other waste, hay, straw, and other forage products, ropes, covers for cows, and stable implements. Exemption may be granted by the mayor on conditions stipulated by the chief of the veterinary district.

MILK

The law of March 26, 1920, now in force, prohibits the removal from infected farm or area, of any article the exportation of which has been prohibited by the Government. According to royal decision of February 23, 1922, this prohibition includes milk.

Upon application of interested persons the mayor, with the consent of the proper veterinarian, may grant exemption and, if necessary, prescribe special conditions for the removal.

During the severe epizootic of 1924 exemption became the rule, at least when the disease had gained a more general spread. New milk was delivered immediately to the creameries, when it had been prescribed that it might not be shipped from the farm in other containers than those of the creamery. The milk was placed in these containers at the limit of the isolation area of the farm.

Damage caused by measures prescribed for preventing the spreading of the contagion may be wholly or partly reimbursed by the public treasury in accordance with the decision of the minister of agriculture. Compensation may be paid, also, for milk that can not be sold, but such procedure is applied to a very small extent.

HORSES, DOGS, CATS, AND POULTRY

No restrictions are prescribed in the laws with regard to the use of horses. It is stipulated that instructions may be issued prescribing the tying or locking up of dogs, cats, and poultry.

DURATION OF QUARANTINE

No period of time is fixed during which a farm shall be considered infected or suspected of being infected. It is only stipulated that the mayor shall remove the posters as soon as the chief of the veterinary district has sent him a written statement to the effect that he is in favor of such a measure.

GENERAL PRECAUTIONARY MEASURES

When there is risk of the spreading of an infectious disease or when such disease is prevalent the Government may issue certain regulations for the prevention of the spread of the infection, Pasteurization of milk, treatment or marking of animals that shall be transported, and conditions for the transportation of milk, meat, and other commodities. Such regulations may apply to the whole country or parts thereof.

INOCULATION

According to law the Government decides which scientific institutions shall be made available in the combat of infectious diseases. Such institutions are the veterinary college in Utrecht and the national serum institute in Rotterdam.

NOTES AND COMMENTS

The Dutch laws of March 26, 1920, revised March 17, 1923, with accompanying royal decrees, are concise. There is a noticeable endeavor in the Netherlands to adapt the governmental measures to existing conditions. This is expressed in a stipulation mentioning that measures which might be taken on account of foot-and-mouth disease should be practical according to science and practice.

UNITED KINGDOM

METHOD OF REPORTING

Report must be made to the local police, who shall report to the secretary, board of agriculture, London, and also to the inspector of local authority (as a rule, a veterinarian). Under obligation to report are the possessor and attendants of animals and the inspector of local authority.

EXTENT OF INFECTED AREA

According to authorization of the Minister of Agriculture and Fisheries a veterinary inspector declares a farm, the livestock of which has been infected with foot-and-mouth disease, an infected place, and in addition, according to the decision of the minister, an area around the same may be declared an infected area. For these two different kinds of infected areas there are different regulations.

SLAUGHTER AND APPRAISAL OF ANIMALS

The local authority, when advisable, may cause the slaughter of (1) cattle, sheep, and pigs infected or suspected of being infected with foot-and-mouth disease; (2) cattle, sheep, and pigs which in some way have been in contact with animals infected with the disease, or which otherwise may be considered as having been subject to infection. Compensation is paid for slaughtered animals infected with the disease according to their value immediately preceding the infection, and for other (exposed) animals according to their value at the time of the slaughter.

The determination of compensation takes place in the following manner in England and Wales:

The inspector of the Department of Agriculture or some other official thereof gives the owner of the animals or his representative a written statement regarding the value the department has fixed for the animals to be slaughtered. If a written protest against this value is not submitted within 14 days thereafter, the compensation is paid. In case of protest the matter is settled by an arbitrator who is appointed by the court, unless agreed upon by the parties. Such arbitrator must render his decision within seven days after being appointed. If the arbitrator decides a higher value than that fixed by the department, the department pays the cost of the arbitration; otherwise the owner defrays the cost. In case the local authority ordered the slaughter, what is stipulated with regard to the department applies to the local authority.

If the local authority ordered the slaughter, the compensation is paid from local tax funds but the amount paid out is afterwards refunded by the government.

Nobody may, without the permission of the proper authority (inspector of the department or local authorities) enter stables, fields, or other places within the infected place. Any one entering a place where an infected animal or one suspected of being infected is or has recently been kept shall wear suitable overall clothing approved by the inspector, and shoes that can be disinfected. Whoever leaves such place shall take off the overall clothing and carefully disinfect hands and shoes. Persons who attend animals infected or suspected of being infected must not attend another animal without the permission of the inspector. With regard to inspectors, appraisers, practicing veterinarians, and others who have to visit an infected place, it is prescribed that they shall put on overall clothing, etc., and undergo disinfection before leaving the place. At the infected place there shall be kept at a suitable place a container with some prescribed disinfectant.

From a farm to which an animal has been transferred according to (3) and (4) (see Quarantine of Animals) the animal may not again be moved until a period of 28 days has expired, with certain exceptions for breeders.

Animals brought to a slaughterhouse according to permit shall be slaughtered within 96 hours after arrival. Prior to being transferred to such place they shall be marked: Cows, by a clip 6 inches long on the left side toward the back and by cutting the hair off the end of the tail; sheep, with an M 6 inches high painted or stamped in red or blue color on both sides of the animal; and pigs, with the

letter M painted or stamped in red or blue color on both sides of the body.

A permit granted is not valid more than six days. As a condition for the permit a certain manner of transportation may be prescribed. A railway shall preferably be used. The person who transports the animals shall bring the permit with him.

The foregoing stipulations relative to the transportation of animals in an infected area shall not prevent the transportation of animals through this area by railway, provided the animals do not leave the cars.

QUARANTINE OF ANIMALS

INFECTED PLACE

Without the permit of the department inspector, cloven-footed animals may not be brought into or removed from an infected place.

INFECTED AREA

No animal shall be moved out of an infected area. All transfers of cloven-footed animals from a farm within an infected area are practically prohibited, unless permission is obtained from the veterinary inspector. Animals may not be brought into such an area for any other purpose than slaughter or to a farm situated within 2 miles of an infected place, and then only by permit. Market or other exhibitions of cloven-footed animals may not be held, except that a market for animals intended for immediate slaughter may be permitted. The veterinarian shall then examine the animals. Likewise the sale of animals on farms may be permitted. Permit to hold such market as just mentioned is not given for any place within 5 miles of an infected place. The sale of animals on a farm is not allowed if such farm is within 2 miles of an infected place.

Permission for the moving of cloven-footed animals into or within an infected area is granted for transfer: (1) From a farm outside of infected area to slaughterhouse within infected area; (2) from a farm outside of infected area to a farm within the infected area; (3) from a farm within the infected area to an authorized market of animals for slaughter or slaughterhouse sale (at farm) in the same infected area; (4) from an authorized market for animals for slaughter or slaughterhouse sale in one infected area, or from a farm in one infected area to a slaughterhouse in the same or in another infected area; (5) from one farm to another within the same infected area, or between different parts of the same farm in such infected area, if the inspector considers the removal necessary or useful.

RESTRICTIONS ON GOODS

Forage, litter, manure, implements, feathers, basket work, and other articles may not be removed without the permission of the department or of the inspector of local authority, which permission is not granted until the articles in question have been disinfected. Manure may not be taken to any other place than an incinerator or depository approved by the inspector. It may not be taken to other districts (county or city) without permission of the local authorities.

Muck and litter shall be carefully disinfected at the expense of the owner before it is brought or conducted from the place where there are animals infected or suspected of being infected.

No carcass may be removed unless it has been butchered and permission for removal granted by the inspector of the department or of the local authority. The carcass, together with head and hoofs, shall be examined by the inspector, and for the removal a certificate is required from him to the effect that the carcass shows no symptoms of foot-and-mouth disease. The entrails shall be burned or destroyed in some manner at the farm. Hides from such carcasses may not be removed from the premises without permission and without having been immersed in a disinfectant. The carcass of an animal which has not been slaughtered but has died of some other cause than foot-and-mouth disease shall be destroyed at the expense of the owner, but, with the permission of the inspector, the hide may be removed after disinfection.

Carcasses of animals that have died of foot-and-mouth disease shall either be buried with hide at a suitable place at a depth of not less than 6 feet and be covered with quicklime or other disinfectant, or else brought to an abattoir for horses and there destroyed chemically or by exposure to high temperature.

INFECTED AREA

Waste from slaughterhouses may not be shipped from infected area or be brought into contact with cloven-footed animals within such area.

MILK

Milk may not be removed from an infected place without permission either of the inspector of the department, or of an official in the department, or of the inspector of the local authority. The laws give no information as to what period after the outbreak of the disease such permission may be granted. It appears from the report of the committee on the foot-and-mouth epizootic in England of 1922 that practice has been fluctuating in this respect. According to this report complaints have occurred inasmuch as permits have been refused at such a late stage that no danger of the contagion's being transmitted through the milk could be considered possible. Complaints have particularly been directed against health authorities which have refused to permit the removal, through their health inspectors and medical officers. With respect to the long period of time during which the restrictions prescribed in connection with the isolation are in force, the stopping of the sale of milk must in the opinion of the committee seriously affect the owner of the animals; and the committee has therefore expressed the opinion that it should be allowed to dispose of the milk for human food, in which case necessary precautions should be observed. If an animal has recovered from the disease according to certificate from the veterinary inspector, but the health officer nevertheless refuses permission for the exportation from the farm of milk from such animal, the owner, in the opinion of the committee, should receive compensation from the health authority.

HORSES, DOGS, CATS, AND POULTRY

There are no special regulations with regard to horses and cats.

In an area within a radius of 2 miles from an infected place dogs which are not kept in dwelling houses shall (1) be kept in kennels or other places from which they can not escape, or (2) be kept tied on the farm, or (3) be accompanied by their owners or other persons so that they are under control.

With respect to both dogs and poultry the inspector of local authority or of the department may issue the necessary control regulations. Poultry are not slaughtered but must be kept penned.

DURATION OF QUARANTINE

It is stipulated regarding infected places that, if at such a place all the infected animals have been slaughtered, it may cease to be considered infected when 28 days have elapsed from the day when the slaughter was completed, provided final disinfection has been performed and approved by the inspector of the department. Otherwise no regulations have been found pertaining to the period during which the declaration of infection shall remain in force. (According to the report for 1923–24, paragraph 38 (*34*), the owner of the animals is free from all restrictions after about eight weeks.)

GENERAL PRECAUTIONARY MEASURES

The inspector of the department or of the local authority may isolate farm, field, or other place within an infected area, which may then be entered only by the owner or his servants for the treatment of the animals.

The local authority may prescribe or, in case of need, cause disinfection of buildings, farm, or other place where animals have been received for sale, exhibition, or the like, as well as of means of transportation. This disinfection takes place at the expense of the owner.

The local authority may issue instructions regulating the removal of animals by land or by sea to their district or within the district, provided, however, that these instructions may not be contrary to those stipulated with regard to removal of animals from infected places or within an infected area. Furthermore, for the prevention of the spreading of foot-and-mouth disease the local authority may issue regulations intended to postpone or regulate the exhibition or sale of cloven-footed animals at markets, and other places.

When an animal is found infected with foot-and-mouth disease at a market place, railway station, or other place which is not under the control of the owner of the animal, the veterinary inspector may cause the animal to be seized and brought to a suitable place and stabled there. The animal may not be removed afterwards without the permit of the Department of Agriculture. The place, however, may be declared as infected only by the department. The veterinary inspector prescribes the disinfection of the market or other place, before new animals are brought there. The cost of these measures is to be defrayed by the owner of the animal.

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