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FOOT AND MOUTH DISEASE

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INTRODUCTION

Fifty years ago (in 1897) Loeffler, Frosch, and Uhlenhuth established the filterable nature of the incitant of foot-and-mouth disease. This had been preceded by the remarkable discovery of the Russian researcher D. I. Ivanovskiy, who, studying mosaic diseases of tobacco, first established that certain incitants of diseases in plants pass through bacterial filters.

Scientific data in the domain of the study of foot-and-mouth disease, after it was established that the incitant of this infliction is of filterable nature, was enriched by a whole series of discoveries of great theoretical and practical significance. It suffices to recall such an outstanding discovery of the French investigators Vallée and Carré as that of the plurality (mnozhestvennost') of types of the foot-and-mouth virus. These investigators discovered two immunologically different types of the foot-and-mouth virus, indicated provisionally by them with the letters "O" and "A".

This was not only confirmed by subsequent research, but even supplemented by the discovery of a new type "C". In addition, there were revealed numerous variants which could not be associated with any of the indicated types of the foot-and-mouth virus.

At present it is considered that the existence has been proved of three immunologically different types of the foot-and-mouth virus "O", "A", and "C", and of numerous variants of these types.

Thanks to these new data, problems of immunity and problems of immunization for foot-and-mouth disease are reflected in a somewhat different light. It has become possible in practice to solve problems of specific bioprophylactics somewhat differently. Obviously, many valued bio-preparations should give more effective results in the battle against foot-and-mouth disease than preparations of single value. This is also the present trend in the solution of the problem of methods of active and passive immunization against foot-and-mouth disease.

The problem of methods of anti-foot-and-mouth vaccination may be considered to be conclusively solved at the present moment.

In a number of foreign countries aluminum-hydroxide vaccine, proposed by Danish investigators and perfected in Germany, is being applied successfully in the struggle against foot-and-mouth disease.

Great light has now been shed on the problem of virus-carrying in connection with foot-and-mouth disease, which is a very serious epizoological factor. It must be considered to be proved unconditionally that a certain portion of animals which have contracted the foot-and-mouth disease remain virus-carrying over a long period. From this is derived the necessity of quarantine-restrictive measures with respect to animals which have contracted foot-and-mouth disease. In many countries the periods of such quarantines which have been adopted with respect to animals which have contracted foot-and-mouth disease remain without adequate scientific foundation. Practical precautions bear a certain contrast to scientific data with respect to virus-

carrying in connection with foot-and-mouth disease. It is essential to continue with still greater urgency the study of this problem and accumulate further data from practical experimentation, in order to form final conclusions on the epizootological significance of virus-carrying and resulting quarantines with respect to animals which have contracted foot-and-mouth disease.

Science has also brought much that is new to bear on the questions of the stability of the foot-and-mouth virus in an external medium, which has substantially broadened our concepts with respect to the epizoology of foot-and-mouth disease. Interesting work on this question has been carried out by our Soviet investigators.

Contemporary investigators have as yet done very little in the area of research on new and perfection of old methods of foot-and-mouth disease therapy. This is explained by a certain underestimation of this extremely important problem, whose successful solution would help prevent numerous complications of the basic foot-and-mouth disease, and would help to decrease losses from this affliction.

The study of the epizoology of foot-and-mouth disease has been completely inadequate. This has happened because many questions of this affliction have been decided in the laboratories of institutes and experimental stations, and not under conditions of the epizootological medium itself. In this connection practical

workers, observing and taking into account the influence of various epizoological factors on the development of foot-and-mouth epizootics, could be of great assistance to scientists. Such observations should be brought to light in the press and taken into consideration by investigators.

Many questions of the foot-and-mouth disease problem can be decided only through active cooperation between science and practice.

CHAPTER I

THE SPREAD OF FOOT-AND-MOUTH DISEASE

GENERAL DATA

Foot-and-mouth disease is encountered in all countries of Europe, in Asia, Africa, and South America.

In a whole series of countries epizootics of foot-and-mouth disease have by no means decreased during the past 10 or 15 years, but remain at almost the same level, as is evident from data collected by Flückiger for the period from 1925 to 1933 in 33 countries. This is true, at least, with respect to Germany, Italy, Poland, Czechoslovakia, Yugoslavia, France, Spain, Rumania, and Holland.

How wide is the spread of foot-and-mouth disease may even be seen from the fact that in 1935, according to data of the Bureau of Animal-Husbandry Industry of the US (Mohler), 66 countries were declared affected with respect to foot-and-mouth disease.

Below are adduced certain data on the trend of foot-and-mouth disease in individual countries.

US. Outbreaks of foot-and-mouth disease epizootics in the US have been observed in the years 1870, 1880, 1884, 1902, 1908, 1914, 1924, 1925, and 1929.

The outbreak of foot-and-mouth disease in 1908 was the most substantial of all epizootics in the US for the previous 27 years.

In liquidating the epizootic more than 150,000 head of cattle were killed, at a cost of about 6 million dollars.

Great Britain. A considerable outbreak of foot-and-mouth disease was noted in England as early as 1839, after which this sickness did not appear for 2 or 3 years. From 1877 to 1884 the foot-and-mouth disease was noted annually, the number of infected farmsteads at times reaching 18,732 a year, and the number of infected cattle 400,000 head (Hutyra and Marek).

In the period from 1892 to 1912, in spite of the compulsory killing of stock on all afflicted farmsteads, 158 more outbreaks were recorded.

Strong outbreaks in England were observed in 1922, 1923, and 1924. The epizootic of foot-and-mouth disease in 1922-1924, which broke out first on the eastern coast of England and subsequently spread to the south and to the north of the country, was the most serious epizootic for the preceding 30 years (Zwick). During this epizootic 1,140 separate outbreaks of foot-and-mouth disease were recorded and in this connection 55,599 head of cattle were compulsorily killed.

In 1923, 1854 outbreaks of foot-and-mouth disease were recorded and 125,098 head of stock were put to death; in 1924, 1,440 outbreaks of foot-and-mouth disease and 88,726 head of

stock were killed (American Commission on the Study of Foot-and-Mouth Disease); in 1926, 204 outbreaks; and in 1927, 19 outbreaks. In 1922 considerable mortality from foot-and-mouth disease was observed -- up to 2.4 percent on the average. In 1923 this mortality was still higher and made up to 3.1 percent.

France. The first large outbreak of foot-and-mouth disease in France evidently occurred in 1893, after which the disease was observed annually.

In 1921 foot-and-mouth disease affected, in 53 departments, 628 population points with 2,673 farms affected by the disease. In subsequent years, from 1925 to 1933, an average of 134,175 new cases of the sickness was noted annually (Flückiger).

There was a large outbreak of foot-and-mouth disease epizootic in France in 1937-1938, the disease having been brought via Marseilles from North Africa, together with imported vegetables and hogs. Almost the entire country -- 72 of 89 departments -- was seized by the epizootic in a very short period.

Spain. Foot-and-mouth disease in Spain is evidently static in character. For the period from 1925 to 1933, according to Flückiger's data, an average of 14,519 new infections were recorded annually.

Denmark. In the period from 1904 to 1912 Denmark was free of foot-and-mouth disease (Hutyra and Marek). -

In 1912 foot-and-mouth disease was brought into Denmark and acquired a considerable spread there — 408 farms were attacked by it. Toward 1919 the epizootic was eliminated. In 1924 foot-and-mouth disease was again brought into Denmark, in all probability from Germany, and spread there with exceptional rapidity. It infected 50 percent of all herds.

Toward the beginning of 1925, 8,050 cases were recorded. Subsequently there began a decrease in the number of farms affected by foot-and-mouth disease, but in 1926 there began a new increase in the epizootic wave. This time the sickness spread to Jutland, where in one month alone (May) 13,000 new cases were recorded. Incidentally, about 50 percent of all animals which had become infected developed foot-and-mouth disease again the following year. This is evidently explained by the difference in types of the virus which causes various epizootics.

Germany. In Germany foot-and-mouth disease is observed continuously as a static sickness. Epizootics had already begun to be reported from year to year in 1886. The strongest outbreaks in Germany were in 1888-1892, 1899-1901, 1910-1912, 1919-1921, and 1937-1938. During the foot-and-mouth disease epizootic of 1892, 4 million head of stock developed the disease (Zwick).

After a brief interruption, in 1896-1897, a large outbreak occurred again, during which about 3 million head of stock became infected. Following, a rather protracted period of "calm" occurred,

but in 1910-1911 a new wave of the epizootic began, as a result of which 29,877 population points were declared affected, and 3,366,369 head of cattle, 602,927 sheep, 53,674 goats, and 2,555,371 pigs were attacked by foot-and-mouth disease.

In spring of 1914 a new increase in the development of the foot-and-mouth disease epizootic began. Toward the end of this year it attacked 7,045 population points with 17,964 farms.

In 1919-1921 such a "severe" epizootic broke out that its proportions and effects cannot be compared with any foot-and-mouth disease epizootic observed at any earlier time in Germany. It suffices to point to the fact that in the fall of 1920 the disease attacked 23,369 population points with 181,067 infected farms.

During the period 1919-1921, 4.5 million head of cattle were attacked by foot-and-mouth disease (25 percent of all stock owned at that time in Germany). In 1926 in Germany, 187,000 farms infected by foot-and-mouth disease were counted, in 1927, 15,000 farms, in 1928, 23,000 farms, and in 1929, 4,000 farms (Waldmann). For the period from 1925 to 1933 an average of 46,090 infected points was noted annually (Flückiger).

In 1937-38 a substantial outbreak of foot-and-mouth disease occurred again in Germany, affecting a large area of the country. In this instance the foot-and-mouth disease had been brought in from France.

Hungary. In Hungary foot-and-mouth disease exists continuously.

In 1920-1921 numerous instances of a malignant wave of foot-and-mouth disease were noted, accompanied by massive stock-mortality. In one large herd the mortality among adult cattle reached 50 percent.

Rumania. In Rumania foot-and-mouth disease is of static character. For the period from 1925 to 1933 an average of 38,566 new cases of symptoms of the disease were recorded annually (Flückiger).

Russia. In prerevolutionary Russia foot-and-mouth disease was a static sickness. In essence, no struggle with this widely spread sickness was carried on then. The first statistical data on the spread of foot-and-mouth disease in Russia are found in the annual reports of the Veterinary Administration of the Ministry of Internal Affairs. (Data on the spread of foot-and-mouth disease for the period from 1881 to 1912 have been compiled on the basis of reports of the Veterinary Administration of the Ministry of Internal Affairs, published annually in the magazine Arkhiv Veterinarnykh Nauk.) We submit these data in two tables.

In individual gubernias, stock mortality substantially exceeded the average data submitted in Table I.

TABLE I

SPREAD OF FOOT-AND-MOUTH DISEASE IN RUSSIA IN 1881-1887

Years	Number of Gubernias and Oblasts Attacked by Foot-and-Mouth Disease	Number of Head Infected		Number of Mortalities As a Percent of the Number Infected	
		Cattle	Sheep, Goats, Swine	Cattle	Sheep, Goats Swine
1881	21	30,573	18,172	1.8	1.4
1882	40	164,968	19,651	1.7	2.8
1883	38	40,679	20,613	3.6	4.1
1884	18	2,992	721	12.9	66.2
1885	30	72,683	813	1.54	6.3
1886	52	214,468	25,540	2.03	1.1
1887	52	167,895	7,145	0.72	3.9

From analysis of these data (Figure 1) it may be seen that foot-and-mouth disease in prerevolutionary Russia was recorded annually as a considerable epizootic, attacking almost all types of animals susceptible to foot-and-mouth disease. In 1896, according to very approximate reckoning, about 2 million head of stock (1,705,322) were attacked by the foot-and-mouth disease epizootic, 246,444 head of reindeer alone being attacked by foot-and-mouth disease, of which 118,717 died and 32,085 were killed; in all, 150,802 head of reindeer perished from foot-and-mouth disease.

In 1901 a new intensification of the foot-and-mouth disease epizootic was noted, which persisted until 1905 inclusively, attaining

TABLE 2

Spread of Foot-and-Mouth Disease in Russia in 1888 - 1912

Years	Number of Gubernias and Oblasts attacked by Foot-and-Mouth Disease	Number of Head Infected				Number of Mortalities as a Percent of the Number Infected			
		Cattle	Sheep	Goats	Swine	Cattle	Sheep	Goats	Swine
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
1888	57	326,258	82,038	--	6763	0.4	0.6	--	3.3
1889	65	555,080	104,961	--	3407	0.3	0.5	--	0.8
1890	70	447,824	141,163	--	31742	1.4	0.8	--	1.0
1891	54	225,789	48,682	--	6557	0.8	1.5	--	2.3
1892	54	231,645	10,160	--	1171	0.5	1.02	--	0.9
1893	60	306,297	141,368	--	25165	0.3	0.3	--	0.9
1894	59	286,355	9,764	--	4216	0.3	0.9	--	2.9
1895	62	222,765	31,605	--	9000	0.08	0.1	--	0.09
1896	60	1,415,314	129,323	1121	12918	0.1	0.2	--	1.6
1897	69	628,005	60,021	815	17535	0.05	0.2	--	0.3
1898	67	513,643	66,012	407	5420	0.5	2.5	3.9	2.1
1899	63	291,004	19,114	--	7912	0.3	0.5	--	5.0
1900	67	627,690	120,048	454	52345	0.4	0.7	--	1.6
1901	71	829,030	112,795	65	19645	0.1	0.4	1.5	0.3
1902	68	1,885,869	351,713	78	25458	0.2	0.2	--	1.0

TABLE 2 (Continued)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
1903	60	456,394	7,754	—	2317	0.1	3.0	—	2.8
1904	79	1,032,985	76,725	—	5470	27.7	21.8	—	0.2
1905	77	2,145,025	547,089	—	15911	35.3	42.5	—	0.2
1906	65	225,509	15,460	—	6241	20.9	27.0	—	0.1
1907	73	548,410	38,193	—	22576		No Data		
1908	77	275,906	15,821	—	4643	0.2	18.4	—	1.3
1909	74	501,154	57,900	—	17380	0.2	0.3	—	0.4
1910	86	3,137,447	379	10671	91400	0.1	0.07	—	2.7
1911	83	2,153,587	666,023	—	129456	0.3	0.3	—	1.4
1912	82	224,756	57,461	—	10631	—	—	—	—

a maximum in 1905, when 2,141,025 head of cattle, 112,795 sheep, 12,645 swine, and other animals were infected with foot-and-mouth disease. After this there came a certain lessening of the epizootic, but after 1909 a new intensification of the epizootic wave began, foot-and-mouth disease spreading with exceptional rapidity and attacking a considerable area of the country.

In 1910 foot-and-mouth disease attacked 40,986 population points, in which 3,137,447 head of large cattle alone were infected with foot-and-mouth disease, not counting other kinds of animals infected with the disease.

Figure 1. Spread of foot-and-mouth disease in Russia for the period 1881-1912.

During the imperialist war no statistics on foot-and-mouth disease were compiled, but during these years it was hardly less widespread.

Some conception of the proportions of the spread of foot-and-mouth disease and the losses caused by it during the war may be imparted by the following official communique. In 1915 on only one of the fronts 24,623 head of cattle, or 6.43 percent of the overall number of head consigned for the supply of the front, perished from foot-and-mouth disease.

USSR. Foot-and-mouth disease, like many other infectious diseases, was a bitter legacy from the old, prerevolutionary

Russia. In the first years of the civil war real struggles against foot-and-mouth disease were not carried on, not counting several quarantines connected with the transportation of infected stock.

At present the incidence of foot-and-mouth disease among stock in the USSR, by comparison with the incidence of foot-and-mouth disease among stock in the countries of western Europe, is very small, if taken with respect to the overall number of stock susceptible to foot-and-mouth disease in these countries and the size of territories on whose expanse this stock is located.

In the period 1927-1930 stock in a considerable area of the country was attacked by foot-and-mouth disease. The veterinary organization in these years proved to be poorly equipped for campaigning against foot-and-mouth disease. After this, with regard to the spread of foot-and-mouth disease, an interval of "calm" began, which continued until autumn of 1935. After this new outbreaks of foot-and-mouth disease appeared, which thanks to widely developed anti-epizootic and prophylactic measures were quickly eliminated.

Soviet veterinary workers, using contemporary scientific data and experience accumulated under practical conditions in the struggle against foot-and-mouth disease, are continuing to cope successfully with the problems of its elimination. (Prof. Magnusson (Sweden), who visited the USSR in 1932, where he became acquainted

with the administration of the struggle against foot-and-mouth disease, said: "In the field of struggle against foot-and-mouth disease the Soviet Union now stands in the first rank of the leading countries of Europe". See A. L. Skomorokhov's article, "Our Achievements in the Field of Study of Foot-and-Mouth Disease and Methods of Struggle against it in the USSR". Sov. veterin., No 1, 1934.)

HISTORICAL DATA

In existing literature on foot-and-mouth disease we find extremely sparse information concerning the history of the rise of foot-and-mouth disease epizootics.

The first description of the sickness among animals apparently similar to foot-and-mouth disease was made by Hieronymus Fracastorius in 1546 in Venice. The sickness which he described had appeared then in Upper Italy in 1514 (Waldmann and Trautwein). And this was not accidental, since Italy in those days represented one of the leading commercial centers in Europe.

Commerce in animals and products of animal husbandry, and transportation of animals and animal materials, were doubtless at that time the basic cause of the spread of foot-and-mouth disease. This may be seen from the fact that as early as the sixteenth century there was introduced in Italy a system of special attestations given by owners of animals as to the state of health of these latter upon their removal from safe localities.

Sagar in 1764 observed a similar sickness in Moravia among cattle, sheep, goats, and hogs, and also among various wild beasts. This sickness he considered to be infectious also for human beings. He considered that the cause of this sickness was fodder infected with smut (Necard and Leclainche).

Brussonius and Siegel assert that in the period 1878-1886 16 epidemics of foot-and-mouth disease were observed among human beings using raw milk from sick cows. In 1778, in one of the Austrian monasteries, an outbreak of epidemic occurred among its inhabitants, by its symptoms similar to the foot-and-mouth disease sickness (Stallybrass).

In the seventeenth through nineteenth century there appeared descriptions which were more detailed and closer to the facts of foot-and-mouth disease epizootics, which attacked southern Germany, Switzerland, Italy, and France. These assumed enormous proportions at that time, and the course of the disease itself often bore such a malignant character that it was confused with cattle-plague.

Foot-and-mouth disease in those days was already considered infectious, many authors seeing the cause of the sickness in a peculiar "contagium", which could be transmitted under certain conditions from one animal to another (Tscheulin, 1811; Waldinger, 1813; Tamberluchi; and others). There were even authors who considered that this sickness was not infectious, ascribing its origin to atmospheric factors (Husard and Girard, 1827; Broche, 1820; and Sauter, 1822). However, with the passage of time facts

confirming the infectiousness of foot-and-mouth disease, which was revealed by numerous authors, were accumulated in even greater quantity. Levarat, apart from his observations on the infectiousness of foot-and-mouth disease, induced the sickness in animals by means of artificial infection (inoculation).

In the second half of the past century Bollinger succeeded in proving that foot-and-mouth disease occurs as the result of a particular infection. And, finally, the German investigators Loeffler, Frosch, and Uhlenhuth established in 1897 the filterable nature of the foot-and-mouth disease incitant. These authors' discovery was also confirmed in the work of Hecker, 1899. (The discovery of the filterable nature of the incitants of infectious sicknesses of animals and plants was first made by Russian scholars. In 1886 Gamaleya announced that he had been successful in infecting a calf with blood from an animal sick with cattle-plague, this blood first having been filtered through a Chamberlain filter. In 1892 Ivanovskiy first established that the incitant of tobacco-mosaic sickness can pass through bacterial filters and again cause sickness of these plants. These remarkable investigations of Russian scholars cannot remain unnoticed in foreign countries, although there has been great tardiness in beginning to discuss them.)

The investigations of the German scientists were soon duplicated by the French investigators Nocar, Roux, and others.

Hoof-and-mouth disease, as the result of different localized afflictions, was frequently called by the most diverse names: aftosa fever, stomatite, gloss-anthrax, in-between-hoof exanthema, hoof-and-mouth disease, etc. (Gins and Krause).

CHAPTER II

THE FOOT-AND-MOUTH DISEASE INCITANT

THE NATURE OF THE FOOT-AND-MOUTH DISEASE INCITANT

The incitant of the foot-and-mouth disease belongs in the category of filterable viruses (ultraviruses). The question of the nature of this causal agent has as yet not been conclusively settled by science, just as it has not been settled with respect to other filterable viruses.

The outstanding Soviet scholar Academician Gamaleya considers that the attribute, inherent for viruses, of autonomy, selectivity, adaptability, and, finally, their ability in the process of reproduction to preserve their own individuality, which is manifested in the specific character of the sicknesses caused by them, provides a basis for the reckoning of viruses as living beings — microorganisms. Such a view on the nature of filterable viruses is confirmed also by the fact that there exist so-called "corpuscular viruses" (elementary bodies) which secrete specific soluble antigens. This connects viruses still closer with microbes. (Boycott, wishing to define the position of viruses in the natural system, arrived at the conclusion that they stand at the boundary of the transition from inanimate to animate - quoted by Gamaleya, N. F.)

The question of filterable viruses was clarified at the Twelfth International Veterinary Congress (1934) in the report of

Gerlach, who also considers that viruses are living organisms. Grassia, on the basis of his numerous investigations of the biological behavior of viruses, exposed to severe criticism the theory of the "arbitrary occurrence of phagous cells from normal bacteria". This theory, as Grassia affirms, has as yet been confirmed experimentally by no one. The great number of phagous cells, their autonomy, specific nature, and mutability only confirm their living nature.

The American commission for the study of foot-and-mouth disease (Olitsky, Traum, and Shöening), on the basis of its investigations, also expressed the assumption of the live, corpuscular nature of the foot-and-mouth disease. (The American commission for the study of foot-and-mouth disease was organized through the Bureau of Animal Industry of the Department of Agriculture of the US in 1925, composed of Dr. Harry Shöening of the Bureau of Animal Industry, Dr. Jacob Traum of the University of California, and Dr. Peter Olitsky of the Rockefeller Institute.)

Opposite views with respect to the nature of viruses are held by certain foreign and Soviet investigators.

Doerr, Frenkel, Janssen, Morphy, and others defend the hypothesis of the endogenic origin of viruses. According to the stand of Doerr and his followers, viruses are enzyme-like agents, which cause changes in the metabolism of the cell itself, as a result of which the cell produces the substances of metabolism.

"Many viruses", says the Soviet investigator Dunin, "can be regarded as a special inanimate substance which possesses the properties of a unique catalyst. Only in distinction to normal catalysts does the virus, arriving in a new medium (the healthy organism), cause a unique catalytic (biochemical) reaction, as a result of which an accumulation of the virus takes place. In very simple form, the participants in a catalytic process are the original substance, the catalyst, and the product of catalysis. In the case of development of virus disease and the 'reproduction' of the virus the process is simplified and we have: the original substance, the virus (as a catalyst), and, the same virus as the product of catalysis."

In this connection the discovery of the American investigator Stanley in 1935-1936 is of particular interest. Stanley, studying virus diseases of tobacco, isolated a crystalline protein processing all the properties of a virus. Certain investigators have been inclined to see in Stanley's discovery of parasitic albumins one of the proofs of the inanimate nature of viruses.

Ryzhkov considers that the "most remarkable thing in the phenomena described is the fact that they do not quite fit the usual conception of animate and inanimate. The crystalline albumin of the mosaic disease must not be called an organism. These newly found parasitic albumins stand as it were on the border between animate and inanimate."

Doubtless, the discovery of parasitic albumins possessing the properties of pathogenic incitants somewhat strengthens the position of the partisans of the "dead", inorganic nature of viruses, but it in no degree resolves the problem itself as to what specifically is represented by viruses -- "being" or "matter".

Pyl, on the basis of his investigations, classifies the foot-and-mouth disease virus as a hydrophilic, highly-dispersive colloid.

Janssen, using the methods of colloidal chemistry, took the virus to be an insoluble active particle, composed of a slightly denatured globulin and the protein containing the virus. In Janssen's experiments the dehydration of albuminal substratum and, as a result of this, the altering of the structure of the albumin, did not have perceptible influence on the activity of the virus. The author has drawn from this the conclusion that the foot-and-mouth disease virus is an inanimate protein substance of the ferment type.

Ratner, and also Solov'yev, as a result of study of the biology and ascertainment of the nature of the virus, classify it as a molecular dispersion and deny the animate nature of the foot-and-mouth disease incitant. However, the data of these authors, secured by very limited preliminary investigations, seem unconvincing to us. On the basis of them it is impossible to draw a conclusion as to the dead or living nature of the virus.

The basic proofs of the partisans of the inanimate natures of viruses may be summed up by the facts that (1) viruses do not possess an independent metabolism and do not grow in an inanimate (artificial) culture medium, (2) they do not produce specific antigens, (3) inactivated viruses can be restored under known conditions (reversibility of inactivation), and (4) certain viruses are of too small a magnitude, not exceeding that of one or several albumin molecules.

In refutation of all these arguments Academician Gamaleya adduces a whole series of extremely convincing pronouncements and theses.

"In proportion to the increase of parasitism they (pathogenic bacteria -- A. Z.) gradually lose the substance and the enzymes which are possessed by independent living creatures and borrowed by parasites from the host organisms. This, understandably, applies particularly to viruses which have adapted themselves to intracellular parasitism. It is therefore not surprising that viruses purified of tissue cells, and a bacteriophage purified of bacteria, do not reveal metabolisms. In the absence of such auxiliary resources, which they receive in the host-cells, 'purified' viruses are in a state of anabiosis. They can grow and multiply only in using those substances and enzymes which they receive in host organisms. Only inside the latter can viruses reveal all the properties inherent to a complete organism."
(Gamaleya, N. F. "The Present-Day State of the Virus Problem. Virus Sicknesses of Plants and Measures of Struggle Against Them." Trudy soveshchaniya po virusnym boleznyam rasteniy. Izd. Akad. nauk SSSR, 1941, pp. 10-13.)

Rivers long ago spoke of the fact that the majority of virus diseases exhibit a close interaction between the etiological beginning and the cells of the host, regarding this as a particularly intimate type of parasitism. According to Gardner viruses live only in the cells, and not in the fluids of an organism.

Myromtsev does not consider insoluble the problem of the fact that viruses cannot be cultivated in artificial culture media. In reconciling culture media on the basis of their physio-chemical properties with the fluids and tissues of an organism it will become practically possible to cultivate viruses in artificial culture media. "In attempts to cultivate ultraviruses", says Muromtsev, "it is possible with equal foundation to go both backward toward full-value tissue cultures and forward toward artificial media without living cells."

At the present time the ability of corpuscular viruses (elementary bodies) to secrete specific antigens must be considered proved. At least this ability to produce antigens has been proved for the phagous cell, the virus of vaccinia, the yellow fever virus, and others.

On the question of the reversibility of the inactivation of viruses the following statement of Academician Gamaleya may be cited: "It is necessary at the same time to renounce resolutely the last survivals of vitalism, which supposses that upon the termination of life some vital principle disappears irrevocably.

Staphylococcus can be killed with corrosive sublimate and then become capable of life again when the corrosive sublimate is precipitated with hydrogen sulphide. The same thing is observed for a phageus cell under the action of safranine". (Gamaleya, N. F. "Textbook of Medical Microbiology." Medgiz. 1943, p. 317.)

The excessively small size of viruses does not give us foundation for denying the animate nature of these, since "in essence we have no concept of the minimum degree of organization essential for the lowest living creature. It is extremely probable that all the requirements for the sustenance of a given virus are satisfied by the cell of the host in which it lives, so that all of its proper function is limited to adsorption and division. It is possible that a few protein molecules are sufficient for this." (Gamaleya)

Finally, the discovery of "parasitic albumins" in crystalline form in virus sicknesses of plants does not contradict proof of the animate nature of viruses. Crystalline virus proteins are also secreted at the present time from the tissues of animals infected with virus (foot-and-mouth disease and others). These crystalline proteins "are formed of an accumulation of an enormous number of the same elementary bodies, which have, however, the form of little sticks rather than spheroids, which is subsequently confirmed also for plant viruses". In the opinion of Academician Gamaleya, "the ability of elementary bodies to form, composing themselves in crystalline formations, in no way contradicts the fact that elementary bodies are animate microbes".

The views on the nature of viruses cited here on the part of representatives of various scientific trends give us basis to come out completely and definitely in favor of the animate nature of viruses. Consequently, the incitant of foot-and-mouth disease must also be considered a "creature", possessing a living, organized nature.

The discovery of the incitant of foot-and-mouth disease is connected with the names of Loeffler, Frosch, and Uhlenhuth. These investigators established that the incitant of foot-and-mouth disease is a filter-passer. Due to its very small magnitude it passes freely through bacterial filters. At the same time, the virus recovered in the filtrate preserves the ability to cause sickness in animals susceptible to foot-and-mouth disease. Thus the etiology of foot-and-mouth disease was first determined conclusively (1897) and the filterable properties of the foot-and-mouth disease incitant were discovered.

This outstanding discovery of Loeffler, Frosch, and Uhlenhuth did not solve the problem of the nature itself of the foot-and-mouth disease incitant, but after this discovery new paths for the further study of its biology were projected.

SIZE OF THE VIRUS

Determination of the magnitude of particles of the foot-and-mouth disease virus is carried out by the methods of filtration and ultrafiltration. Data of different investigators with respect

to the magnitude of particles of the virus are divergent, which may be explained also by non-uniform experimental conditions in studying this question.

According to Waldmann's data, acquired by him in experiments in filtration of the virus through a Gallert filter, the magnitude of its particles is within the range 1μ to 200 mμ, i.e. it approximates the magnitude of colloids.

Galloway and Elford determined the average diameter of particles of the virus as 8 to 12 mμ, and Levaditi determines it as 3 to 5 mμ. Elford, in determining the magnitude of particles of the virus, uses a special correcting formula, which has been named the Elford formula. According to Elford's data, only particles of a virus pass through a filter whose size is 2 to 3 times less than that of the pores of the filter. The virus does not pass through filters whose pores are of 25 mμ, and consequently the size of its particles is equal to 8 to 12 mμ.

Krasnov and Reinie determine the size of particles of virus type O as more than 7 mμ and less than 16 mμ. These results almost coincide with results obtained in the experiments of Galloway and Elford. These authors, on the basis of their investigations, reached the conclusion that the limit of ultrafiltration of the virus is between 48 and 13 mμ, and that it is closer to 13 mμ than to 48. In their experiments they used virus from the epithelium and "lymph" of aphthae. After [redacted]

preliminary filtration through paper and sand the virus was dilated with Heartlay's medium in a proportion of 1:100.

Galloway, using the colloidal membranes of Ford, reached the conclusion that the dimensions of particles of the foot-and-mouth disease virus are less than those of all known viruses, with the exception of the virus of poliomyelitis in human beings. The diameter of particles of the foot-and-mouth disease virus is approximately equal to 8 to 12 mμ, which is 0.01 to 0.02 of the diameter of the smallest microbe.

According to data of the American commission, the magnitude of particles of the virus is 3.6 mμ. Measurement of particles of the virus on the basis of the system of so-called "molecular" filtrations through Bechold ultrafilters shows that its active particles have, by comparison with other particles of identical charge, a diameter between 20 and 1000 mμ(Rivers).

According to Bechold, and also Modrow, the average diameter of particles of virus type O is 20 mμ; while particles of virus A are considerably larger and are closer in magnitude to the size of elements visible in the microscope. These authors affirm on the basis of their investigations that the magnitude of particles of the foot-and-mouth disease virus in different varieties (types and variants) is non-uniform and that with the help of the ultrafiltration method it is possible to separate one variety of the virus of another, particularly virus type O from virus types A and C. Galloway then, on the contrary, considers

that by the method of ultrafiltration it is impossible to establish any divergence in the magnitude of different types and variants of the virus.

For study of the magnitude of particles of the virus Schlesinger and Galloway applied the method of precipitating it with the help of a "Sharpless" superpower centrifuge, with subsequent filtration through colloidal membranes. On the basis of their experiments they determine the size of virus particles at 16 to 23 mμ. Virus particles of all three types proved uniform in their experiment. In the same authors' later experiments in ultrafiltration the magnitude of virus particles was 8 to 12 mμ. In determining the magnitude of particles of the virus by means of weighing, the diameter of these particles is equal to 25 mμ, if it is assumed that they are spherical in form.

Ardenne and Pyl with the help of the electron microscope obtained a photograph of a smear of fluid from the foot-and-mouth disease vesicle. This photograph makes it possible to detect minute particles having a diameter of 25 to 30 mμ. In all probability, these particles are molecules of the virus. According to data of the same authors, the specific gravity of the virus is approximately 1.40.

Pyl assumes on the basis of his investigations that the foot-and-mouth disease virus in solutions, or in aphthous lymph, is not in the form of an individual particle, but consists of a whole aggregate of such individual virus particles ("virus

aggregate"). The magnitude of these aggregates depends on the temperature and concentration of the culture, since at temperatures from 0 to 20 Centigrade dissociation ("rasshcheplenive") of the virus aggregate into small virulent particles and back evidently takes place. All these data, obtained as the result of numerous investigations, give only a limited conception of the magnitude of particles of the foot-and-mouth disease virus; conclusive solution of this problem must be the object of further investigations. Until more perfect methods of purifying the virus from the bulk albumins are found, and consequently, until it is obtained in pure form, it will be impossible to solve positively the problem of the magnitude of particles of the foot-and-mouth disease virus.

In many ultravirus infections special intracellular formations have been discovered, the so-called "inclusion bodies". The specific nature of these formations for many ultravirus infections has not yet been proved. These inclusion bodies have diagnostic significance for such sicknesses as hydrophobia (Negri's body), Borna disease (the Joest-Degen corpuscle), and small-pox (small-pox-diphtheria) in fowl (Paschen's body). Similar inclusion bodies have also been repeatedly described for foot-and-mouth disease.

Gins has described inclusion bodies discovered by him in the epithelium of the tongue of guinea-pigs which had developed foot-and-mouth disease. Trautwein, and likewise Ruhl, also discovered inclusion bodies in the epithelium of the tongue of

healthy guinea-pigs. The peculiar inclusions in the erythrocytes animals which had developed foot-and-mouth disease described in their time by Cosco and Aguzzi have not been given further confirmation by anyone.

Gerlach, with the help of ultra-violet microscopy, discovered granular forms of the type of elementary virus bodies in the contents of foot-and-mouth disease aphthae. For the time being the auther refrains from any conclusions with respect to the origin and nature of these bodies.

FILTRATION AND ULTRAFILTRATION

The filterability of the foot-and-mouth disease virus, as has been said already, was first established by Loeffler, Frosch, and Uhlenhuth. They proved that the foot-and-mouth disease virus passes freely through the Berkefeld and Chamberlain filters. With this in mind they made use of the virus in the form of the contents of aphthae ("virus-lymph"), which had been preliminarily diluted with a 1:40 physiological solution of sodium chloride. The non-admissibility of bacteria through the pores of the filter was controlled by the addition to the filterable liquid of bac. prodigiosus with subsequent straining of the filtrate into culture media. The presence of virus in the filtrate was verified by means of infecting calves intravenously.

While the strainings in the culture media did not produce growth, the calves became sick with foot-and-mouth disease with

typical clinical symptoms, just as control animals which had been infected with the virus ("lymph") before its filtration. Loeffler, Frosch, and Uhlenhuth also proved that even after repeated filtrations the virus was in the filtrate and capable of causing disease in animals susceptible to foot-and-mouth disease. The more dense (narrow-pored) Kitasato filters have partially stopped the virus.

The results of the investigations of Loeffler, Frosch, and Uhlenhuth on the filterability of the virus have been confirmed by Hecker in Germany, Nocard and Roux in France, and other investigators.

The filtration of the virus in connection with study of its biology has also engaged other investigators. Abe has filtered through a Berkenfeld candle virus-lymph from aphthae of a guinea pig, the virus-lymph having first been diluted with a 1:100 physiological solution of sodium chloride.

Stockmann and Minnet, (the English commission on study of foot-and-mouth disease was organized in 1924 under the Ministry of Agriculture and Fisheries, and was composed of Prof. I. Martin (chairman), Dr. S. Stockmann, Dr. Arkwright, and others), for purposes of filtration, took the virus in the form of small pieces of the epithelium of the walls of aphthae ("epithelial virus"), ground them in a porcelain mortar, and then strongly diluted this ground mass with a physiological solution of sodium chloride. In filtering such a virus "suspension" negative results were

obtained. Filtration was carried out through Berkefeld N and Chamberlain F_4 and L_5 bougies, and also through Seitz asbestos plates.

The failures of Stockmann and Minnet with filtration are explained by the fact that evidently the virus in this case was adsorbed by the filter, or, which is still more probable, the pores of the filter were blocked by the cellular elements of the ground epithelium. Subsequently, Stockmann and Minnet left off such treatment of the virus. For filtration they took the virus in the form of lymph from primary aphthae of the guinea pig, likewise given preliminary dilution with a 1:50 to 1:00 physiological solution of sodium chloride. In this case the virus passes freely in filtration through Berkefeld N and Chamberlain F_4 and L_5 bougies, which was confirmed by the infection of experimental animals. The virus also passed freely through Seitz asbestos plates. The filtrate of foot-and-mouth disease virus obtained by these same investigators caused the infection of experimental animals in dilution of 1:20,000.

Bedson and Maitland obtained good results with filtration of the virus through a Mandler candle under pressure of 100 millimeters. A 1:500,000 dilution of the virus was taken. Galloway and Nicolau also used a Mandler candle for filtration of the virus. They used each time only new candles which had not yet been put in use, in order thus to determine the degree of absorption of the virus in repeated filtrations. They used for filtration the virus

("lymph") from the guinea pig and diluted it with a 1:50 buffer solution of pH 7.6. The filtering took place at low pressure.

In their experiments Galloway and Nicolau used titrated virus exclusively. They produced infection of experimental animals with various dilutions of the virus, after filtration as well as before. With two- and three-fold filtrations under the method indicated the quantity of virus in the filtrate did not diminish considerably, while in the course of five successive filtrations the virus disappeared completely.

Olitsky and Boez made a filtration of the virus through Berkefeld V and N candles, and also through Chamberlain L_1, L_2, L_3, L_5, L_7, L_9, L_{11}, and L_{13} bougies.

They took the foot-and-mouth disease virus in the form of the epithelium and "lymph" of aphthae. To the diluted virus was added before filtration a buffer solution of phosphate pH 7.5, 7.6 (for 1 liter of distilled water they used 2.5 Kalium biphosphoricum). The virus, prepared for filtration, was first filtered through filter paper, and then again through Seitz asbestos plates. In Olitsky's and Boez' experiments virus treated in this manner passed freely through Berkefeld candles and Chamberlain bougies. It began to be blocked only upon filtration through a Chamberlain L_5 bougie, passed with difficulty through L_7 and L_9 bougies, and, finally, did not pass at all through L_{11} bougies.

In filtration through the Seitz filter the foot-and-mouth disease virus is adsorbed on the asbestos discs of the filter.

In Yudin's experiments with filtration of the virus with the help of a Seitz filter it was established that a dry asbestos disc EK adsorbs the virus. After steam sterilization, when the EK asbestos disc is still wet, or after preliminary filtration through the discs of a buffer solution pH 7.6 without glycerine, or of a buffer solution pH 6.8 with glycerine, adsorption of the virus also takes place. Adsorption can be prevented only through preliminary filtration through these EK asbestos discs of a one percent solution of peptone of pH 7.6 or pH 6.8.

The ease with which the foot-and-mouth disease virus passes through filters depends, as has now been established by several investigators, on the filtering material itself, the degree of its preliminary preparation for filtration, the concentration of hydrogen ions, on the size of the pores of the filter, the electric charge, the amount of pressure in filtration, the temperature in which it is accomplished, and, finally, on a number of other conditions which often cannot be taken into account.

Abe was evidently the first to achieve ultrafiltration of the virus, for which he used Haen membranes. In his experiments the virus passed through membranes from No 1 to No 100 and was completely blocked upon filtration through membranes beginning with No 200, under pressure of 25 centimeters. Galloway, for filtration

of the virus, as has been indicated earlier, used the collodion membranes of Ford.

Levaditi, Galloway, and Nicolau, toward the same end, applied collodion sacs, prepared from a 6 percent ether-alcohol solution of collodion. In these investigators' experiments the virus passed through triple collodion sacs, with positive results being obtained in 8 out of 9 cases. Stockmann and Minnet, on the other hand, did not succeed in filtering the virus through a double layer of 5 percent collodion sacs.

Olitsky, Traum, and Schoening accomplished filtration through sacs of 5 to 12 percent collodion. The passing ability of such a collodion filter was increased after dipping it in 95 percent alcohol. However, this method of filtration was acknowledged by the authors themselves to be fruitless and incapable of always giving positive results. In a second instance, when they prepared the collodion sacs with another emulsion, the virus was blocked completely. Therefore it was impossible to draw any conclusions from these experiments.

For ultrafiltration of the virus Olitsky and Boez used Bechold collodion membranes prepared from various concentrations of collodion in solution in solid acetic acid on filter paper. The virus always passed freely through filters of 1½ collodion. In one case the virus passed through a filter of 3 percent solution of collodion, but in all remaining instances it was blocked by the greater percentile concentration of collodion. In their experiments

on filtration these authors used a buffer solution of phosphate at pH 8.5.

Modrow accomplished filtration of the foot-and-mouth disease through a Zigmondi ultrafilter, but she was unable to obtain constant and uniform results. More satisfactory results were obtained by Modrow in filtration through a Bechold ultrafilter. In her experiments the virus was blocked in filtration through a Bechold ultrafilter of 9 percent solution of collodion in solid acetic acid, and, on the other hand, passes easily through membranes of 1 percent solution of collodion in the same solid acetic acid.

Galloway and Elford, using the methods of ultrafiltration, applied Elford's corrective formula in order to determine the most probable dimensions of the virus. The work of Ledaviti, Pike, Krasnov, and Wood, and also of Krasnov and Reinie, was also dedicated to this question.

The method of ultrafiltration is used by investigators first of all to determine, although approximately, the size of virulent particles of the virus. However, as Modrow points out correctly in one of her works on the subject of filtration and ultrafiltration: "Determination of the magnitude of the pores in ultrafilters is for the time being an extremely doubtful matter, and the ability of a collodion filter to pass particles of the foot-and-mouth disease virus depends on many factors which often cannot be taken into account".

In any case, the question of the size of the incitant of foot-and-mouth disease for the time being cannot be answered by the method of ultrafiltration.

CENTRIFUGATION OF THE VIRUS

The method of centrifugation is applied widely in bacteriological practice for the precipitation of various particles in one suspension or another. By the same method certain investigators have been attempting to precipitate the foot-and-mouth disease virus and thus prove its corpuscular nature. Other investigators have been trying with the method of centrifugation to obtain the virus in a more or less pure and concentrated form.

Experiments have shown that to precipitate the foot-and-mouth disease virus from its medium by the centrifugal method has in essence not been successful for anyone as yet. This, of course, cannot serve as an indication that the virus in these cases was in a dissolved state. Evidently an incitant of extremely small dimensions is involved. Incidentally, Duclaux and certain other physicists consider that the method of centrifugation is in general unfitted to the precipitation of such small particles as the ultravirus.

ADSORPTION OF THE VIRUS

The method of adsorption is usually applied for accumulation of the foot-and-mouth disease virus.

Pyl succeeded in this way in increasing the concentration of the virus 1000-fold. The adsorbed virus can be eluted back by changing the concentration of hydrogen ions. The process of adsorption and elutriation of the virus evidently has in itself no harmful effect on it. Charcoal, kaolin, aluminum hydroxide, and albumin are most often used as adsorbents. Charcoal is a good adsorbent, and in addition with this adsorbent the pH of the medium has no substantial effect on the process itself of adsorption of the virus.

For adsorption of the virus Gin and Krause used kaolin in combination with centrifugation, but they did not succeed in this manner in isolating the virus from its medium.

Abe succeeded in adsorbing the foot-and-mouth disease virus on kaolin, and particularly on "animal" charcoal.

In Dahmen's experiments adsorption of the virus neither succeeded with kaolin, starch, nor red blood corpuscles. He tried to use wood charcoal but the results were likewise negative.

Vallée and Carré succeeded in adsorbing the virus on red blood corpuscles, and also on living and dead microbe bodies. The adsorbing ability of red blood corpuscles was proved also by de Blieck and Winkel.

On the basis of their experiments Badson and Maitland reached the conclusion that the erythrocytes of sheep, guinea pigs, and big cattle adsorb the virus in small quantity and easily release it.

Pyl used aluminum hydroxide, which adsorbs the virus in alkaline solution (kaolin adsorbs the virus only in a neutral medium).

According to Haudurcy, adsorption of the virus can be used as a method of concentrating it. This is essential for proof of the presence of the virus in a preparation which has proved to be avirulent in inoculations. The essence of this method is as follows. To the material being investigated one adds the adsorbent substance, then collects the precipitate and introduces it in a susceptible animal. By this method detection of the virus has succeeded even in a solution of 1:10,000,000. In this solution it was inactive, but contained virulent elements.

With the help of the adsorption method certain investigators have attempted to isolate the virus from its medium. None of these investigators has succeeded as yet in doing this (Waldmann and Trautwein).

Pyl succeeded with the method of adsorption in freeing the virus from the body albumins to the extent that the albumin produced a negative reaction.

Janssen, with the goal of purging the virus from the body albumins, applied various methods of adsorption and elution, but did not succeed in obtaining it in a pure form, i.e. completely free of globulins. Later, with the help of a special method of adsorption, Janssen succeeded in obtaining the virus supposedly completely free of the body albumins. As an adsorbent he used gypsum -- calcium sulphate ($CaSO_4$).

Janssen assumes that with the help of the method of chemical precipitation of the albumins it is fully possible to free the virus from the normal proteins. Janssen's investigation with respect to obtaining the virus in pure form, completely free of the body albumins, holds great interest for the study of its biology.

The Danish investigators S. Schmidt, Hansen, and Schmit-Jensen, with the help of the method of adsorption of the virus with aluminum hydroxide ($Al(OH)_3$) obtained a "complex": foot-and-mouth disease virus-hydrate of aluminum oxide, possessing antigenic properties.

CULTURE OF THE VIRUS

The first attempts to cultivate the foot-and-mouth disease virus were undertaken by Loeffler and Frosch (1897) and also by Loeffler and Uhlenhuth (1898-1905), but in all cases negative results were obtained. Cultivation of the virus engaged other investigators, also unsuccessfully: Pfeiffer and Grugel, Hecker, Reinhard and Grugel, and others.

Tietze's announcement in 1922 that he had succeeded in cultivating the virus in a liquid artificial culture medium discovered by him (Martin's broth with addition of 15 to 20 percent serum of large cattle at pH 7.8 to 8.0) was not confirmed by further investigations.

The excitement-provoking discovery of the incitant of foot-and-mouth disease by the German investigators Dahmen and Frosch (1924) also was confirmed by no one. Dahmen prepared a dense culture medium (Martin's broth with the addition of 50 percent serum of horses, sheep, or guinea pigs and 3 percent agar at pH 8.0). Frosch, with the aid of a Köhler ultra-violet camera, which doubles the resolving power of the objective, discovered in the "culture" unaccustomed small, short sticks (smaller than 0.1μ), situated in pairs. The authors named the "incitant" discovered by them Loeffleria Nevermanni. However, a commission specially created for the verification of Dahmen's and Frosch's discovery of authoritative representatives of German veterinary science (Giese, Gildemeister, Richter, Klein) did not confirm the discovery of Frosch and Dahmen. Subsequently Cins, Waldmann, and Trautwein, having studied the virus culture described by the authors, did not confirm it. Dahmen's and Frosch's discovery was finally checked by the English commission (Bedson and Maitland in 1925, Stockmann and Minnot in 1926) and by the American commission (Olitsky and Boez in 1927), and negative results were invariably obtained in all cases.

De Blieck and Winkel cultivated the incitant of foot-and-mouth disease in culture media of blood-serum with the addition of sugar and glycerin, and also obtained negative results. Pfeiler (1922-1926) repeatedly announced successful experiments in the cultivation of the foot-and-mouth disease incitant in a complicated culture medium specially prepared by him, but the composition of

this medium was not published, and therefore his discovery was not checked by anyone and does not deserve credence. Large-scale experiments in the cultivation of the virus were carried on by the English commission (M. and G. Maitland), the American commission (Olitsky, Traum, and Schoening), and, finally, in the Riems Institute in Germany (Waldmann and Trautwein), and in all cases negative results were obtained. (Incidentally, in 1924 the English Ministry of Agriculture, attaching exceptional importance to the discovery of the foot-and-mouth disease inciteant, announced a prize of 150,000 pounds for whoever first discovered the inciteant of foot-and-mouth disease and indicated methods of cultivating it in artificial culture media.)

After a method of tissue cultures was developed, ultraviruses began to be cultivated in explanted tissues (Parker and May, Carrel and Rivers, Rauren and others). Later a very simple and advantageous method of cultivating viruses on the chorioallantois of the chicken embryo was applied toward the same end (the method of Goodpasture and Woodruff).

All other methods of cultivating viruses, as we shall see later, proved of little value.

The English investigators M. and G. Maitland (1929) applied the method of tissue cultures to the reproduction of the virus of foot-and-mouth disease. (One of the first attempts to cultivate the foot-and-mouth disease virus with the aid of tissue cultures -- the method of explanation -- was made in the USSR by Prof. Revo in

1927, although the fact of reproduction of the virus, rather than its survival, was not convincingly proved.)

In connection with the specific peculiarities of the foot-and-mouth disease virus the Maitlands introduced certain changes in the method of tissue cultures itself. Embryonic tissue of the guinea pig (lips, tongue, extremities, hairy part of the skin) serves as their culture medium. In such tissue cultures the Maitlands proved the reproduction of the virus, succeeding in passing it through 17 successive passages and maintaining it in the subject cultures for the duration of 10 months.

The virulence of the virus in the process of cultivation, as shown by the Maitlands' researches, did not decrease. The maximum increase of the virus in the majority of cases was noted by the authors on the third or fourth day of cultivation in the thermostat. The quantity of virus increased 1000 times during this time, and in one case as much as 10,000 times. The greatest titre of virus which the Maitlands attained was 1:100,000. In explanted tissues of the adult guinea pig, increase of the virus was not observed. The authors likewise established that the degree of increase of the virus does not depend on the character of growth of the tissue itself. On liver-tissue reproduction of the virus did not succeed.

In cultures of tissue of the chicken embryo the virus did not multiply, which the Maitlands associate with the virucidal action of the serum of embryos (the chicken, as is known, is not susceptible to foot-and-mouth disease). This likewise does not

depend on the pH of the medium and, in all probability, is conditioned by the specific peculiarities of the embryonic tissue itself and of the plasma of the chicken.

The Maitlands used the following technique. To 0.66 cubic centimeters of embryo-tissue of the guinea pig which had been ground in preliminary they added from 1.33 to 2.0 cubic centimeters of virus-"lymph" and left all this at a temperature of 5 degrees Centigrade for 1.5 hours. After this they added to the ground embryonic tissue 5 cubic centimeters of fresh liver plasma of the guinea pig and the same quantity of Tirod's solution. The embryonic fluid did not combine with this mixture. Cultivation was carried out in Carrel cups in a thermostat with temperature of 37 degrees Centigrade. Good cultures were also obtained in Roux flagons, in which up to 50 cubic centimeters was placed. The original virus material was used in the form of virus-"lymph" in M/50 phosphate-buffer solution at pH 7.6 in dilution of 1:50, after first filtering it through a filter. This method of the Maitlands, proposed by them for multiplication of the foot-and-mouth disease virus in the presence of animate cells, has found brilliant confirmation in the work of many other investigators.

Gecke in 1930-31, and likewise Striegler in 1933-34, carried out large-scale investigations on study of the method of tissue cultures applied to the cultivation of the foot-and-mouth disease virus.

Gecke and Striegler used virus-"lymph" from 24-hour primary

aphthae of the guinea pig as original material for cultivation of the virus. They diluted the virus with 1:20 or 1:50 buffer solution of phosphate pH 7.6 and then passed it through a Seitz filter. As tissue for its cultivation the authors used the skin (epithelial tissue) of the embryo of the guinea pig. For the same purpose they also used small pieces of lung embryo. As a culture medium for the tissue culture they used blood plasma of the animal from which tissue for cultivation of the virus had been taken. Striegler also used serum of the guinea pig as a culture medium. The embryo fluid which was added to the tissue culture for stimulation of growth of the tissue itself was prepared from the embryo of the same type of animal from which tissue had been taken for cultivation.

Cecke, in cultivating the foot-and-mouth disease virus on the tissue of guinea pigs, used embryo fluid of the embryo of the hen instead of embryo fluid from the same type of animal, but obtained scarcely satisfactory results.

Frenkel and Van Vaveren cultivated the virus on the epithelial tissue (skin) of embryos of cattle. The culture virus which they obtained in this instance possessed stronger antigenic properties for spontaneously susceptible animals than the same virus obtained on tissue of embryos of the guinea pig. In experiments by these investigators, foot-and-mouth disease virus of type A, after 27 passages in the course of 104 days, at a temperature of 37 degrees Centigrade, did not change its type properties.

In experiments on cultivation of the virus on tissue of sheep embryos good results were also obtained. As a culture medium the authors used: (1) plasma of the chicken embryo with the addition of Tirod's liquid, (2) serum of cattle with the addition of 0.75 percent agar-agar, (3) cattle serum with the addition of Tirod's liquid, and (4) Tirod's liquid -- in all cases they obtained good results.

In testing the culture virus on cattle and hogs by means of artificial infection and with subsequent intravenous injection of the virus it was established that the experimental animals had an immunity to foot-and-mouth disease, and serum obtained from them possessed high anti-body content. Frenkel and Van Vaveren attempted to substitute for the chicken plasma, which they had used instead of calf plasma, agar-agar (0.75 percent agar-agar in Ringer's solution). In the presence of agar-agar growth of tissues took place very slowly, while in the presence of chicken plasma it may be very well observed.

According to Striegler's data, in the Riems Institute one strain of culture virus was maintained, with the help of tissue cultures, for two years, for which it was propagated through 118 passages. As a result of this passing the virulence of the foot-and-mouth disease virus reached a magnitude of 24×10^{174}, instead of the initial virulence of 10^{6}.

In multiplying the virus in tissue cultures great importance is attached to the temperature and concentration of hydrogen ions

of the medium in which the culture is carried out. At a temperature of 37 degrees Centigrade both virus and tissue multiply, while at a temperature of 30 degrees Centigrade the virus multiplies very slowly and the tissue almost does not multiply. And, finally, at a temperature of 26 degrees Centigrade neither the virus nor the tissue multiplies at all. As far as the concentration of hydrogen ions in the medium where multiplication of the virus takes place is concerned, pH 7.6 -- 6.9 is most favorable, and below pH 6.3 the virus does not multiply at all. In changing the pH of the medium from weak-alkaline to neutral multiplication of the virus increases.

Pyl and Köbe, for activation of the virus in multiplying it in tissue cultures, used extract of seminal glands (Reynals' factor), and also extract from the epithelium of the plantar surface of the extremities of guinea pigs, both those which had developed and those which had not developed foot-and-mouth disease. However, perceptible increase on the virus which could be attributed to the specific action of the extract could not be established by the authors. They noted only the favorable action of the extract on the pH of the medium itself. After addition of the extract this remained at a level of pH 7.6.

Frenkel and Van Vavaren cultivated the virus in a fluid medium in the presence of fibroblasts of rabbits, hogs, and calves, but they obtained negative results.

For cultivation of the virus Köbe used fibroblasts of the

tissue of irises (Fisher's method), but did not succeed in proving multiplication of the virus.

The foot-and-mouth disease virus, as shown by the investigations of Gecke, Striegler, the Maitlands, Köbe, Frenkel and Van Vavaren, and others, multiplies best of all in explanted epithelial tissues. Incidentally, in Köbe's experiments the epithelial cells of the stomach, intestines, kidneys, and mammary glands proved of little advantage for cultivation of the virus.

For the time being there remains uninvestigated the extremely interesting question as to where exactly in tissue cultures the virus multiplies: inside the cell itself or on its surface, or, perhaps, only in the medium in the presence of elective tissue. It also remains unknown as to whether the tissues are some substances essential to the life of the virus itself. Gecke, on the basis of his investigations, reaches the conclusion that the virus evidently multiplies inside the cells themselves and that its multiplication depends not on growth of the cells themselves, as several investigators hold, but from the activity of the latter. The presence of the virus in cultures outside the tissue cannot serve as evidence of its multiplication. This can be the result of its diffusion from the cells into the surrounding medium.

In the Riems Institute the culture virus was applied to the hyperimmunization of cattle for the purpose of obtaining anti-foot-and-mouth disease serum. Earlier virus for the same purpose was

obtained from hogs, which considerably complicated the whole process and increased the cost of the anti-foot-and-mouth disease serum produced. In the Riems Institute, according to Striegler's announcement, up to 2000 cubic centimeters of culture virus with a titre of 1:1,000,000 was prepared annually.

Striegler and Nagel experimentally immunized cattle with culture virus, but did not obtain from it anymore, or less, firm immunity, even to natural infection. These results are in some contradiction to the data on application of the culture virus in the Riems Institute for purposes of hyperimmunization in obtaining anti-foot-and-mouth disease immunity serums.

Frenkel and Van Vaveren engaged in cultivating the virus on the chorioallantois of the chicken embryo (Goodpasture and Woodruff's method). For this purpose they used chicken's eggs after preliminary incubation for 3, 9, 10, 11, and 13 days. As a result of their investigations the authors could not confirm the multiplication of the virus on the chorioallantois of chicken embryos. In their experiments the virus kept its viability on chorioallantois for only 48 hours in all, at a temperature of 40 degrees Centigrade. However, in two cases they succeeded in obtaining subcultures of the virus.

Peragallo, using the same method, on the other hand, obtained positive results, and succeeded in propagating the virus through 20 passages. He produced a sowing of the virus on the chorioallantois of a chicken embryo, and a re-sowing 36 to 48 hours after placing the virus in the thermostat. (Certain investigators -- Galloway

and Elford, Burnet and Galloway -- have cultivated the virus of vesicular stomatitis on the chorioallantois of chicken embryos without particular difficulty.)

The Soviet investigators Zil'ber and Vostrukhova developed a method of cultivating ultraviruses in symbiosis with plant microorganisms, based on the phenomena of allobiophory. These authors succeeded in cultivating certain viruses (of typhus and others) on yeast cells and yellow sarcina. Subsequently similar experiments in the cultivation of viruses were carried out in several foreign countries.

Frenokl and Amies in Holland cultivated the foot-and-mouth disease virus on yeast cells, but obtained negative results.

Poppe and Busch in Germany, on the basis of their experiments on cultivation of the foot-and-mouth disease virus in symbiosis with bacteria cells, on the other hand, reached somewhat different conclusions. In individual instances these authors succeeded in multiplying the virus on "red yeast" (Torula rubra). The presence of the virus in such symbiotic cultures was proved by them after 60 generations in a 10^{62} dilution. In one case the authors succeeded in obtaining cultivation of the foot-and-mouth disease virus in symbiosis with Staphilococcus albus.

In the foot-and-mouth disease laboratory of the Kazakh Scientific Research Veterinary Institute (NIVI) experiments in the cultivation of the virus of yeast cells according to Zil'ber's and

Vestrukhova's method, with certain modification of the latter, were undertaken. In cultivating the virus on yeast cells (brewer's yeast, kefir yeast, and others) in Tirod's solution with addition of 10 percent serum of guinea pigs negative results were obtained. On a semi-fluid sugar medium together with yeast cells the presence of the virus was noted in the culture no farther than the second generation, which can be attributed to the survival of the virus, and not its multiplication. In repeated experiments negative results were obtained.

The varying results of their experiments are explained by the authors by the high sensitivity of the virus to the composition of and alterations in the culture medium, the pH of the latter evidently having decisive importance.

It seems to us that the method of symbiotic cultivation is promising and can find a known application first of all in study of the biological properties of the virus.

RESISTANCE OF THE VIRUS

The foot-and-mouth disease virus, as has been established by numerous investigators, is characterized by a rather high resistance with respect to all kinds of physico-chemical reactions, and also to the influence of various factors in the surrounding medium. The resistance of the virus depends mainly on the properties of the medium in which it is situated (epithelial tissue, aphthose lymph, blood, milk, urine, saliva, and others). The degree of

virulence of the virus itself also has significance. In a form more or less free of body albumins the virus possesses considerably less resistance.

Pyl has made the observation that the virus purified of body albumins becomes more labile, its quantity always decreasing after purging of body albumins.

Elford, in studying photodynamic action in the presence of methylene blue, established that with respect to the virus it appears stronger as it is released from body albumins with the aid of filtration or ultrafiltration.

Olitsky explains the high resistance of the virus by the coagulation of protein with which it is connected. The coagulation of protein defends the virus from the action of chemical substances, and besides a considerable portion of it is adsorbed evidently on the coagulated protein. With artificial restraint of coagulation or the use of methods preventing this coagulation, the virus becomes less resistant.

Change in the concentration of hydrogen ions of the medium of the virus also produces an extremely noticeable influence on its resistance. It is known that the virus is extremely sensitive to change in the acid-base equilibrium, particularly in the direction of acidity. The smallest inclination in the direction of acidity (pH 6.3) kills the virus (Schlögel). The English and American commissions on foot-and-mouth disease reached the conclusion, on

the basis of their investigations, that the virus keeps its virulence longest at pH 7.5 to 7.6.

In Minnet's experiments the virus in a preservative medium at pH 7.0 to 8.0 and temperature 1 degree Centigrade remained active for a period of 77 to 93 days. Under the same conditions at pH 6.4 to 6.8 it survived at times for 93 to 100 days. And, contrarily, at pH 6.4 to 7.0 and temperature 12 to 20 degrees Centigrade the virus quickly lots its virulence, and under the same temperature conditions but pH 7.2 to 8.0 maintained its virulence considerably longer. The optimum concentration of hydrogen ions in this instance proved to be pH 7.4 (phosphate buffer solution), with which the virus remained active longer than 73 days. In a medium with pH from 4.0 to 6.2 the virus lost its virulence in the first 4 days.

Pyl and Klenk also arrived in turn at the conclusion that the virus remains active longest at pH 7.65 to 7.74 precisely. With a subsequent extremely small change in the pH of the medium in the direction of acidity the period of survival of the virus was considerably abbreviated; this did not occur with a similar small increase in alkalinity.

Pyl and also Minnet succeeded in preserving the virus at pH 9.24 to 9.4 for 24 hours. In an alkaline medium at pH 10 the virus quickly loses its virulence. At pH 6.0 to 6.5 the virus was not preserved successfully for a long time, since this pH of the medium is evidently the limit of its stability. The same

conclusion was reached by Schlegel, in whose experiments the virus perished at pH 6.3, i.e. in weak-acid reaction. However, Busch succeeded in preserving the virus in active form under refrigeration at pH 6.5. The virus, as shown by investigations at pH 6.0, usually perishes almost instantly.

Pyl notes one astonishing fact: in his experiments the virus in an acid medium at pH 2.2 to 3.2 was more stable than even in the usual (optimum) weak-base reaction. Considerable stability, although somewhat less pronounced, was shown by the virus also in a strong-base medium at pH 11.75. Consequently, the virus has apparently several optimum points for length of survival: one at pH 7.6, a second at pH 3.0, and a third at pH 11.75.

High temperature, as was established long ago, acts with lethal effect on the virus. At a temperature of 60 degrees Centigrade the virus ("lymph") loses its virulence in 5 to 15 minutes (Loeffler and Frosch). According to Trautwein's data, under the same conditions it loses its virulence in 5 minutes. At 50 degrees Centigrade the virulence of the foot-and-mouth disease virus is maintained for 30 minutes at a temperature of 80 to 100 degrees Centigrade it dies instantly (Gins and Krause).

The virus in the form of filtrate of the contents of aphthae, somewhat diluted in preliminary, at temperature of 55 degrees Centigrade loses its activity in 15 to 40 minutes (English commission). Taken in the form of virulent blood, it loses its activity at

temperature of 42 degrees Centigrade in 12 hours (Klobouk), at 60 degrees Centigrade in 15 minutes, at 41 degrees Centigrade in 12 hours, at 37 degrees in 120 hours, at 2 to 12 degrees Centigrade in 179 hours; decrease in its activity takes place only 201 hours (Seelemann and others). According to data of the English commission, the virus in defibrinated blood at 55 degrees Centigrade loses its virulence in 20 minutes.

Many investigators (Loeffler and Uhlenhuth, Vallee and Carre, the English commission) point to the fact that the virus rapidly loses its activity at thermostatic temperature. In thermostatic conditions it becomes inactive in 12 to 24 hours, but if it is taken for experiment in filtered and somewhat diluted form it remains active under these conditions for 1 to 3 days.

Loeffler and Uhlenhuth succeeded in individual instances in keeping the virus active at temperature of 39 to 40 degrees Centigrade for 4 days. At temperature of 30 degrees Centigrade, according to data of these authors, the virus lost its activity in 9 days. In experiments of the English commission the virus, taken in the form of virus-"lymph", kept its activity in buffer solution of phosphate at thermostatic temperature for 4 to 5 days, at temperature of 55 degrees Centigrade for 15 to 40 minutes in all. In experiments of the American commission (Olitsky, Traum, and Schoening) the virus at temperature of 30 to 37 degrees Centigrade remained active for 24 to 48 hours, but no longer than 6 days, and at temperature of 18 to 20 degrees Centigrade for 25 days, but no longer than 60 days.

According to data of other investigators, the virus taken in the form of virulent blood, at temperature of 37.5 degrees Centigrade kept its activity for 24 hours, at temperature of 42 degrees Centigrade for 12 hours, at 24 degrees Centigrade for 19 days, and pronounced weakening of the activity of the virus came only in 144 hours (Klobouk). The virus in the form of epithelium of aphthae at thermostatic temperature kept its activity for 7 days in the presence of an already-begun decomposition of tissue and in one case even with putrefaction the virus remained active up to 3 weeks (Rosskopf).

In desiccated state the virus becomes more resistant, particularly with respect to the action of high temperatures, Taken in the form of "lymph" in desiccated condition it keeps its virulence at temperatures as great as 100 degrees Centigrade for 30 minutes, at 116 degrees Centigrade for 7 minutes, and even at 122 degrees Centigrade for 3 minutes (Modrow and others). In Janssen's experiments the virus in the form of virus-"lymph" and virus-epithelium in desiccated state kept its virulence at 70 degrees Centigrade for 30 minutes, and at 100 degrees Centigrade lost it.

The English commission desiccated the virus, taken in the form of "lymph", on a microscope slide at 19 degrees Centigrade, and it remained active for 14 days, and at temperature of 37 degrees Centigrade for 7 days. With rapid desiccation and storage in an exsiccator over sulphuric acid the virus kept its virulence

for 6 months, while under the same conditions, but with 70 percent humidity in the room and under normal storage it lost it in 2 days. In a completely dry atmosphere and at 18 degrees Centigrade the virus in Burbury's experiments was active up to 2 years. In Roux' earlier experiments the virus, in the form of virus-"lymph", with rapid desiccation in a vacuum at normal room temperature kept its virulence for 7 to 18 days, and the desiccated serum containing the virus even for 20 to 105 days.

The high resistance of the virus to low temperatures was noted long ago by many experimenters. Loeffler and Frosch succeeded in preserving the virus in the form of filtered virus-"lymph" in a refrigerated compartment for 8 months. In Nocard's experiment the virus in the form of virus-"lymph" at a temperature of 12 to 15 degrees Centigrade remained active for 2 months. Similar results were obtained by Gins and Krause with the virus in experiments on guinea pigs. Loeffler and Frosch succeeded in preserving the virus-"lymph" in active form for 8 hours at a temperature of 48 degrees Centigrade. The virus in the form of virus-epithelium can be kept active in a refrigerated compartment up to 77 days (Rosskopf). Blood containing the virus obtained without special precautions remains virulent for 5 months in storage in a refrigerated compartment (Gins and Krause). The virus, as shown by investigations, can also be preserved in blood for a long time at low temperatures. Roux, and also Cosco, succeeded in preserving the virus in blood at the temperature of a refrigeration compartment for several months.

In Rosskopf's experiments virulent blood in a refrigerated compartment keeps its activity for 112 days, and the serum for 63 days in all. Gins and Krause succeeded in preserving the virus in blood citrate at temperature from -4 degrees to -9 degrees Centigrade for 72 days. Virulent blood, taken from sick cattle, exposed to freezing and thawing contained the active virus for 72 days. Cosco reached the conclusion on the basis of his experiments that low temperatures generally do not have a harmful effect on the virus in blood and in serum.

In connection with the data on the high resistance of virus with respect to the effect of low temperatures, special attention is deserved by experiments concerning its survival in products of animal origin under various methods of technological preparation and storage.

According to data of the English commission, the virus remained active up to 30 or 40 days in the blood of cattle and hogs killed in the acute (febrile) stage of development of the infection, after rapid chilling and freezing of the carcasses. In the marrow of the corpses of guinea pigs exposed to freezing, the virus kept its virulence for 96 days, in the epithelium of aphtahe for 102 days, and in blood for 36 days. In another instance, the virus in the marrow of the chilled carcass of a guinea pig remained active up to 42 days, and in a frozen carcass for 76 days. In the organs and tissues of the corpse of a guinea pig at 2 degrees Centigrade the virus kept its virulence for 62 to 145 days, specifically, in the tongue for 82 days, in the

lungs for 96 days, in the heart for 62 days, in the liver for 99 days, in the kidneys for 96 days, in the fat for 82 days, in the marrow for 107 days, and in the tendons for 149 days (Andrewes).

Skomorokhov and Popova in their experiments with the survival of the virual at low temperatures established that in guinea pig carcasses frozen at a temperature from -36 to -15 degrees Centigrade the virus keeps its virulence for 21 days. The virus in the form of virus-epithelium of primary guinea pig aphthae frozen in test tubes, under the same storage conditions and at the same temperature, and with light admitted, remained active for 32 days, and in the form of virus-"lymph" for 28 days.

In the second experiment with survival of the virus at low temperatures carried out by Popova it was proved that with storage of frozen guinea pig carcasses at temperature from -30 to -50 degrees Centigrade the virus kept its activity in the muscles for 7 days, in the marrow for 53 days, and in the epithelium for 70 days.

Repeated freezing and thawing also does not kill the virus. In the experiments of the English commission the virus was exposed to 20 alternate freezings to -18 degrees Centigrade and warmings to 18 degrees Centigrade and did not lose its activity. In another instance the virus, together with the marrow substance from a guinea pig marrow was exposed 6 times to freezing over a period of 30 days, then to thawing, and in

all cases kept its virulence. Consequently, on the basis of all these data it is possible to draw the conclusion that low temperatures do not have essential effect on the foot-and-mouth disease virus, and, in any case they do not kill it. Low temperatures are more apt to have a "preservative" effect on the virus, and facilitate its longer survival outside the organism of the animal.

In carcasses left for "ripening" at a temperature of 10 to 12 degrees Centigrade, the virus dies in 24 hours under the influence of an accumulation of lactic acid (Schlegel, Lourens, the English commission, Makarevich, and others).

Interesting data are available with respect to the survival of the virus in milk under various conditions of its storage and preparation.

In Terbrüggen's experiments in fresh milk at 37 degrees Centigrade the virus remained active for 12 hours (pH 4.73), at 17 to 20 degrees for 25 hours (pH 4.66), and 5 degrees for 12 days (pH 5.8). At 37 degrees Centigrade the virus lost its activity in 24 hours, and its presence in milk could not be proved, while its presence was proved at 17 to 20 degrees Centigrade for 34 hours, and at 5 degrees Centigrade for all of 13 days. In skimmed milk at 37 degrees Centigrade the presence of the virus was proved to be greatest for 10 hours, at room temperature for 30 hours, and at 5 degrees Centigrade for 9 days. In cream at 37 degrees Centigrade the presence of the virus was proved successfully for 27 hours, at room temperature for 3 days, and at 5 degrees Centigrade for 10 days.

According to Galloway's data, in milk chilled directly after milking and subsequently stored at 4 degrees Centigrade, the foot-and-mouth disease virus keeps its activity for 15 days. After cooling of the milk immediately after milking, but with storage at 18 degrees Centigrade, the virus dies in 6 days (with the souring of the milk). In milk which has first been pasteurized, according to data of the same investigator, the virus in a dilution of 1:500 keeps its activity at 4 degrees Centigrade for 50 days, and at 18 degrees Centigrade for 30 to 35 days. In Andrewes' experiments in milk at 18 degrees Centigrade the virus stayed active for 9 days, at 4 degrees Centigrade for 16 to 17 days. In milk which had first been sterilized at 18 degrees Centigrade the virus remained still active after 30 days, at 4 degrees Centigrade after 46 days.

Galloway established a considerable resistance of the virus in dried milk. It turned out that in dried milk the virus does not lose its virulence when raised to temperatures of: 100 degrees Centigrade for 10 seconds, to 90 degrees Centigrade for 30 seconds, to 80 degrees for 1 minute, and to 70 to 75 degrees for 5 minutes. The time of survival and its resistance with respect to high temperatures depends also on the time during which drying of the infected milk is carried out.

In the same investigator's experiments with the slow drying of milk the virus at 18 degrees Centigrade lots its virulence in 48 hours; in quickly dried milk under the same conditions it lost

its virulence in 32 to 43 days. Drying of the milk by means of so-called "pulverization", which is carried out at lower temperatures, does not involve the death of the virus; in milk dried under the same conditions after preliminary pasteurization the presence of the virus could not be successfully proved (English commission).

According to Eberts' data, and also those of other investigators, with the souring of milk the virus dies under the influence of lactic and other acids which have formed. According to Galloway's data, the virus in milk under souring rather quickly loses its virulence, which depends, however, on the quantity of it and the temperature of the milk itself. The virus in milk at 18 degrees Centigrade does not lose its activity for 7 to 9 days, and at 4 degrees Centigrade for 15 to 17 days.

In milk at 60 to 63 degrees Centigrade the virus loses its virulence in 30 minutes (Zeller and Wedemann); at 60 to 64 degrees Centigrade in 15 minutes (Ernst). The same thing takes place at a temperature of 70 to 85 degrees Centigrade (Winkler, Frischern, Raffay, and others).

In fresh butter from fresh cream the virus, according to Terbrüggen's data, keeps its activity for 8 days. After preliminary storage of the butter fat for 24 hours in a cool room it remains active for 9 days, and in the cold for 26 days. In sweet butter even with spoiling the virus does not lose its virulence for 45 days. According to Galloway's data, in salted fresh butter the

virus remained active for 14 days. According to Terbrüggen's data, at 5 degrees Centigrade the virus kept its virulence for 18 days, and in storage in a cold room even for 45 days. In sour cream butter the presence of the virus in general cannot be proved. In buttermilk the virus can remain active for 11 hours, and in rinse-water for 35 hours.

In cheese the virus loses its virulence in the very process of production, evidently under the influence of warming and the effect of acid in its ripening. In the finished product the virus remained active no longer than 20 hours. In ripe cheeses the virus dies quickly. Many investigators (Winkler, Frischorn, and Raffy, Mussemeier and others) consider that in products prepared from sour milk, the presence of the virus in general cannot be demonstrated. In sour milk whey and buttermilk (pH 4.29) the virus dies in 5 minutes (Poels, Boors).

Preservation of various products of animal husbandry in practice is often accomplished with the help of sodium chloride or other preservatives. The virus in products preserved with sodium chloride can keep its activity for a very long time. In experiments of the English commission in salted meat under normal storage conditions the virus remained active up to 42 days. In various preservative substances (salt, sugar, saltpetre, etc.) used in the meat industry, the virus at 17 degrees remained active 35 to 49 days (Minnet). In hides preserved with sodium chloride, stored at barnyard temperatures, the virus kept its activity for 46 days.

From the practical standpoint there is great interest in the survival of the virus on other bases and, finally, in complete exposure. Large-scale investigations in this direction were carried out by the English commission. On the basis of their experiments they established that the virus in the form of virus epithelium can keep its activity in a haystack for 56 to 108 days, and clover-hay even for 140 days, in bran for 56 to 140 days, in flour for 14 to 49 days, in the hairy covering of cattle for 28 days, on woolens for 14 days, on silks for 7 days, on filter-paper for 48 hours, and in sand for 14 days. Taken in the form of virulent blood, it lost its activity in hay in 21 to 28 days, and on the other bases in 4 days. In one instance a month after storage of the virus it was possible to cause foot-and-mouth disease sickness by feeding the hay to calves (English commission). Hay gathered during the dry time of year and polluted with the virus at the time of standing in the field can evidently keep its activity for a long time (months). In hay which during drying and gathering has been repeatedly rained on, the virus is preserved for much less time. On hay polluted with the virus in the cold period of the year (autumn, fall), it can last until spring, inasmuch as low temperatures preserve the virus. In literature on the subject there are old indications that the virus on hay can last as much as 6 months (Hecker).

Interesting experiments on the study of survival of the virus on hay have been carried out by Kindyakov in Kazakhstan (Figure 2). For the experiment fresh hay of the swamp type was

used, composed of mixed grasses. The virus used was type O with virulence in dilution of 1:10,000,000 and well adapted to guinea pigs. It was established that on the surface of a hay-stack the virus can remain viable in August for one day, in September for 3 to 6 days, and in October for 8 to 10 days. Placed inside the stack of hay in the summer months (June, August) it died in one month, and in autumn-winter weather remained viable for 185 to 200 days.

Dried under the same conditions, it can keep its viability on straw for 2 months.

In silage the virus dies rapidly under the influence of acetic and lactic acids. In solutions of these acids, taken in the same concentration as in silage (acetic 0.31 percent and lactic 0.063 percent), it lost its virulence in 24 hours (Belogorskiy and Lipatov).

From the practical standpoint it is very important to know how long the virus can remain viable out-of-doors. It is known that the duration of its survival out-of-doors depends on very many factors, of which the most disinfective factor is the sun's rays.

In Nocard's and Leclanche's experiments the virus, dried for 2 days at 22 degrees Centigrade in air under the effect of the direct rays of the sun, lost its virulence in 24 hours. In the English commission's experiments, in the form of a thin layer

of "lymph" on a microscope slide under the action of the direct rays of the sun in summer, it lost its activity in only one hour, and in winter, when the sun shines more weakly, in 2 to 3 weeks. In Trautwein's experiments, in the form of virus-"lymph" under the influence of sunlight (August) it ceased to be active in only 2½ hours.

According to investigations of the same Trautwein, the virus in the form of "lymph" under the action of ultraviolet rays of a mercury-quartz lamp

DESIGNATIONS

———	Survival of virus in meadows on stems of vegetation
- - - -	Survivial of virus on the damp ground
— — —	Survival of the virus on the surface of haystacks
.....	Survival of the virus inside haystacks

Figure 2. Duration of stability of the foot-and-mouth disease virus out-of-doors under Central Asian conditions.

lost its activity in 30 minutes, while no less than 60 minutes were required in individual cases for inactivation of the same virus taken in the form of virus-epithelium. Galloway and Edinow established that the filtered virus in phosphate buffer solution

under the influence of ultraviolet rays lost its activity in 3 to 5 minutes (under ultraviolet rays of wavelength 5720 - 2300); in unfiltered form under the same conditions it kept its activity for 30 minutes.

The English commission, studying the photodynamic effect of methylene blue in a dilution of 1:50,000, established that under the influence of light in the presence of methylene blue the virus becomes inactive.

In Trautwein's experiments the virus, taken in the form of epithelium of aphthae, out-of-doors in the air and under the influence of light, sun, and other factors of the outside, did not lose its activity for 67 days. Dried on cotton tissue at room temperature it remained active for 5 days, on watch-glass in a barnyard building for 7 days, in washings of dung for 7 days, in sand for 7 days, on the surface of sandy soil for 11 days.

Kindyakov has studied the length of survival of the virus in pastures. Placed on the stalks of plants during the pasture season in November, it kept its virulence until February of the following year, i.e. for 88 days. With respect to its stability as a function of the time of year the author observed a definite correlation (Figure 2). In wintertime the virus in Kindyakov's experiments kept its activity for a range of 95 to 105 days, in March - February for 27 to 53 days, in May - April no longer than 7 to 11 days. The stability of the virus on the surface of moist

earth in a pasture changed with the same conformity to principle as with the virus placed on plant stalks, as a function of the time of year. In wintertime the virus kept its activity 146 to 163 days, in March and February for 39 to 75 days, and in May and April for 11 to 35 days.

On the basis of his investigations Kindyakov reached the conclusion that at an average monthly air temperature from 16 to 25 degrees Centigrade in a pasture on the stalks of plants the foot-and-mouth disease virus keeps its activity for a range of 1 to 15 days, and on the surface of moist earth up to 42 days. At an average monthly temperature of the air of 15 degrees and lower the stability of the foot-and-mouth disease virus, as a rule, increases rather strongly. And finally, at an average monthly air temperature below 0 degrees Centigrade the foot-and-mouth disease virus evidently is "preserved". Kindyakov, on the basis of his observations on the duration of foot-and-mouth disease epizootics in Central Asia draws the completely correct conclusion that frequent outbreaks of an epizootic in this case must be connected also with the increased stability of the virus out-of-doors. This is a serious factor in the epizootology of foot-and-mouth disease, which must not be underestimated.

According to Andrewes' data, the virus kept its activity on frosted glass for 27 days, on wood, glass, and brick for 14 days, on leather for 9 days, and on material which had been covered with the flesh of an animal for 45 to 46 days (20 degrees Centigrade).

According to data of the American commission, in garden soil (and likewise in hay) it remained active for 25 to 30 days.

Many researchers have engaged in study of the survival of the virus in feces and the washings of feces. In feces at a depth of 30 to 40 centimeters it dies in 6 days (Hecker, Loeffler, Trautwein, and others). In Wagener's experiments on the surface of feces it remained active for a very long time. On dung-heaps covered with a tarpaulin it did not lose its activity for 28 days and on similar heaps covered with sand for 10 days.

Kindyakov, on the basis of his experiments with the survival of the virus in feces reached the conclusion that in dung-heaps piled up during the warm time of year it dies on the 29th to the 33rd day, under the influences of the processes of decay of the dung. In frozen dung it keeps its virulence for the duration of the whole winter and for the first two spring months, until warm weather comes and decay of the dung begins. In frozen dung the virus kept its activity for 156 to 168 days, until warm weather came.

In Wagener's experiments the virus in the washings of dung of open barnyard sewers remained active for 9 to 16 days and even for 39 days. In closed sewers, on the other hand, it died considerably earlier, in the first 24 hours (at temperatures from 7 to 22 degrees), and in closed drains without free access to air in 38 days. In

winter, when the processes of decomposition of dung as a consequence of low temperature out-of-doors are less intense, the virus kept its activity up to 103 days.

Many investigators have engaged in the problem of studying the resistance of the virus with respect to various chemical substances. It is not killed, as became known long age, by ordinary disinfectant measures applied for the elimination of bacterial causative agents. The American commission on foot-and-mouth disease explains the high stability of the virus with various chemical substances by the congulation of its surrounding albuminal substance, which preserves it from the reaction of a chemical reagent. Many chemical substances, used for the purpose of disinfection, possess just this same ability to congulate the albumin, which restrains or stops entirely the virulent effect of the disinfectant substance.

The resistance of the virus may be particularly strongly revealed with respect to glycerine, which is a good preservative agent for keeping the virus.

Stockmann and Minet, in a chemically pure neutral glycerine taken equally with a physiological solution of NaCl, kept the virus active at 5 degrees Centigrade for 400 days. It was preserved for a somewhat shorter time in 96 percent glycerine for 300 days. At the present time chemically pure glycerine, it being recommended that a phosphate buffer solution be used instead of

physiological solution of sodium chloride, is used in laboratory practise for preservation of the virus. This makes it possible to maintain the pH of the preservative medium at a determinate level. In such a preservative medium the virus can keep its virulence for 6 to 12 months in a refrigerator.

The virus is also distinguished by a high resistance with respect to various fat-solutions, particularly in alcohol, ether, and chloroform.

Abe, in his experiments, as already noted before, used alcohol exclusively for precipitation of the virus, since even a 70 to 75 percent solution of alcohol does not inactivate it for 2 to 3 days. Filtered in preliminary, the virus keeps its activity for 18 hours in 60 percent alcohol, but staphylococci and intestinal bacilli used as a control died in one minute.

According to data of the American commission on foot-and-mouth disease, the virus died in only 26 hours in 60 percent alcohol. In the form of filtered virus-"lymph" under the action of 60 percent alcohol it lost its activity in several minutes.

Bedson and Maitland were not successful in making the virus inactive with a 60 percent alcohol solution for 18 hours. This stability under exposure to alcohol, American investigators explain exclusively by the coagulation of the virus-"lymph" under the influence of the action of alcohol. If a small quantity of sodium hydroxide (up to pH 8.2) is added to the alcohol, coagulation may be prevented, and alcohol can make the virus inactive.

In a 40 to 50 percent solution of alcohol the virus becomes inactive in one minute, and in 20 percent alcohol in 2.5 hours. In Stockmann's and Minett's experiments the virus, in 25 to 50 percent solution of alcohol kept its activity for 3 days, in 10 percent alcohol for 20 days, and in 60 percent solution for 26 hours.

In ether the virus can keep its activity for 3 hours and becomes inactive only in 24 hours at room temperature.

Ether fumes do not kill the virus for 36 hours. In Schmidt's experiments the virus, in the form of an ether-suspension of epithelium of aphthae, loses its activity only after staying in it for 54 days. However, after treatment of the virus with ether for 12 to 16 days its activity becomes perceptibly weaker, but it still causes disease in individual animals. Attempts to weaken the virus by this method and obtain an antigen for active immunization against foot-and-mouth disease produced no results.

High resistance of the virus is known also with respect to chloroform. Dependent on the concentration of the chloroform, it can keep its activity, according to data of the English commission on foot-and-mouth disease, up to 27 days, and according to Khomutov's data up to 17 to 60 days. In saturated chloroform solution the virus remained active for 24 hours (Bedson and Maitland) and even for 41 hours (Stockmann and Minett). With a chloroform concentration of greater than 16 percent Khomutov succeeded in making foot-and-mouth diseaseinactive for 48 hours. On the basis

of his investigations he recommends using chloroform for preserving the virus. Virus inactivated by chloroform was used by him as an antigen for active immunization against foot-and-mouth disease in experiments on guinea pigs.

With respect to the resistance of the virus to disinfectants of all kinds there are conflicting data. In their time Loeffler and Uhlenhuth reached the conclusion on the basis of their investigations that 1 percent solution of carbolic acid, 2 percent solution of formalin, 3 percent solution of soda, 1 percent solution of hydrochloric acid, 1 percent solution of phosphoric acid, and also milk of limestone kill the virus. However, in Loeffler's and Frosch's experiments the virus in the form of filtered virus-"lymph" in 0.5 to 1 percent solution of carbolic acid and thymol kept its activity in a refrigerator for 3 to 4 months, and in individual cases even up to 5 months. The same filtered virus, but in dilution of 1:10 in 1 percent solution of carbolic acid lost its activity considerably sooner: in the course of only 11 weeks in all.

The disinfectants recommended by Loeffler and Uhlenhuth were for a long time applied in practice in the struggle against foot-and-mouth disease, as completely "effective" disinfectants. All of these questions as to the resistance of the virus with respect to disinfectants have been seen in a completely different light in connection with the investigations of the American and English commissions on foot-and-mouth disease, and also of Trautwein, Winkel, Ovsyannikov, Ratner, and Tonigs.

In the experiments of the American foot-and-mouth disease commission the virus, taken in the form of "lymph" of aphthae in dilution of 1:100 in 0.1 percent solution of corrosive sublimate, remained active for 3 hours, and in 1 percent solution of corrosive sublimate and also in 3 percent creosol and other creosol compounds did not lose its activity for 6 hours. Similar results were obtained by Minett. Phenol, creosol, and all derivatives had an extremely weak virucidal effect on the virus in his experiments. According to Minkel, in 5 percent solution of creoline can keep its activity for 22 days. According to data of the English commission on foot-and-mouth disease, sodium fluoride, toluol, phenol, lysol, hydrogen peroxide, chlorine, iodine, chloropicrin, chloroform, ether, and acetone in concentrations which kill ordinary bacteria do not deprive the virus of activity. In a saturated solution of sodium chloride, sodium sulphate, and magnesium sulphate at low temperature the virus did not lose its activity for 3 months.

Galloway studied 90 different chemical substances from the standpoint of virucidal properties, 36 of them being paints and 54 compound substances (arsenic preparation and various compounds of bismuth, cupreine, copper, cobalt, chromium, nickel, lead, antimony, gold, platinum, iron, vanadium, cesium, cerium, chromic acid, colloidal metals, sodium salicylate, hexyresorcinol, methylene hydrochloride, itramin, Bayer 205, Fournaud 309, and others) and not one of them proved sufficiently effective from the standpoint of virucidal effect on the virus.

Helm and Wedemann tested the virucidal effect of a 1 percent solution of sulphurcresol solution mixture and obtained satisfactory results. However, Trautwein could not confirm the data of these authors. In Weiss' experiments the virus in 2 percent solution of soap-cresol mixture the virus did not lose its activity for 24 hours. According to old data of Loeffler and Uhlenhuth, it kept its activity in milk of lime for one hour. In Minett's experiments with the virus, taken in the form of epithelium of aphthae, milk of lime proved to be a weakly virucidal agent. It evidently could have a virucidal effect but only in direct contact with the virus, which is impossible to obtain under practical conditions of disinfection.

Sorbestvenskiy considers that chlorine is an extremely energetic agent for destruction of the virus, exceeding in this respect many other chemical substances, such as phenol, creosol, and others. He supposes that chlorine can be used for disinfection (chlorimating) of buildings with foot-and-mouth disease.

In the experiments of the American commission on foot-and-mouth disease in the study of the effect of various disinfectants the best results were obtained with sodium hydroxide and antiformine. In 0.25 to 0.5 percent solution of sodium hydroxide the virus lost its activity in 3 minutes, in 2 percent solution of sodium hydroxide, and also in 1 percent solution of antiformine, the virus, taken in the form of epithelium and "lymph" of aphthae in dilution of 1:10, lost its activity in one minute.

In Magnusson's experiments the virus became inactive in 3 percent solution of sodium hydroxide in 5 minutes.

The virucidal effect of sodium hydroxide was confirmed by the experiments of the English commission on foot-and-mouth disease, and also by Trautwein and Reppin, Winkel and others. Incidentally, in the experiments of Ovsyannikov the virus, taken in the form of epithelium of aphthae of the guinea pig in 2 percent solution of sodium hydroxide, did not lose its activity for 30 minutes. According to the data of Waldmann and Trautwein potassium hydroxide is almost undistinguishable from sodium hydroxide for its virucidal properties. Ratner and Tonigs in studying the virucidal properties of potassium hydroxide established that in 5 percent solution of this preparation the virus loses its virulence in 30 minutes.

However, the practical significance of caustic bases recommended as disinfectants for foot-and-mouth disease is somewhat decreased by the fact that in the presence of organic and inorganic substances, and also from the effect of air they lose rather rapidly their virucidal properties. According to Minett's data, well defined virucidal effect with respect to foot-and-mouth disease virus is possessed by formalin, taken in the form of a 1 percent solution. It keeps its virucidal effect for 24 hours out-of-doors, but is completely harmless for animals and human beings, and therefore, in Minett's opinion, can be used for practical disinfection. Incidentally, the disinfection of hay, either piled in stacks or baled, with 0.1 to 1 percent solution of formalin does

not alter its properties of taste or nourishment, which is very important from the farm-management standpoint.

Trautwein and Reppin adopt a critical attitude to the evaluation of the virucidal effect of formalin, since it acts on the virus very slowly, killing it only in 6 hours. Its effect, as affirmed by Trautwein and Reppin, is equal to that of a creosol mixture and 30 percent dilution of chloride of lime.

In Ovsyannikov's experiments 1-, 1.5-, and 2 percent solutions of formalin did not kill the virus taken in the form of epithelium of aphthae, even after 3-hours exposure. The question of the virucidal effect of formalin on the virus has unfortunately been given little attention so far.

According to Trautwein's data, sulphuric acid possesses high virucidal properties with respect to the virus. (It is known that it possesses very weak bactericidal properties.)

Trautwein studied the virucidal effect of sulphurous gas (SO_2), which in his opinion can be a good means of disinfection for foot-and-mouth disease. The same conclusion with respect to sulphurous gas was reached by Steidle.

In Dobson's experiments good results were obtained with the application of sodium carbonate (common washing soda). The virus, according to the author's data, lost its activity in 1 percent solution at ordinary room temperature in 15 minutes, and at temperature of 50 to 60 degrees Centigrade in 5 minutes. This is

particularly important in washing utensils and cleaning buildings where there are products of infection.

In connection with the extremely limited assortment of disinfectants which have more or less promising virucidal properties, there have been great difficulties until present with the disinfection of leather materials exposed to foot-and-mouth disease. Minett on the basis of his investigations recommends for the disinfection of hides that they be soaked for 5 hours in a solution of sodium bisulphide (1:10,000) or for 2 hours in a solution of sodium fluoride (1:20,000). These solutions, as affirmed by the author, do not spoil the hides and kill the virus reliably.

Belogorskiy recommends for the same purpose soaking the hides in brine solution with 0.1 to 0.2 percent concentration of sodium hydroxide, or with 0.5 percent concentration of bisulphite or 5 percent solution of carbonate of soda. Such concentrations of all these agents have absolutely no effect on the quality of the leather.

Consequently, of all chemical agents which have been studied with respect to the virucidal effect on the foot-and-mouth disease virus, only those may be recommended which do not coagulate the albumin. The best of these will doubtless be substances which contain a base (NaOH, KOH).

The virucidal effect of caustic bases (sodium hydroxide and potassium hydroxide) with respect to the virus is explained evidently by the fact that they cause a rapid process of hydrolysis and break-

down of the components of the albuminal substance with which the virus itself is connected.

The virus, as a result of the destruction of its protective covering in the process of hydrolysis, as certain investigators suppose, becomes accessible for the reaction of the active group of hydroxide ions, there being created a pH medium favorable to its survival (pH 11).

Sodium hydroxide can be applied for practical disinfection together with lime in the white-washing of stalls, troughs, and walls. A 1 percent solution of sodium hydroxide harmless to animals can be used also for disinfection of external coverings and hooves (Trautwein and Reppin). The non-uniform results obtained by many investigators with respect to the resistance of the virus must be associated evidently with the different conditions under which these investigations were carried out.

Greatest resistance with respect to various chemical substances as well as out-door exposure, as may be seen from the data given above, is possessed by the virus in the form of "aphthous epithelium", while it is much less stable when taken in the form of aphthous "lymph". Loeffler and Uhlenhuth used a just less resistant virus, in the form of the contents of foot-and-mouth disease aphthae, and therefore they could not obtain such results as were obtained later by other investigators.

In studying the resistance of the virus there is doubtless

also great importance in its virulence, which, in its turn, is subject to considerable fluctuations. And, finally, a whole series of other conditions, which often cannot be taken into account, also can have an influence on experimental results. In spite of all the difference of opinion with respect to evaluation of the degree of resistance of the virus, it remains proved that the latter, being secreted by a sick organism into the out-of-doors in the form of a "colloidal substratum", possesses very high resistance with respect to the reaction of various factors of the out-doors, and also with respect to various physio-chemical agents.

VIRULENCE OF THE VIRUS

The foot-and-mouth-disease virus possesses unusually high virulence. For infection of susceptible animals an insignificantly small quantity of the virus is necessary. In the old experiments of Loeffler, Frosch, and Uhlenhuth it was proven sufficiently active in dilution of 1:50,000 to cause foot-and-mouth disease in calves. Stronger concentrations of the virus -- 1:[illegible] and 1:[illegible] -- caused very severe sickness in calves. The same results were obtained in his day by [illegible] with a dilution of the virus of 1:[illegible]. Stronger dilutions -- 1:[illegible] and 1:100,000 -- in the experiments of these authors gave negative results. [illegible] caused infection with foot-and-mouth disease with a dilution of the virus in the form of aphthous "lymph" of 1:250,000; stronger solutions also gave negative results in his experiments. [illegible] was also confirmed in the experiments of [illegible] and Krause, Abe, [illegible], and others. (In this respect the foot-and-mouth-disease virus can be compared only with the virus of fowl plague, which in dilutions of many millions also proves to be extremely active.)

[illegible] and Trautwein, [illegible], [illegible] and [illegible], Bedson and [illegible] caused infection of animals with a dilution of the virus of 1:500,000. In the experiments of the American commission it caused infection with a dilution of 1:10,000,000. The virus was taken for experiment in this case from 24-hour aphthae of guinea pigs and then a phosphate-buffer solution was added to it (pH 7.5). Infection was produced by the intracutaneous method in the plantar surface of the rear extremities of the guinea pigs. The virus was filtered

through a paper filter to free it of coarse particles.

In the Kiems Institute, during study of the virulence of the foot-and-mouth-disease virus, still more striking results were obtained. The virus, taken in the form of aphthous "lymph", proved capable of causing infection in dilution of 1:1[illegible],000,000 and even in dilution of 1:2[illegible],000,000.

[illegible] and other investigators established that the virus in the form of aphthous "lymph" in primary aphthae attains maximum virulence in the course of the first 24 hours after infection. After this the virulence gradually decreases. [illegible] succeeded with virus taken from 24-hour primary aphthae in causing infection when it was diluted to 1:[illegible], [illegible] with virus taken [illegible] it was necessary to take 100 times as much of the virus, and from 72-hour aphthae [illegible] times as much. [illegible] days after the appearance of aphthae it ordinarily loses its virulence. Therefore, it is necessary for diagnostic purposes to collect the lymph from aphthae only before 48 hours have elapsed ([illegible]).

Alekseyeva studied the activity of the virus at various stages in the development of the aphthous process. She succeeded in proving that the virus type O from primary 24-hour aphthae of hogs can cause infection in dilution of 1:5,000,000, while the same virus, taken from 30-hour aphthae possessed insignificant virulence.

According to the data of other investigators, the virus completely disappears from primary aphthae and blood in 3 to 4 days after infection, and frequently its presence cannot be proved even under experimental conditions.

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Consequently, the virulence of the virus at various stages of the development of the foot-and-mouth-disease process is not always uniform. [illegible] in particular calls attention to this on the part of the various investigators in the field of foot-and-mouth-disease study, in order to avoid possible errors, particularly in [illegible] mutation of the virus.

In conditions of the natural development of foot-and-mouth-disease epizootics the virulence of the virus is subject at times to considerable fluctuations. This is indicated by the varying nature of the course of the disease, which depends not only on individual peculiarities of the animal or the general condition of the entire herd [illegible], but also on the concentration (accumulation) of the virus in the medium surrounding the animals (the "[illegible]" [illegible] "[illegible] factor").

[illegible] conclusions [illegible] on the basis of the experiments that the severity of the course of the infection itself and the duration of the incubation period depend also on the concentration of the virus. The virus, taken in the form of [illegible] in dilution of 1:[illegible], caused severe infection in the course of a single day in all guinea pigs used in the experiment. With 1:200,000 dilution of the same virus the sickness came in 3 days, with 1:2,000,000 dilution in 4 days, and finally with 1:4,000,000 dilution in very weak form and only on the ninth day following infection. On the example of this experiment the influence of the quantitative factor on the severity of the disease and the duration of the incubation period may be seen with special vividness. At the same time there are doubtless available various

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factors, often not subject to consideration, which influence the nature of the course of the epizootic of foot-and-mouth disease itself.

Many investigators (Loeffler and Frosch, Ernst and Drescher, Titze, Waldmann and Trautwein, and others) noted long ago, and subsequently proved experimentally, that rapid alternate passage of the virus in various types of animals susceptible to foot-and-mouth disease produces an intensification of its virulence.

Loeffler and Frosch succeeded in increasing the virulence of the virus by means of alternate passage of it in calves and hogs. Waldmann and Trautwein attained the same results with alternate passage of the virus in guinea pigs and white rats. Burbury introduced to the ranks of passage animals the rabbit as well. Waldmann and Trautwein increased the virulence of the virus for cattle, but subsequently, in order to cause infection with it in guinea pigs, the authors had to pass it first through the organism of a hog.

Many investigators, particularly Ernst and Drescher, have established that prolonged passage of the virus in the same type of animal intensifies its virulence with respect to the given type of animal, while with respect to other types of animals it appears weaker. However, this phenomenon is temporary, and the virus, due to its rapid adaptability, can in one or two passages again establish its virulence for a given type. Ernst and Drescher established also that passage of the virus in animals which had in preliminary been immunized (passively or actively) leads to the gradual weakening of its virulence and, in the end, such virus ceases completely

to cause infection in animals susceptible to foot-and-mouth disease.

In Schleswig-Holstein in 1927 and in Hanover in 1928 outbreaks of an epizootic of foot-and-mouth disease were observed only among hogs and did not spread to cattle even in cases where both types of animals were in the same building (Trautwein). Similar phenomena have been noted several times on certain farms in our country, when foot-and-mouth disease of cattle, in the absence of appropriate preventive measures, still did not spread to other times of animals susceptible to foot-and-mouth disease. This unique "selectivity" of the virus for a definite type of animal can be explained by its insufficient virulence for other types of animals, which has been the result either of prolonged passage in the same time of animal or, possibly, a consequence of the mutability of the virus, which is inherent in its very biology.

Such selectivity of the virus can be influenced by other factors which in conditions of the natural development of the infection can bear on the entire epizootic process as a whole, often not lending themselves to any kind of accounting in practice.

Many investigators, (Hecker, Loeffler, Frosch and Uhlenhuth, Schipp, Ernst and Drescher, Waldmann and Trautwein, and others) have attempted to weaken the virus for spontaneously susceptible animals by means of prolonged passage of it through the organism of animals of the same type. However, all the investigators' attempts in this direction ended unsuccessfully. In the Riems Institute there was one strain of the virus which had been propagated through guinea

pigs for more than 3000 passages without any weakening of its virulence for spontaneously susceptible animals (Waldmann and Trautwein). Similar results were obtained in the experiments of Ernst, De Blieck, and other investigators.

In cutaneous infection, as has been established by a number of investigators, the virus can be propagated lengthily through any one type of animal without weakening of its virulence.

With prolonged passage of the virus its virulence may be weakened at times for another type of animal, but this weakening remains of little duration, since the virus easily adapts itself to a new type of animal and becomes infective for it. Consequently, for weakening of the virus' virulence and subsequent securing of these properties the method of prolonged passage has not justified itself and has no practical significance.

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

The French investigators Vallee and Carre first announced in 1921 the assumption of the existence of a plurality of viruses. In 1922, in the experimental study of the immunological properties of foot-and-mouth-disease viruses of French and German origin they discovered two immunologically different types of the virus, indicated by them with the letters O and A. Type O is a foot-and-mouth-disease virus of French origin from the department of the Oise and Type A is of German origin from Eastern Prussia (Allemagne).

The essence of this noteworthy discovery is in the fact that each of these two types causes immunity only with respect to the

same type of virus which has caused infection. Consequently, animals which have developed sickness for the first time as a result of infection with virus type O and have become immune to repeated infection with virus of this same type can be infected a second time with virus type A. Such infection is also impossible in reverse order, i.e. animals which have developed foot-and-mouth disease of type A and which are immune to it can be infected with type O virus (Figure 3).

Figure 3. Hoof of a pig after development of foot-and-mouth disease as a result of experimental infection with virus types O, A and C (after Waldmann and Trautwein).

Type O and A viruses cause a completely uniform clinical picture of the disease, and are distinguished not by their pathogenic action on the animal organism, but exclusively by the immunological properties. For proof of the existence in nature of various types of foot-and-mouth-disease virus present-day investigators use the method of cross-immunization almost exclusively. Nevertheless, there are attempts to apply for the same purposes serological reactions (PCK) by means of preparation of foot-and-mouth-disease type antigens (Solov'yev, Helm, Galea, and others); however, these investigations have not yet left the stage of laboratory research.

While Vallee and Carre were founding experimentally the existence of a plurality of types of viruses, another investigator in France, Schein, announced the hypothesis of a "plurality of foot-and-mouth-disease sicknesses", as a consequence of the possible

co-existence of two variant incitants of foot-and-mouth disease. He assumed the existence of at least two independent forms of foot-and-mouth-disease sickness -- "aphthous fever" (Fievre aphtheuse) and "aphtheidal fever" (Fievre aphtheide) which in Schein's opinion are usually confused in practice.

Before Vallee's and Carre's discovery of the plurality of types of the foot-and-mouth-disease virus certain investigators attempted to explain the etiological essence of these phenomena by an insufficiently firm immunity or a complete absence of immunity following development of the foot-and-mouth-disease (Pokshishevskiy, Kovach, Strebel, Rudovskiy, Moore, and others).

Vallee's and Carre's discovery shed definite illumination on questions of immunity to foot-and-mouth disease and at the same time attracted the attention of other investigators to the question of study of the plurality of types of viruses.

Waldmann and Mayer in the Riems Institute could not confirm Vallee's and Carre's discoveries on the basis of experimental study of the immunological properties of viruses of German, French, and Italian origin. Bedson, Maitland, and Burbury, checking the discovery made by Vallee and Carre, also reached negative conclusions on the basis of their experimental investigations. And only much later did Waldmann and Trautwein (1926) in studying 30 strains of viruses of various origin confirm the discovery of the plurality of foot-and-mouth-disease viruses made by Vallee and Carre.

Waldmann and Trautwein in turn discovered still a third type of virus, which they called type C. Thus, the French investigators

Vallee and Carre, on the one hand, and the German investigators Waldmann and Trautwein, on the other, conclusively established and confirmed experimentally the existence in nature of at least three immunologically different types of the foot-and-mouth-disease virus.

Waldmann and Trautwein called the three types of virus which they studied A, B, and C (German A corresponded to French C, and German B to French A).

The discovery of the plurality of types of viruses was afterwards confirmed experimentally by Lebailly in France (1926), by Bedson, Maitland, and Burbury (1927) in England, by Olitsky, Traum, and Schoening (1926) in the USA, by Magnusson and Germansson in Sweden, by Lignieres in the Argentine (1929), by Winkel in Holland (1928-1930), by Piworarzyk in Poland (1928-1930), and by other investigators. The plurality of viruses was confirmed also by numerous epizootological observations by Burgi in Switzerland, by Jensen in Denmark, by Casper in Austria, by Stazzi in Italy, and others. Only Ernst, Gut, and Hopfengaertner (1927) continued, on the basis of their small investigations, to deny the plurality of virus-types. Incidentally, they studied a total of only 6 strains of virus of German, French, and Swedish origin, which actually could have been virus of the same type. And finally, these experiments were carried out only on guinea pigs, which makes them for this reason extremely unconvincing.

In 1928 the International Epizootic Bureau in Paris, on the basis of Vallee's report "On the Plurality of Types of Foot-and-mouth-disease Viruses" introduced a resolution to the effect that

it was now necessary to consider proven the existence of three immunologically different types of virus. In this connection a single international nomenclature for types of the virus was introduced: type O, type A, and type C.

Vallee and Carre, and also Waldmann and Trautwein affirm that the types of viruses discovered by them possess fully constant immunological properties. Vallee and Carre studied for 8 years the type characteristics of strains of type O and A viruses, exposing them to the reaction of the most diverse factors. They propagated them in passages through various forms of animals, stored them at various temperatures, altered the pH of the medium, froze them, desiccated them, caused chemical agents to react with them, and in all cases these viruses either fully kept their original type characteristics or died.

Waldmann and Trautwein discovered another whole series of atypical strains of viruses which could not be classified as a single one of the three types discovered earlier by Vallee and Carre, and also by themselves. Evidently atypical strains are rather frequently encountered.

They were discovered also by Vallee and Carre in France, by Manninger in Hungary, and by Magnusson in Sweden.

Trautwein, in differentiating 78 strains of viruses received from different places, established well-defined type characteristics for only 29 strains (25 strains were classified as type A (O), 3 strains as type B (A), and 1 strain as type C, but the remaining 49 strains proved to be atypical strains (variants). Of them, 25 strains

were variants of type A (O), 6 strains variants of type B (A), and 18 strains variants of type C. Atypical strains were discovered in 1929, during the epizootic of foot-and-mouth disease in Switzerland (Burgi) and in the same year in Germany (Waldmann). In Pirbright (England) 86 strains were investigated, collected from various regions of England. Of them, 51 strains were classified as type O, 22 as type A, 2 as type C. The remaining 13 strains proved atypical and were not differentiated.

Waldmann and Trautwein established also that among strains of the same type of virus considerable differences are frequently observed. In Vallee's and Rinjard's experiments individual strains of viruses with very high virulence for spontaneously susceptible animals (cattle and hogs) could not be transferred to guinea pigs with any success at all. In Germany in 1927 and 1928, as noted already, an epizootic of foot-and-mouth disease was observed which affected only hogs and did not spread to cattle, even when the latter were in a single building with sick hogs. The epizootic among hogs was caused in this case by foot-and-mouth-disease type O virus. Waldmann succeeded easily in infecting guinea pigs and hogs with this virus, but with great difficulty in infecting cattle.

In the Riems Institute 4 strains received from Argentine were investigated. They possessed high virulence for spontaneously susceptible animals, but only two of them were transferred successfully to guinea pigs. One of them (a strain of virus type C) was transferred successfully to guinea pigs only in the course of half a year, by means of alternate passages through cattle and hogs. In his day Lignieres reported similar facts from Argentina, evidently

having dealt with the same strain of the virus.

In this instance investigators observed the known selectivity of the virus for one type of animal and its weakly manifested virulence for other types of animals.

Present-day science does not yet give a conclusive answer to the question in what manner various immunological differences occur among different types and variants of virus. However, it may be considered established that repeated passage of the same strain of virus through definite types of animals can alter its biological properties and even alter its type classification (English commission on foot-and-mouth disease).

Manninger affirms that the virus in the organism of animals highly susceptible to foot-and-mouth disease (cattle and hogs) undergoes considerable changes. In his opinion this is explained by the large number of atypical strains, discovered in the Riems Institute in the differentiation of virus taken from cattle and received from various countries.

Very interesting data on the mutability of the virus in passage in various types of animals was reported by the English commission on foot-and-mouth disease. The virus R. V. 1-6, obtained from Southern Rhodesia, was classified as type O in the process of determination of type characteristics. In cross-infection with one of these strains of the virus guinea pigs acquired in all cases a well-defined immunity to type O virus. Cattle infected with one of the R. V. 1-6 viruses did not acquire, on the other hand, immunity to type O virus. Animals which had developed foot-and-mouth

disease and were immune to type O virus again became sick with foot-and-mouth disease upon infection with one of the R. V. 1-6 viruses.

Trautwein and Reppin in turn made a very interesting observation, that after passages in guinea pigs in their experiments atypical strains began to revealthe properties of type O (variant of type O). The authors, in differentiating types of viruses in the Riems Institute, distinguished 45 variants of type A, of which 22 strains of these viruses after passage in guinea pigs acquired all the properties typical of type A.

A similar observation of the "fixation" of viruses in passage through the organism of guinea pigs was noted also by the English commission. Strains of virus Nos 7 and 8 in differentiation according to their properties approached type A; after 19 passages in guinea pigs this resemblance to type A became still more sharply defined. A similar observation was also made by the English commission on foot-and-mouth disease. In Lincolnshire in 1933 virus was taken from a sick ox, the virus having proved, following original differentiation, to be a variant of type O. Subsequently, in passage in guinea pigs this strain of the virus acquired all properties typical of type O virus. Animals which had earlier developed foot-and-mouth disease caused by type O virus did not become sick upon artificial infection with virus of the same type.

The English investigators Ecoles, Longley, and Tomson, in defining the type classification of foot-and-mouth-disease virus of cattle which had contracted the sickness naturally, investigated

the virus simultaneously with both cattle and guinea pigs. In differentiating virus which had been propagated in cattle, it was found that the virus had a definite kinship with two types -- O and A. Upon the first passage of this strain in guinea pigs the same properties were observed as upon passage of it in cattle. But after the 10th passage in guinea pigs it acquired all the properties of type O, and after the 14th passage was differentiated as type C. In the intervening 12 th passage the antigenic properties of all three types -- O, A, and C -- were discovered.

In England in 1931 considerably more strains of type A were differentiated by comparison with the remaining types of viruses. In individual strains of type O and C viruses certain properties with respect to type-immunological properties were observed. The English Commission on Foot-and-mouth Disease in one of its reports cites a case when of 470 head of cattle immune to all three types of the virus -- O, A, C -- 44 head of stock became sick upon infection for the fourth time with a new strain of the virus. In Germany in 1930 there was recorded a second development of foot-and-mouth disease by stock in the same herds, caused by virus which upon differentiation had in both cases been classified as the same type. It is difficult to explain the sickness of stock in this instance by a "falling off" of immunity resulting from repeated contraction of the disease. It is most probable that the fourth time the stock were infected with virus far removed in its immunological properties from type O, A, and C virus.

Waldmann affirms that atypical strains are encountered all the more often as the tendency to wide spread of an epizootic becomes more strongly manifested.

During the large epizootic of foot-and-mouth disease in Germany in 1926, when a colossal number of population points (187,000 farmsteads attacked by foot-and-mouth disease) were affected by foot-and-mouth disease, all three types of the virus were differentiated: O, A, and C, and their variants. Beginning in 1927, when epizootic of foot-and-mouth disease started gradually to subside, only a single type O and its variants could be differentiated successfully.

According to Waldmann's data, in the years when foot-and-mouth disease has a sharply defined tendency to rapid epizootic spread all three types -- O, A, and C -- are encountered, and in years of relative "calm" only the single type O and its variants are encountered. On this basis Waldmann attempted to find a causal connection between the appearance of new types and variants during epizootics and the character and severity of the course of the epizootics themselves.

Not all investigators share Waldmann's point-of-view in this question. In particular, Kindyakov, during the foot-and-mouth epizootics in Central Asia, observed a reverse phenomenon: in the years (1938-1940) with smallest spread of the foot-and-mouth-disease epizootic he differentiated variants of types O and A, and in the years (1941-1942) of greatest increase of the epizootic wave he differentiated only one variant of type O.

Waldmann in Germany, and also Rinjard in France, in an experimental investigation of the foot-and-mouth-disease epizootic of 1937-38 reached the conclusion that the virus in conditions of

natural development of the epizootic can change its antigenic structure under the influence of an ever-increasing number of animals immune to foot-and-mouth disease. Having changed its antigenic properties, it becomes afterwards newly capable of overcoming immunity existing in animals, acquired by them from the original type of virus. The question of the causes and conditions of the occurrence of various types and variants of the virus in the period of development of epizootics continues to attract the attention of present-day investigators. Meanwhile, science gives us no conclusive answer to this question, which has great theoretical and practical significance.

The appearance of atypical viruses may most easily be observed at the very beginning of an outbreak of epizootic. Manninger explains this phenomenon by the rapid passage of the virus in animals highly sensitive to foot-and-mouth disease -- cattle, hogs, sheep, etc. Trautwein regards the atypical strains as "transitional forms" -- variants. In the author's opinion, they represent nothing more than "an expression of the physiological adaptation of the foot-and-mouth disease virus to a determinate medium". The English Commission on Foot-and-mouth Disease considers the following the most accurate explanation of the origin of atypical strains -- viruses with different type-immunological propensities: "All strains of foot-and-mouth-disease viruses consist of the same immunological components, but the quantity of the latter can vary. A strictly defined quantity of these immunological components is expressed only in viruses of the standard types O, A, and C". This commission assumes that in conditions of natural development of a foot-and-mouth

disease epizootic infection with mixed viruses of two different types is possible. Subsequently then, in a later passage, one of them, as a rule, crowds out ("squeezes") the other. Trautwein, in experimental conditions, infected guinea pigs with mixed viruses of several types, only the one type O remaining always in further passages in guinea pigs, and the other types seemingly crowded out by the latter.

In the opinion of English investigators (Eccles, Longley, and Tomson), certain types of viruses have a very complex immunological structure in conditions of natural development of epizootics. They assume that the virus in the organism of the guinea pig can alter its antigenic structure and dissociate, and, as a result, new "mutations" of strains in various stages of transmission of the virus in the process of its passage can occur. Manninger and Laszlo succeeded, in the process of passage of Bavarian type A virus and Milan type C virus in guinea pigs, in converting them into type O after the 9th passage.

According to data of the same investigators, the Riems viruses of types A and C, after prolonged storage in a glycerine preservative medium and without passage in guinea pigs, acquired the properties of type O, it being no longer possible after this to convert these strains back into types A and C. Manninger affirms that viruses of type A and C can go over into type O. The latter is the most constant type of foot-and-mouth-disease virus. Viruses of types A and C, according to the author's data, as a rule are observed only at the beginning of an epizootic, and at its end are newly transformed into type O. The

type O virus, on the other hand, does not change its type properties and does not reveal any tendency toward transformation into other types.

Incidentally, in Trautwein's experiments may also be found the phenomena of mutability of viruses, which can be interpreted as transformation of one type into another. In one instance a strain of type A virus twice acquired the properties of type O virus in 137 and 186 passages. In another instance a strain of type C virus, after 385 passages in guinea pigs, lost its immunizing properties with respect to itself. And finally, in a third case the foot-and-mouth virus of the "Murri" strain first caused sickness in animals which had earlier developed three different types, and on the 19th passage showed the properties of a pure strain. Skomorokhov succeeded in observing an instance when Riems standard-type viruses A and C, after a year-and-a-half of passage in guinea pigs and storage in a glycerine preservative medium, lost their original type-properties and were transformed into atypical strains with the properties of type O. Manninger affirms that foot-and-mouth-disease viruses in general are not strictly constant types in the immunological sense, but are only different kinds -- variants of virus type O.

Type O virus, by comparison with types A and C, actually possesses the most sharply defined constant-type properties, while the type A and C viruses can be transformed into type O under conditions which have not yet been determined. J. Lignieres of Argentina considers that in general there do not exist strictly determined types of the virus, but that there exist only variants of the same

type. In experiments type O and A viruses have become completely identical after two-year passage in guinea pigs.

Observations of no lesser interest were made by the English Commission on Foot-and-mouth Disease with respect to the possible "splitting" (dissociation) of the foot-and-mouth-disease virus in the process of passage in animals. One of the strains with atypical properties (strain No 126) remained stable until the 20th passage, and then "split" into two "subsidiary" strains, of which one possessed all characteristic properties of type O, the other the properties of type A. For splitting of this strain the authors used the following method. Two groups of guinea pigs were infected with the virus, of which one group was immunized to type O and the other to type A. In differentiation of the virus the A virus was distinguished in the group of guinea pigs immunized to type O, and the O virus was distinguished in the other group, immunized to type A.

In the light of these data the very question of the existence in nature of only three relatively constant types of foot-and-mouth-disease viruses (O, A, and C) and of atypical strains, variants of these same types, is in some doubt.

The English Commission on Foot-and-mouth Disease considers that in Great Britain the quantity of types and variants of viruses is considerably greater and that they are more diversified than has so far been established. In its opinion, together with types of the virus which are stable in the immunological sense there are also "intermediate", or transitional, forms which could be called unstable types. However, the majority of strains still does not depart from the limits of the standard types O, A, and C which are known to us.

(From personal correspondence with Professor Manninger.)

Manninger's announcement that he had succeeded in observing the transformation of one type of virus into another was made first at the International Veterinary Congress in London in 1930, and next at a session of the International Epizootic Bureau in Paris in 1931.

Waldmann, in connection with Manninger's action, expressed at the Veterinary Congress in London the idea that it is theoretically impossible to refute the mutability of types of the virus and in connection with this to refute the possibilities of formation of new or of the disappearance of old types during even one epizootic, but that such a statement, in Waldmann's opinion, is still premature.

Further experimental proofs of the transformation of one type of virus into another are needed. Vallee and Carre also do not deny a mutability of types of viruses which can facilitate the transformation of one type into another.

The question of the plurality of virus types was one of the central problems at the 11th International Veterinary Congress in London, where the reports of Andrewes, Waldmann, and Lignieres on problems of foot-and-mouth disease were presented. In its conclusions the Congress acknowledged that the existence of a plurality of viruses has tremendous significance for accurate understanding of the epizootology of foot-and-mouth disease, and also for solution of the problem of active and passive immunization. The Congress acknowledged it as desirable that in all countries of the world

there should be invoked a differentiation of types of viruses causing epizootics of foot-and-mouth disease.

Great interest from the theoretical as well as the practical standpoint is offered in the study of the geographical distribution of virus types.

Waldmann and Trautwein consider that it would be possible thus to investigate the movement of epizootics and gather material which would make it possible to judge the possibility of occurrence and disappearance of virus types in various territories. In this connection the International Epizootic Bureau introduced in Paris in 1929 a special resolution which obliged countries entering into the membership of the bureau to study foot-and-mouth-disease viruses with the purpose of revealing their type characteristics. On the basis of material gathered it was proposed to publish a general map of the spread of virus types in all countries affected by this disease. (Unfortunately this extremely interesting step was never realized.)

According to the International Epizootic Bureau's data for 1930, the plurality of viruses was established in the following countries: Germany (Waldmann), Denmark (Jensen), Italy (Bizanti), Sweden (Magnusson), Switzerland (Burgi), and Austria (Casper).

On the basis of study by Waldmann and Trautwein of 76 strains received by them from various countries, it is possible to form some conception of the geographical distribution of types for individual countries. The 172 strains of foot-and-mouth-disease virus differentiated by them are distributed among countries in the

following manner: USSR -- 4 strains of type O; Germany -- 91 strains of type O, 9 strains of type A, and 9 strains of type C; Italy -- 10 strains of type O and 5 strains of type C; England -- 2 strains of type O; France -- 10 strains of type O and 1 strain of type A; Switzerland -- 8 strains of type O and 3 strains of type C; Yugoslavia -- 1 strain of type O and 1 strain of type A; Rumania -- 3 strains of type O; Hungary -- 2 strains of type O; Sweden -- 2 strains of type O and 1 strain of type C; Denmark -- 2 strains of type O; Holland -- 2 strains of type O; and Argentina -- 5 strains of type O and 3 strains of type C. From this data it is obvious that the predominant type in all countries is type O (82 percent), while type A has been discovered in only 3 countries in all (6 percent), and type C in 5 countries (12 percent).

In the USSR the question of the plurality of types of the foot-and-mouth-disease virus and its geographical distribution on the territory of the country is being given elaborate study. The first attempts to study it date from 1927, when Revo, at the time of the foot-and-mouth-disease epizootic in the Ukraine, first differentiated two immunologically different types of the virus. With the help of the method of cross-immunization in guinea pigs, 9 strains were classified as type O, and the tenth strain proved to resemble the German type (OF). Incidentally, Savel'yev observed in 1928, on one of the leading hog-fattening farms, observed a thrice-repeated sickening with foot-and-mouth disease of the same hogs in winter, summer, and late fall) over the course of one year. At that time the author did not yet connect this phenomenon with the plurality of types of the virus.

Questions of the plurality of types of the virus have also been studied in the foot-and-mouth-disease institute on the island of Gorodomlya in Lake Seliger. Beginning in 1927 16 strains in all were studied, received at different times from the northern Caucasus, the Middle and Lower Volga, the Ukraine, Siberia, and other districts of the Union. All of them were classified, on the basis of transverse immunization in guinea pigs, as type O, and in only two instances were variants of type O established.

At that time this served as a basis for the conclusion that for the time being there were no scientific proofs that several types of virus were distributed on the territory of the Union, also "it may be assumed that foot-and-mouth-disease epizootics are caused not only by the one type O (as we have indicated), but also by other types and variants".

In 1940 Ratner and Sirotkina announced that in comparative study of two strains of viruses, originating in two localities, they had differentiated them as two immunologically different types. They studied type differences of these strains in guinea pigs by means of the method of cross-immunization, and also by means of study of the protective properties of serums of animals which had developed the disease. The data obtained in the experiment were also confirmed in experiment on animals naturally susceptible to foot-and-mouth disease -- cattle and hogs. Ratner and Sirotkina consider that it is proved beyond question that the two strains of virus studied by them turned out to be immunologically different. Unfortunately, neither strain was differentiated by them with respect to type classification, and the biological properties of these viruses in

the process of propagation in animals susceptible to foot-and-mouth disease were not given further study.

Large-scale and interesting work in the study of the plurality of the virus has been carried on by Kindyakov in the territory of Central Asia. In the period from 1937 to 1942 he studied with reference to type classification 44 strains, (in differentiation of viruses Kindyakov used type viruses received from France) of which 22 strains were differentiated.

The type classification of viruses collected by Kindyakov was determined by the method of cross-immunization in guinea pigs, for which he used 3000 experimental animals. Immunized groups of guinea pigs (6 to 8 apiece) were first infected with "passage" (test) virus, and then, after full recovery, they were newly infected on the 21st to the 30th day with French viruses of type O, A, and C. Clinical observation was carried on for 8 days in this instance, after which the results were taken into consideration on the basis of clinical data.

In cases where the "passage" virus caused a condition of complete immunity in guinea pigs (absence of primary and secondary aphthae) to one of the typed (French) viruses, Kindyakov classified it as a "pure" type. If the virus being investigated caused only partial immunity (presence of primary and absence of secondary aphthae on any part of the experimental guinea pig), he then classified it as a corresponding variant of a certain type.

As a result of Kindyakov's study of 22 strains, obtained at various times of the year in the course of 6 years, he differentiated

type O virus and its variants. In addition, he also repeatedly differentiated a variant of type A. The data obtained permitted Kindyakov to draw the conclusion that in the territory of Central Asia he had established "immunologically different types of the foot-and-mouth-disease virus" (type O and its variants and a variant of type A). Consequently, on the basis of experimental data of Soviet investigators (Ratner and Sirotkina, Kindyakov), and also of epizootological observations (Savel'yev and others) it may be considered proved that in the territory of our Union there may appear at the time of epizootics, together with the constantly encountered type O, other, less broadly distributed types of the virus and, in particular, variants of types O and A.

Vallee and Carre used the method of cross-immunization exclusively for differentiation of types of viruses, cattle serving as their experimental animals. They consider that cattle are the best subjects for differentiation of viruses, although use of them for this purpose involves great difficulties as compared with guinea pigs. They recommend carrying out differentiation of virus in animals which have recovered from primary infection, and not in animals which have been treated experimentally for this purpose. In testing immunity to one of the types of virus a whole group of guinea pigs, first inoculated with serum of the test animal, is taken for experiment.

The method of differentiating types of viruses by means of cross-immunization of guinea pigs was first worked out by Waldmann and Meyer (1924) and then perfected by Waldmann and Trautwein (1926). Somewhat later it was checked and confirmed by Stockmann

and Minett (1927), and also by the American commission on study of foot-and-mouth disease, and by many other investigators.

In studying the plurality of types of viruses Trautwein used the method of cross-immunization. He first accomplished cross-immunization with guinea pigs, and then with cattle and hogs. Waldmann and Trautwein consider the guinea pig the most useful experimental animal for study of foot-and-mouth disease and particularly for differentiation of types of viruses. The guinea pig does not become infected with foot-and-mouth disease spontaneously, as is known, but this infection succeeds rather easily under experimental conditions.

For differentiation of each strain Trautwein recommends using no less than six groups of 6 guinea pigs apiece, thus 36 guinea pigs in all. All viruses obtained in the laboratory from cattle which have developed foot-and-mouth disease spontaneously, must be passed in preliminary in guinea pigs for no less than 6 to 10 times. This accomplishes "acclimatization" of the virus to the organism of the guinea pig, and consequently more constant results will be obtained upon infection. For differentiation of viruses guinea pigs immunized with respect to O, A, and C types must be available. To this end they are prepared beforehand by means of infection with typed viruses, and after recovery, i.e. in 20 to 30 days, they are used for experiment. In each group there must be no less than 6 guinea pigs, or in other words 2 guinea pigs for each test. Guinea pigs immune to typed viruses are infected in this case with unknown virus which has been propagated beforehand in guinea pigs. Differentiation of types may also be carried out in guinea

pigs by means of checking the presence of immunity in these animals with the typed viruses, but for this guinea pigs which have developed foot-and-mouth disease of an unknown type must be available. The experimental guinea pigs must be observed in all cases for no less than 8 to 10 days, and the results of observation are taken into consideration daily and preserved in the appropriate records.

In studying immune serums from guinea pigs which had contracted disease Trautwein established a strict specificity of immune bodies in the blood of these animals. It developed that the serum of guinea pigs treated with virus of one type possesses immunizing properties only against that same type of virus.

Consequently, in the serum of guinea pigs infected with type O virus only homologous immune bodies can be proved, i.e. of type O. Such serum proves no longer effective against the other two types A and C. On the basis of the type specificity of serum of guinea pigs which have developed foot-and-mouth disease, which type specificity is well defined for them, it is possible to accomplish differentiation of virus with the help of tests of the monovalent action of serums.

However, this strict monovalence of immune serum has been established only for the guinea pig, while in other animals it has considerable deviations. In Trautwein's experiments heterogenous antibodies were observed in the immune serum of other animals: for cattle in 52 percent of cases, for pigs in 36 percent. Similar phenomena were established by the author with respect to the serum of reconvalescents for these types of animals.

Burbury carried out differentiation of types of viruses on guinea pigs which had first been vaccinated with formol-vaccine. Test of immunity was carried out in 8 days, by means of infection with the appropriate typed serum. For each test attempt the author recommends taking 8 guinea pigs. Virus from cattle must be passed in preliminary in guinea pigs no less than 8 to 13 times. Only after this is it possible to begin differentiation of it. Using this method, Burbury succeeded in proving, on one hand, the type specificity of viruses and, on the other hand, the possibility of using it for differentiation of viruses.

Sakvarelidze, Solov'yev, Siuka, Helm, and also Galea prepared specific type antigens for the reaction of complement-fixation. With the help of the complement-fixation reaction and the reaction of conglutination they succeeded in determining the type classification of viruses. Other investigators (Waldmann and Trautwein, Olitsky, Traum and Schoening, Ernst, Ascoli, Meisinoesn, Boram, and others) adopt a critical attitude toward the complement-fixation reaction as a method of differentiating types of foot-and-mouth disease virus.

Zinck, using the complement-fixation-reaction method for differentiation of virus types, obtained negative results.

Solov'yev applied the reaction of conglutination for differentiation of virus types, using for this purpose specific type antigens prepared according to Sakvarelidze's method. According to the author's data, obtained on the basis of rather extensive material (1329 serums of various animals), the reaction of conglutination gave in all only 0.3 -- 1.2 percent doubtful showings,

while the same author obtained 4.8 percent in FCK.

Use of FCK for differentiation of virus types can have some meaning only as a supplementary method of laboratory investigation in the study of the biological peculiarities of virus.

The development of disease in animals, as is known, involves the formation in the organism of the latter of antibodies not only with properties of a strictly determinate type of virus which causes the sickness of the animal (homologous antibodies), but also the formation of heterogeneous antibodies. In this instance serological reactions (complement-fixation reaction, complement reaction, PK, and others) cannot give precise indications of the presence in the serums of animals which have developed foot-and-mouth disease of antibodies with strict type properties. This detracts from the significance of serological reactions as methods of differentiation of types of viruses.

Consequently, of all methods of differentiation of virus types proposed by various investigators, the only reliable one is the method of cross-immunization in animals susceptible to foot-and-mouth disease -- guinea pigs, cattle, and swine.

The question of the origin of types and variants of the virus remains as yet not conclusively solved. Most acceptable is the point of view of those investigators who assume the formation of new types and variants as a result of dissociation (mutability, as is known, is observed not only for ultraviruses, but also for bacteria. It has been proved that there is a "possibility of the formation of variants leading to the appearance of permanent and

hereditable types and forms...Bacteria change form rather easily due to environmental conditions and can transmit these changes to their offspring. Griffith has shown the possibility of the transformation of one type (lancet-shaped streptococcus-pneumococcus) into another in the test tube as well as in a live organism". Gamaleya. "Infection and Immunity", Medgiz, 1959.) of the virus in the process of development of the foot-and-mouth-disease epizootic.

It may be considered proved that there is a possibility of transformation of one type into another in consequence of the mutability of viruses which is so broadly distributed in nature among the world of pathogenic microbes.

It has also been established conclusively that the dominant basic type of virus in all countries is type O. It possesses the most manifest constant biological type properties. It seems to us that the point of view of those investigators who consider viruses of types A and C as "offshoots" of type O is the most correct. Questions of the study of the plurality of the virus continue to remain among the pressing questions of the solution of the foot-and-mouth-disease problem.

PRESERVATION OF THE VIRUS

Chemically pure neutral glycerine, taken equally with a phosphate buffer solution of pH 7.5 -- 7.6 is used as a preservative medium for the foot-and-mouth-disease virus. Under laboratory conditions the virus is kept best of all in a glycerine preservative medium which has a stable concentration of hydrogen ions, close to neutral pH 6.8 -- 7.6.

Observance of this condition is attained by application of a special buffer solution of sodium and potassium phosphate salts. (The basic property of buffer (regulator) solutions, i.e. of mixtures of weak acids and their salts, consists of the fact that their pH is almost entirely unchanged by the state of general concentration of solutions and by the appearance in them of foreign acids and bases.)

This has also been confirmed further by the English Commission on Foot-and-mouth Disease (Minett) and the American Commission on Foot-and-mouth Disease (Olitsky), and also by many other investigators.

Pyl and Klenk established that there is no difference in the length of survival of the virus in a preservative medium from pH 7.55 to 7.7. According to the data of these investigators, with a small increase in acidity the length of survival of the virus is sharply decreased. With a small increase in alkalinity this does not take place. Vallee, Carre, and Rinjard affirm that with alteration of the pH of the preservative medium consisting of glycerine and phosphate-buffer solution, even at low temperatures (--8--10 degrees C), a weakening of the virulence of the preservative medium takes place.

In Lipatov's and Alekseyeva's experiments the virus in glycerine taken in equal parts with M/15 buffer solution of phosphate at pH 7.6 and at room temperature from 15--20 degrees C, remained active for 1 to 3 months; at pH 6.8 in the same medium and under the same conditions it remained active for 5--6 months, and in a refrigerator at temperature --1--3 degrees even up to 6 months.

With storage of the virus in glycerine with M/15 phosphate-buffer solution at pH 6.8, and also at pH 7.6 in a refrigerated compartment, the activity and length of survival of the virus was uniform in both cases. With lowering of the temperature to 3--1 degree C and storage of the virus in a preservative glycerine medium at pH 7.8, the length of its survival increases considerably.

On the basis of their experiments the authors consider the best preservative medium for foot-and-mouth-disease virus to be glycerine taken in equal parts with M/15 phosphate-buffer solution. Volkova obtained best results with a preservative medium of chemically pure neutral glycerine with phosphate-buffer solution at pH 7.6--6.8. She succeeded in preserving the virus in this medium in a refrigerator for more than 7 months (until the end of the experiment). Under laboratory conditions we succeeded in keeping the virus active for a year and more in this medium with pH 7.6--6.8. A preservative glycerine medium can be prepared likewise without the phosphate-buffer solution. In that case instead of the buffer solution a simple physiological solution of sodium chloride at the same pH 7.6--7.6 is used. However, this medium is less stable with respect to pH, and, consequently, a change in the pH of the medium is possible, which will be reflected in the length of survival of the virus in the preservative medium.

For preservation of the virus in the glycerine medium ordinary glass test-tubes of the bacteriological type, prepared to special order with ground stoppers, are very advantageous. No more virus (virus-epithelium) must be put in each such test-tube, filled with preservative fluid, than that from two to four extremities of

guinea pigs, or 0.2 to 0.5 grams of virus in the form of the epithelium of aphthae of cattle or swine. (For storage in preservative media the virus is taken in the form of small pieces of epithelium which are well developed but which have not ruptured.) It is recommended that the virus be kept in a refrigerator at temperature ranging from 0 to 2 degrees. In preparation of the preservative medium it is absolutely necessary that the quality of the glycerine be checked; it must be free of all foreign matter and of neutral reaction.

Bedson and Maitland explain the high resistance of the virus to glycerine by the fact that the covering of the cell itself in which the virus is situated, due to the glycerine, becomes impenetrable and, consequently, is protected from the destructive influence of the medium.

River explains this in another manner: viruses are very sensitive to autolysis taking place in tissues; glycerine acts as a desiccator and thus prevents autolysis of the tissue. This explanation seems more correct to us. From this consideration it follows that all preparation and the very process of placing the virus in the preservative medium must be done with maximum observance of cleanliness, in order to avoid soiling of the medium with foreign microflora, which can lead to germination in the medium of other microbes, the collapse of the tissue itself, and an alteration in the pH of the medium.

PASSAGE OF THE VIRUS IN GUINEA PIGS

Under laboratory conditions the foot-and-mouth disease virus is usually maintained in guinea pigs. Viruses obtained from other

types of animals are likewise transferred to guinea pigs. In adapting the virus to guinea pigs, the type of animal from which material has been taken during the natural development of the epizootic, and also the type classification of the virus, are evidently of no essential significance. Such a conclusion was reached by Kindyakov on the basis of his investigations, as a result of study of viruses distributed in the territory of Central Asia.

It is inherently obvious that not all viruses from animals naturally susceptible to feet-and-mouth disease can be uniformly pathogenic for guinea pigs, insofar as virulence is not a strictly permanent and immutable property of viruses, but on the contrary is subject to considerable variation. This obviously explains the loss of virulent properties of individual viruses in the process of passage in guinea pigs. The technique of infection of guinea pigs consists in the following: The virus-epithelium is first of all ground in a porcelain mortar with a small quantity of sand or ground glass, is diluted with a physiological solution of sodium chloride, and after this is transferred to the skin, scarified in preliminary, of the plantar surface (sole) of the rear extremity of a guinea pig (Figure 4). Instead of scarification the "tunneling" method can be used. A tunnel is made with the help of a syringe needle. (Figure 4. The foot-and-mouth-disease process in the guinea pig on the plantar surface of the rear extremity (after Waldmann and Trautwein.) Instead of this it is possible to prick with the same needle the surface of the planta of the extremity with simultaneous transference to this place of an injection of the virus.

It is not always possible after the first passage to transfer to the guinea pig virus from cattle spontaneously infected with foot-and-mouth disease.

Very often such a "field" virus "becomes accustomed" gradually and lengthily to the organism of the guinea pig. Rinjard recommends in such cases the use of guinea pigs which have already been sensitized. For this purpose he injects 0.1 cubic-centimeters of normal cattle serum intraperitoneally for 2 weeks before infection.

For primary passages in guinea pigs it is recommended that for each test of the virus no less than six guinea pigs be used, since often no more than 20-30 percent develop the disease (Trautwein).

It is very seldom that in primary passages a well-defined development of the infection on the site of injection of the virus and a subjequent generalization are obtained at once with guinea pigs.

Passage of virus from other types of animals which have developed foot-and-mouth disease as the result of natural infection must be carried out no less than 8 to 10 times, until development of primary aphthae in the course of 18--24 hours and generalization of the infection in the following 24-48 hours are successfully achieved. In cases where virus from cattle cannot be transferred successfully to the guinea pig, it must be used to infect swine and then taken from the swine for infection of guinea pigs. In such a case virus from cattle can almost always be transferred successfully to guinea pigs with the aid of alternate passage. Observation of inoculated guinea pigs must be carried out for 8 to 10 days with daily registration of the condition of the animal and corresponding recording with respect to the clinical aspect of the disease.

CHAPTER III

EXPERIMENTAL STUDY OF THE INFECTION

THE SUSCEPTIBILITY OF ANIMALS TO FOOT-AND-MOUTH DISEASE

Foot-and-mouth disease attacks all types of cloven-footed animals. Under conditions of natural infection cattle, swine, sheep, and goats are most susceptible to foot-and-mouth disease, and buffalo, camel, and reindeer are somewhat less susceptible. Among other domestic animals, foot-and-mouth disease can infect dogs and cats. With respect to the susceptibility to foot-and-mouth disease of horses, the opinions of investigators, and also of practical workers, are divergent. Among animals kept in captivity (zoological parks, gardens, etc.), giraffes, llamas, antelopes, bison, zebu, yaks, chamois, roe-bucks, hinds, red deer, and elephants are susceptible to foot-and-mouth disease.

Wild cloven-footed animals in natural conditions evidently develop foot-and-mouth disease very rarely, and have hardly any degree of epizootological importance (Edelmann). An exception to this general rule, as we shall see further on, is the antelope. During foot-and-mouth-disease epizootics in Mongolia repeated cases of natural infection of antelopes ("dzeren") with foot-and-mouth disease have been observed. Cases of foot-and-mouth disease among fur-bearing beasts not in captivity are very seldom encountered, according to Stroh's data, and other investigators affirm that in general they do not develop foot-and-mouth disease, even when they are in contaminated localities. In the opinion of certain

investigators, fur-bearing animals have no significance in the epizootology of foot-and-mouth disease (Olt and Strose).

Fowl are considered unsusceptible to foot-and-mouth disease and are not affected by it in natural conditions, but it has been possible using artificial methods in the laboratory to infect, for example, domestic ducks.

Among rodents, successful artificial infections with foot-and-mouth disease have been accomplished with marmots (Popova, Klobouk), wild rats (Beatty and Pedden), hamsters (Galloway), and field mice (Busch, the English commission). The mole proved to be unsusceptible to artificial infection with foot-and-mouth disease (Frenkel). Edwards succeeded with artificial infection of bats. Among small experimental laboratory animals, the guinea pig, the rabbit, the white mouse, and the hedgehog are susceptible to foot-and-mouth disease. Man is unconditionally susceptible to foot-and-mouth disease.

The degree of susceptibility to foot-and-mouth disease of various types of cloven-footed animals is non-uniform. On the one hand, not all animals of the same type, susceptible to foot-and-mouth disease, are equally sensitive to the disease in the same epizootic situation. Individual resistance, connected with the biological peculiarities of the organism (constitution, sex, age, degree of feeding), also has significance. Highly bred pedigreed stock are considered more sensitive to foot-and-mouth disease than local indigenous stock.

As a general rule, young agricultural animals are more sensitive to foot-and-mouth disease than animals of more advanced age. Such a statement is true at least with respect to younger cattle up to 4 or 6 months in age (milk calves) and suckling pigs up to 2 months in age. During an outbreak of foot-and-mouth-disease epizootic in one of the leading swine-raising farms, Orlov observed a mortality of suckling pigs which in individual farrows reached 100 percent, while among grown swine the disease ran its course easily, without losses.

Similar observations with respect to the high sensitivity of suckling pigs to foot-and-mouth disease have been observed repeatedly in many foreign countries (Waldmann and Trautwein). Guinea pigs exhibit a reverse relationship with respect to sensitivity to foot-and-mouth disease, as established by Waldmann and Pape: young guinea pigs weighing no more than 200 to 250 grams are little sensitive to foot-and-mouth-disease, and older ones weighing 300 grams and more, on the contrary, are very sensitive to this infection. Later, Edwards reached the conclusion on the basis of his investigations that young guinea pigs and rats artificially infected with the virus either do not become sick at all or become sick with a manifestation of only primary aphthae on the site of injection of the virus. Adult and old animals, on the other hand, were more susceptible to the infection than young animals during the period of growth. Gins and Weber, in their day, also pointed to the fact that guinea pigs of young age are distinguished by a rather high resistance to the virus.

The influence of age on an animal's resistance to foot-and-mouth disease can change under various conditions in the other direction. In localities long free of foot-and-mouth disease, where stock have not come into contact with the infection during their individual lives, animals of relatively young age become sick rather easily, while adult and old stock are uniform in that they become sick with difficulty. Thus, the view has been generally accepted that animals in the first period of the adult state (working age) are, under the same conditions, more resistant to foot-and-mouth disease than animals of advanced age.

In Pirbright (England), and also in other experimental stations, the observation has been made that large and well-fed animals of mature age become sick with considerably more severity than small and poorly fed stock. This observation has been made by many investigators with respect also to guinea pigs and hogs. In his experiments with rats Edwards established that "grown and well-fed animals are most easily infected and become sick with greatest severity, while worn-out, poorly fed animals very often either do not become sick at all or the infection passes through them in latent form". He obtained full confirmation of this phenomenon also with cattle and small laboratory animals.

It is possible, in Edwards' opinion, that the variable results of aphthization (artificial infection) applied in various countries (France, Southern Rhodesia, and others) depend not on the "quality" (virulence) of a strain of the virus, but above all on the degree of nutrition of animals and on certain other vital factors. Edwards also feels that the quality and character of feeding are

extremely important factors, on which depend the seasonal variation of the occurrence of foot-and-mouth disease in India. Numerous observations from our Soviet practice also indicate that well-fed animals always become more severely diseased with foot-and-mouth disease than those which are run-down. Various complications of the basic foot-and-mouth disease are most often noted in well-fed animals, and considerably less frequently in run-down animals (Skomorokhov, Savel'yev, and others). Waldmann and Trautwein affirm that the percent mortality due to foot-and-mouth disease in highly bred and fattened swine is always very considerable. Pierot connects the more severe sickness due to foot-and-mouth disease in well-fed and high-producing cows with the intensive rate of nitrogen exchange.

With respect to the influence of the sex of animals on susceptibility to foot-and-mouth disease, there are almost no indications in literature on the subject. There is acceptance, however, of the view that males are more sensitive to foot-and-mouth disease than females, although no one adduces any experimental proofs of this.

In literature on the subject several cases have been noted where sickness appeared only among one type of animal (cattle, hogs) and did not spread to other types of animals susceptible to foot-and-mouth disease. This is not connected with the increased or decreased resistance of the animals to foot-and-mouth disease.

In this case, an "accustoming" of the virus to a definite type of animal is evidently manifested (English commission). In Pirbright, after repeated passage of certain strains of virus in swine it was possible, with great difficulty, to infect cattle

with these viruses. In another instance it was entirely impossible to infect swine with a virus from cattle which had been obtained from Southern Rhodesia.

Of all the types of cloven-footed domestic animals, cattle are the most sensitive to foot-and-mouth disease. This is confirmed by numerous practical observations, and is also adequately founded on experimental data (study of pathogenesis, clinical manifestations, immunity, and others). In all epizootics of foot-and-mouth disease, as a rule, the greatest number of stock affected are cattle, with very few exceptions in cases where only a few hogs become diseased. In practice it must be considered that almost all cattle which do not have acquired immunity to foot-and-mouth disease as a result of infection sustained during their individual lives are exposed to infection independently of breed, age, and sex.

Individual cases where a certain animal remains clinically healthy during an epizootic must be ascribed, in our opinion, to "suppressed forms", or in other words to invisible infection, and not to inherent or natural immunity, as has been considered up to now. (Hutyra and Marek. "Special Pathology and Therapy of Domestic Animals". 1939, Volume I, page 413.)

Swine, like cattle, are distinguished by extremely high susceptibility to foot-and-mouth disease. With the appearance on the farmstead of foot-and-mouth disease in cattle, and with the presence on the same farmstead of swine, the latter also become diseased. It is very difficult, even with the strictest isolation, to protect them from foot-and-mouth disease. Hartenschtein asserts that Mangalic swine are considerably more resistant to foot-and-mouth

disease than Galician and German swine. Primitive breeds of swine are evidently characterized by a higher resistance to foot-and-mouth disease than highly bred and improved breeds.

From the practical standpoint, there is great interest in instances of an epizootic of foot-and-mouth disease among swine, when it did not spread to other forms of animals susceptible to foot-and-mouth disease, and even in an instance when all these animals were kept in a single building together with sick hogs. Waldmann and Trautwein, as indicated before, described two such epizootics of foot-and-mouth disease among swine (Schleswig-Holstein in 1927 and Hanover in 1928). Similar cases of sickness of one type of animal were noted even before, but no serious importance was attached to this. In Germany epizootics of foot-and-mouth disease which attacked only hogs were earlier encountered repeatedly. In 1931 a similar outbreak among swine was noted at the end of the epizootic, when the curve of occurrence among cattle dipped sharply.

Sheep and goats are characterized by high susceptibility to foot-and-mouth disease, although somewhat less than that of cattle and swine. Among sheep and goats there are known to have been also cases of malignant epizootics, accompanied by great mortality. In Germany -- in Hanover, Saxony, Bavaria, and Wurtemburg -- during the epizootic of foot-and-mouth disease in 1920 -- 1921 the mortality of sheep and goats caused by malignant foot-and-mouth disease attained colossal proportions in individual provinces. Not infrequently a few heads in all have remained in large herds after sickness among sheep (Francke and Gaertler). During the course of a mild epizootic of foot-and-mouth disease the mortality of sheep and goats is usually no greater than 0.3 -- 017 percent.

With respect to the susceptibility to foot-and-mouth disease of buffalo a few indications may be found in literature on the subject; however, the degree of susceptibility of these animals remains the subject of little investigation. In the reports of the Veterinary Administration of the Russian Ministry of Internal Affairs for 1901 and 1902 there are references to numerous cases of foot-and-mouth disease sickness for buffalo as well as other animals.

Foot-and-mouth-disease in camels, judging by the reports of the above-noted Veterinary Administration, began to be recorded as early as 1896. Individual cases of foot-and-mouth-disease sickness in camels were noted in almost every epizootic. Kovalevskiy (1913) was one of the first to make an attempt to describe the clinical aspect of foot-and-mouth disease in camels, but he did this without any reference to the general resistance of these animals to foot-and-mouth-disease infection. The degree of resistance of camels to foot-and-mouth disease under conditions of the natural development of an epizootic remained uninvestigated.

Krasovskiy examined this matter experimentally during one of our anti-foot-and-mouth-disease expeditions in 1928. Camels, under conditions of natural infection, did not develop foot-and-mouth disease. Infection of experimental camels was successful only as the result of artificial infection with intravenous injection of large quantities of virus (1--1.5 cubic-centimeters of highly virulent aphthous "lymph"). A case of spontaneous foot-and-mouth-disease sickness in camels has been described also, by the veterinarian Kobets (1936). The practical observations and experimental data of Krasovskiy with respect to the susceptibility of camels to foot-and-

mouth disease provides a foundation for drawing the conclusion that under conditions of natural infection they are rather resistant to foot-and-mouth disease and become infected rather infrequently.

The susceptibility to foot-and-mouth disease of reindeer has long been known. Epizootics of foot-and-mouth disease among reindeer have repeatedly been described in literature on the subject (Ekkert, Hering, Spinola, Esser, Barroni, Shuts, and others). Domestic as well as wild reindeer are susceptible in equal degree to foot-and-mouth disease (Ekkert). In the former Arkhangel' gubernia 246,444 head were attacked by foot-and-mouth disease in 1896, of which 32,085 were killed and 118,717 died (61.3 percent).

In a National Park in California (US) in 1925 22,214 head of reindeer were killed in connection with the appearance of foot-and-mouth disease. (At the moment of compulsory slaughter of reindeer, the number of those which were sick was 10 percent of the whole.) Spontaneous foot-and-mouth-disease sickness of reindeer was also observed by Gryuner on the zoological farm of the Omsk Veterinary Institute (1928).

The first attempts at experimental study of foot-and-mouth disease in reindeer in Russia were undertaken by Ekkert and Bevel' (1900). They reached the conclusion that: (1) reindeer are susceptible to spontaneous as well as artificial infection; (2) the disease, transferred from cattle to reindeer, can run its course in them nonmalignantly, as in other animals; (3) foot-and-mouth disease is transmitted from sick reindeer to people as well as cattle. Magnusson, on the basis of experimental data which he had

obtained from reindeer, reached the conclusion that they are susceptible to foot-and-mouth disease through natural as well as artificial infection. Incidentally, in contemporary literature on the subject there are indications that the serum of reindeer which have developed foot-and-mouth disease is characterized by a high antibody content.

Horses are evidently not susceptible to natural infection with foot-and-mouth disease, although in existing manuals on epizootology there have been encountered, right up to present, indications that horses in infrequent instances develop foot-and-mouth disease (Vysheleeskiy, Hutyra and Marek, Frener and Zwick, Franeke and Gaertler, and others).

Large-scale experiments in the study of the susceptibility of horses were carried out by the American commission on foot-and-mouth disease (Olitsky, Traum, and Schoening). They did not succeed in a single case in infecting horses with foot-and-mouth disease. One horse was exposed to hyperimmunization, attempts to prove the presence in serum of antibodies also being unsuccessful. (Ramon established as the result of experimental investigations that horses can be used as producers for obtaining anti-foot-and-mouth-disease serum. In Ramon's experiments horse-serum was just as effective as hyperimmune serum of cattle (C. R. Acad. S., Paris, 1942). The authors reached the conclusion that in places foot-and-mouth disease is evidently confused with contagious vesicular stomatitis of horses, inasmuch as the clinical aspects of these sicknesses are in close resemblance to each other. Ovsyannikov did not succeed in his experiments with the infection of horses with foot-and-mouth

disease through injection of considerable quantities of the virus.

The susceptibility of dogs to foot-and-mouth disease was proved in Hecker's experiments of some time ago. Spontaneous infection of dogs was observed by Loeffler, Fersch, Uhlenhuth, and also by Albrecht. At a later period the susceptibility of dogs to foot-and-mouth disease was also studied by Galloway, who likewise confirmed the susceptibility of dogs to foot-and-mouth disease.

The susceptibility of cats to foot-and-mouth disease has been studied by many investigators. It was confirmed by Hecker's experiments of some time ago, and also those of Esser and Lucas, at a later period by Minett and also by Galloway (English Commission) and Howe. Adult cats are more resistant to foot-and-mouth disease, kittens dying upon infection with the disease (Minett). Howe succeeded in the infection of an experimental cat under conditions of naturalcontact. Cats have proved uniformly susceptible to all three types of foot-and-mouth-disease virus (Galloway). Spontaneous infection of cats with foot-and-mouth disease is fully possible through feeding them infected milk (Esser, Ester, Wormbotter, Mette, and Hauptmann).

Edwards observed a case of spontaneous infection of a hedgehog. He succeeded in turning up the hedgehog on a farm not far from Pirbright, where there was foot-and-mouth disease. It was killed and consigned to the Pirbright experimental station for investigation. There the presence of foot-and-mouth-disease virus was proved in the skin of affected extremities, and also in the epithelium of the tongue and, finally, in the blood and internal organs. The presence of virus in this hedgehog was also confirmed

in experiments on still other animals susceptible to foot-and-mouth disease. However, we are not inclined to overestimate the importance of the hedgehog as a possible reservoir and carrier of the foot-and-mouth-disease infection. In Dubyankskiy's and Khomenko's experiments with hedgehogs it was not possible to confirm the high sensitivity of hedgehogs to foot-and-mouth disease which was observed by the English investigators. It is possible that there are individual breeds of hedgehogs which actually possess very high sensitivity to foot-and-mouth disease.

Wild and house rats proved susceptible to artificial infection with foot-and-mouth disease, while under natural conditions they evidently become infected very infrequently. At the Pirbright experimental station (1932) and in its environs 162 wild rats were caught, of which 123 were studied by means of infection with material from guinea pigs, and in only two cases were positive results obtained. In another case, of 144 rats caught on farms contaminated with foot-and-mouth disease not a single rat sick with foot-and-mouth disease could be observed. For the time being there is evidently no basis for ascribing a given role to rats in the epizootology of foot-and-mouth disease.

Edwards proved experimentally that bats are susceptible to foot-and-mouth disease. In his experiments he succeeded in transferring the foot-and-mouth-disease virus from one experimental bat to another, by means of intramuscular injection of a small amount of blood containing the virus. Sickness in bats ran its course in mild, nonmalignant form, with the appearance of aphthae on the tongue. Incidentally, Edwards used bats raised in captivity.

The question of the susceptibility to foot-and-mouth disease of wild animals, and the determination of their role in the epizoology of foot-and-mouth disease remains the subject of little study. Elton, for the purpose of observing wild animals, travelled specially to places contaminated with foot-and-mouth disease. On the basis of his observations the author considers that for the time being there are no proofs with respect to any connection between wild animals and outbreaks of the foot-and-mouth-disease epizootic among cattle and swine. There are, however, observations of a reverse order. Antelopes ("dzerony") in Mongolia, during foot-and-mouth-disease epizootics, themselves develop foot-and-mouth disease and, travelling from one place to another, transmit it at times over very great distances (Skomorokhov). Galloway succeeded in proving by means of experimental infection that among fur-bearing animals squirrels are susceptible to foot-and-mouth disease. This, however, does not give the right to talk of any epizoological role of these animals.

Of animals kept in captivity, it is curious to consider foot-and-mouth-disease sickness in the elephant. Ramiah described a case of spontaneous foot-and-mouth-disease sickness of the female elephant during a foot-and-mouth-disease epizootic in cattle in the environs of a zoo. Foot-and-mouth-disease sickness in the elephant was accompanied by a refusal of food, irritation of the tongue and upper jaw, strong salivation, and also by lameness. Aphthae and erosions were noted in the buccal cavity and on the extremities. Recovery came in 6 to 8 weeks. In 15 months this elephant again became infected and sick. This time aphthae and erosions were observed only on the extremities, peeling of the

skin on the soles of the feet taking place during the recovery. Recovery came in 8 weeks. A similar case of foot-and-mouth-disease sickness in the elephant was described much earlier by Lepin (1900).

In contemporary literature on the subject there are extremely conflicting data with respect to susceptibility of fowl to foot-and-mouth disease. Nevertheless, the majority of investigators consider that fowl, at least under spontaneous conditions, are non-susceptible to foot-and-mouth disease. In old reports it is possible to find indications which verify the susceptibility of fowl to foot-and-mouth disease (Spinola, Behla, Kitt and Hobmaier, and others). According to Spinola's data, he observed a spontaneous infection of hens and geese. Beatty, Sacco, and Pedden succeeded under experimental conditions in infecting sparrows, in whom the presence of the virus was improved in the blood and the cardiac muscle. Hoelsbergen indicates that in fowl sicknesses are at times encountered which resemble foot-and-mouth disease. Becker, incidentally, also speaks of this. In manuals on epizoology the authors usually circumvent this question with silence (Hutyra and Marek, Nocard and Leclanche, and others). Others consider fowl susceptible to foot-and-mouth disease, at the same time pointing out that sickness among them is encountered very seldom (Frener and Zwick, Vyshelesskiy and others).

In studying the susceptibility of fowl to foot-and-mouth disease under experimental conditions Loeffler, Frosch, and Uhlenhuth, and at a later date Waldmann, Wagener, Fink, Borisov, and finally the English Commission, obtained negative results.

All data of an experimental order, and also practical observations, make it possible at the present time to express the firm conviction that fowl, at least in natural conditions of infection, do not develop foot-and-mouth disease. But wild birds (starlings, crows, and others), as will be seen later, can be carriers of the infection. From this standpoint they can play a known role in the epizootology of foot-and-mouth disease.

Of experimental laboratory animals used for work with the foot-and-mouth-disease virus the guinea pig is of greatest interest. Hecker (1890) first transferred the foot-and-mouth-disease virus to the guinea pig, but at the time this outstanding experiment was not confirmed by other investigators and remained unnoticed. Waldmann and Pape, in 1920-21, worked out a technique of artificial infection of the guinea pig with foot-and-mouth disease. Hobmaier, independently of Waldmann and Pape, also succeeded in proving experimentally the susceptibility of guinea pigs to foot-and-mouth disease. The course of foot-and-mouth disease in guinea pigs is similar to foot-and-mouth disease in cattle, with the appearance of primary aphthae on the site of injection of the virus and a subsequent generalization of the infection and, in consequence of this, the appearance of secondary aphthae on the remaining, uninfected extremities and in the oral cavity.

Minett, Chybbs, and also Edwards, infected hedgehogs, which proved to be animals very sensitive to foot-and-mouth disease, under experimental conditions.

During the anti-foot-and-mouth-disease expedition in 1928 we attempted to use marmots as an experimental animal. They are susceptible to foot-and-mouth disease, as shown by the investigations of our woman coworker Popova. However, due to the great difficulties of working with marmots we gave them up as experimental animals.

THE SUSCEPTIBILITY OF MAN TO FOOT-AND-MOUTH DISEASE

The susceptibility of man to foot-and-mouth disease has been proved by numerous practical observations of veterinarians and physicians. It has also been repeatedly verified and confirmed with the aid of experimental methods of investigation.

Hatle, and also Gay, as early as 1681 described two cases of foot-and-mouth-disease sickness in man: one case with aphthous irritation in the buccal cavity, and the other with the appearance of high temperature and the formation of foot-and-mouth-disease aphthae on the fingers. Dewee described in 1682 a case of foot-and-mouth-disease sickness of severe form in a family of two children as the result of using raw milk from a sick goat. Presently the mother of these two children also became ill, likewise after using raw milk. Foot-and-mouth disease in the children passed with symptoms of high temperature, aphthous irritations in the buccal cavity, and diarrhea. For one child the sickness ended in death. In later reports it is also possible to find confirmation that man is susceptible to foot-and-mouth disease and that children are particularly sensitive to it.

Hertwig, Mann, and Villain, for the purpose of determining man's susceptibility to foot-and-mouth disease, drank the milk of sick cows themselves; all became sick with a typical form of foot-and-mouth disease, with symptoms of high temperature and aphthous irritations in the buccal cavity and on the hands. Villain observed a case of foot-and-mouth-disease infection of a nursing infant with milk of a sick cow. This infant infected his mother, on whose nipples aphthae appeared.

Poppe was infected during experimentation, having accidentally injured his hand. His case of foot-and-mouth disease was accompanied by high temperature and aphthous irritations on the hands and legs. A similar instance occurred in the Riems Institute in Germany. During artificial infection of a bull, a herdsman was wounded by the inoculatory scarificator. Following this typical foot-and-mouth-disease aphthae appeared on him on the fifth day at the site of the wound.

Infection of human beings takes place most often either in caring for sick animals or in consumption of raw milk. Rosenberg observed repeated instances of sickness of human beings, mainly as a consequence of the use of raw milk. He considers that in 60 percent of the cases human beings are infected with foot-and-mouth disease through consumption of raw milk and only in 34 percent through contact with sick animals. In our own practice we have also repeatedly observed cases of sickness in human beings, particularly often in children, caused by consumption of raw milk from sick cows.

During foot-and-mouth epizootics it may be taken almost as a rule that acute gastroenteritis in children, accompanied by profuse diarrhea, has a direct relation to foot-and-mouth-disease infection. These sicknesses occur most often in consequence of the use of raw milk from farms contaminated with foot-and-mouth disease. Milk in this instance affects the child's system either in consequence of the virus which it contains or in consequence of an abrupt change in its composition as a result of development of foot-and-mouth disease by the animal.

Stomatitis in children in foot-and-mouth-disease sickness is not always observed. In adult human beings, on the other hand, foot-and-mouth disease occurs with aphtnous irritations in the buccal cavity, in the interdigital areas of the hands and in the region of the pedal extremities.

The clinical aspect of foot-and-mouth disease in man does not differ in principle from the clinical aspect in animals. The incubation period in man can last no longer than 3 to 6 days. On the site of introduction of the virus there develops first a primary aphtha, and then, as a result of rupturing of the aphtha, generalization of the infection comes. Following this, the first attacks of fever appear, and a general depressed condition, weakness, weariness, rheumatic pain in the bones, and disorders on the part of the stomach and intestinal tract.

During aphthous irritation of the buccal cavity the mucous membrane is strongly hyperemic;at first sharply defined red spots appear on it, and then secondary or plural aphthae on the site of these spots. At the very beginning of the appearance of these aphthae their contents are transparent, but then quickly become turbid. Stomatitis symptoms at this time appear very seldom. With the passage of time the aphthae rupture, and are replaced by ulcers.

Figure 5. Aphthae and erosions on the skin of the palm and on the fingers of the hand of a human being (after Trautwein).

Figure 6. Erosions on the skin of the palm and the fingers of the hand of a human being (after Trautwein).

Foot-and-mouth-disease aphthae can be observed not only on the membrane of the oral cavity, but also in the esophagus, in the nasal cavity, on the skin of the face, in the area around the mouth, on the sexual organs, and elsewhere. The "favorite" site of localisation of aphthous irritations in man is the mucous membrane of the oral cavity and interdigital regions of the hands and feet (Figures 5 and 6). Krause has described a case of irritation of the buccal cavity of a human being whose tongue, in consequence of swelling, at one time protruded from the mouth. In irritation of the extremities, primary aphthae appear on the site of introduction of the virus, and then there is a generalisation of the infection.

Most frequently aphthae are observed on the sole of the foot, the foot, and the interdigital regions.

An extremely interesting case of an epidemic outbreak of foot-and-mouth disease in human beings was described in 1927 by the physician Shusterov. In one of the sovkhozes of the Omsk Oblast an epizootic of foot-and-mouth disease broke out in cattle and sickness among the workers who looked after the cattle also appeared simultaneously. Fifteen persons in all developed foot-and-mouth disease. In 12 persons the foot-and-mouth disease passed with aphthous irritation on the feet in the region of the soles, in the interdigital regions, and in only 3 persons was aphthous stomatitis noted. In individual cases aphthae appeared on the rear part of the foot and in the area of the lower third of the shank. Foot-and-mouth disease was developed by just those workers who went barefoot and washed their feet in a trough in which oxen were watered. In

the course of this, sensitivity was maintained for one to two weeks.

Consequently, the susceptibility of man to foot-and-mouth disease is conclusively proved. The early liquidation of foot-and-mouth disease is the task not only of veterinary, but also of medical workers.

THE SICK ANIMAL AS THE PRIMARY SOURCE AND RESERVOIR OF FOOT-AND-MOUTH-DISEASE INFECTION

The sick animal is a primary source and at the same time a natural reservoir of the foot-and-mouth-disease virus in nature. The causal organism of foot-and-mouth disease can multiply only in live tissues of the organism of an animal susceptible to it, and thence penetrate an external medium. With the development of the infectious process, the organism of the sick animal becomes a place of the accumulation and activation of the virus. From the epizoological point of view the sick animal is the most dangerous source of dissemination of the virus in the atmosphere surrounding animals. However, the sick animal is not uniformly dangerous at all stages of development of the infectious process from the standpoint of secretion of the virus into the external atmosphere.

In the first days of sickness the virus is secreted from the organism of the sick animal in greatest quantity and in strongly virulent form, but subsequently it is attenuated with the passage of each day, begins to be secreted in ever decreasing quantities, and toward the 13th day its presence in the secretions of the sick

organism cannot longer be successfully proved. Very interesting data in this respect were obtained by Buchlmann. The virus, which he took in the form of epithelium of aphthae, was capable, 24 hours after infection of the animals, of causing infection of healthy animals in dilution of 1:4,000,000, 48 hours after infection it had to be taken in 125 times greater amount, and 72 hours afterwards in 8,000 times greater quantity.

In their day Loeffler, Frosch, and Uhlenhuth established that for infection of animals susceptible to foot-and-mouth disease with virus taken from a sick animal in the first days of sickness extremely small quantities were needed. After the 13th day, from the moment of the sickness' beginning, even in the presence of individual clinical symptoms caused by foot-and-mouth disease, the sick animal became harmless in practice with respect to secretion of virus from the organism.

The virus is given off from the sick animal into the outer medium with all secretions -- saliva, milk, bile, and others, and also excretions -- urine and feces. From the practical standpoint the greatest danger is embodied in the secretions of the oral cavity (saliva), especially in the first days of sickness. Waldmann and Repsin proved experimentally the presence of the virus in the saliva of cattle sick with foot-and-mouth disease for the first time at the ninth hour after infection (at that time no specific changes in the oral cavity could yet be observed), the virus completely disappearing from the saliva in 6 days following infection. With the presence in the saliva of little pieces (scraps) of epithelium of ruptured aphthae, it was possible to prove the presence of the virus in the saliva even after 6 days, but no longer than the eleventh day from

the moment of infection.

Certain investigators (Lebailly, Waldmann and Reppin, and others) affirm that long before the appearance of the first clinical signs of the disease sick animals begin to give off the virus into the external medium, and consequently at this time they already represent a danger for other animals susceptible to foot-and-mouth disease.

The English Commission on Foot-and-Mouth Disease could not confirm this in its experiments. On its part it considers that the greatest danger from the standpoint of dissemination of the virus in the medium surrounding animals is represented by sick animals at the moment of full development and rupturing of aphthae.

At the Pirbright experimental station not a single observation could be made to the effect that, in 4 days from the moment of rupturing of aphthae, sick animals could infect healthy animals susceptible to foot-and-mouth disease, even in the case where they were in one building in direct contact with sick animals.

According to the data of the English commission, the virus in the blood of cattle can be found in considerable quantity only several hours before raising of temperature. (Stallybrass affirms that "with dermatropic ectoderms there is always a stage of infection of the blood, even though it be short".) In the blood of sick animals the virus, as a rule, can be observed during the whole period of development of the aphthae. Beatty discovered the presence of the virus in glands of the neck before the appearance of the vesicular rash.

The presence of the virus in the milk of sick animals has been proved by many investigators (Terni, Lebailly, Ernst, Göbel, Trautwein, Thomaschoff and Höwe, Skomorokhov, and others). Trautwein, Thomaschoff, and Höwe observed the presence of the virus in the milk of sick goats in the period between the 13th and 113th hours after infection, and in the milk of guinea pigs in the period between the 12th and 77th hours after infection. We have succeeded in proving the presence of the virus in cow's milk in the period between the 36th, 48th, and 51st hours after infection.

The English Commission on Foot-and-Mouth Disease, on the basis of its experimental investigations, assumes that the appearance of the virus in milk does not depend on the period of infection of the blood. Contemporary investigators hold, however, that the virus appears in milk only as the result of generalization of the infection (i.e. by a primary means). This does not exclude that in individual cases external contamination of milk with the virus is possible. Consequently, raw milk containing the virus, on being fed in unpurified form to susceptible animals, can be a source of infection. There is particularly great practical significance in this in connection with the fattening of calves and pigs with milk. Cases are known of foot-and-mouth-disease sickness of cats as the result of feeding them milk from sick animals.

The presence of the virus has been proved in the urine of animals which are sick or have recovered from foot-and-mouth disease. According to Vallee's and Carre's data, during the period of sickness of cattle the virus is given off in considerable quantities with the urine. Trautwein, Thomaschoff, and Höwe proved the presence of

the virus in the urine of cattle and guinea pigs in the period between the 15th and 105th hours, and in swine in 40 to 50 hours after infection. The presence of the virus was proved by them also in the feces of cattle and guinea pigs. Skomorokhov and Savel'yev, in their experiments on guinea pigs (there were 80 guinea pigs in the experiment), verified the research of these authors. There was virus in the urine between the 36th and 46th hours after infection and in the feces in the period between the 12th and 60th hours. Waldmann, Trautwein, and Pyl proved the presence of the virus in urine of cattle in the course of 18 to 146 days after infection, by the method of adsorption. At the Eleventh International Veterinary Congress in London in 1930, Waldmann affirmed that an animal which has developed foot-and-mouth disease secretes the virus from the organism, as a rule, only in the period of illness itself and no longer than 10 to 12 days. Such a statement is in known contradiction with the experimental data of other investigators and of Waldmann himself, and also with numerous data on the rather prolonged virus-carrying with foot-and-mouth disease. The English commission, on the basis of its investigations, considers that between the presence of the virus in blood, milk, and urine no relationship exists. The role of other secretions and excretions which can be sources of infection remains the subject of little study -- secretions of sexual organs, nasal mucous, tears, and others.

Animals which have recovered can be a source of dissemination of the virus in the external medium -- healthy carriers of virus. The epizootological significance of virus-carrying in foot-and-mouth disease is disputed by no one, although up to now it has been

inadequately investigated.

In practice there is significance not only in the direct sources of infection (the infected organism), but also in the indirect sources of infection (transmitters, carriers). The latter evidently play no smaller role in spreading foot-and-mouth disease than the direct sources of infection.

NATURAL INFECTION

Foot-and-mouth disease runs its course in the form of epizootics and is spread with exceptional rapidity. This is explained, as was said before, on the one hand by the high contagiousness of the foot-and-mouth-disease virus and by the high sensitivity to it of spontaneously susceptible animals, and on the other hand by the variety of means and methods of spread of the infection itself. Natural infection of animals takes place usually by means of direct contact, when the virus by one means or another is received by a healthy animal directly from one that is diseased. But natural infection can take place also by means of indirect contact, when the virus is received by animals through some intervening medium -- food and water, buildings, troughs, litter, manure, objects used in caring for animals, and others. As passive transmitters of the infection, some significance may be assigned also to animals not susceptible to foot-and-mouth disease. A particularly great role as passive transmitters of infection is often played by human beings who are in contact with sick animals or other sources of infection.

Infection of animals through direct contact is of principal

significance in the infected herd itself -- in the pasture, the barnyard, and elsewhere -- while all other methods of indirect transmission of the infection have also very great practical significance in connection with the spread of foot-and-mouth disease.

Means of penetration ("entrance gates") for the infection are the digestive tract, mainly the mucous membrane of the buccal cavity, and also the external surface of the animal in the area of the coronary band and the cleft of the hoof, the teats, and the udder. It has also been proved possible to cause infection through the membrane of the external sexual organs (various manipulations in the investigation of the sexual channels, copulation, and other ways), through the membrane of the nasal cavity, and the conjunctiva of the eyes, but these paths of infection have only secondary significance, and their epizoological significance has been given inadequate study.

A necessary condition for penetration and multiplication of the virus in the organism of an animal susceptible to it must be a break, however small, in the continuity of the tissue (Dalmen and Hecker). However, certain investigators in the field of foot-and-mouth disease hold that the virus can penetrate even through intact tissue (Loeffler and Frosch, and others). This statement, however, is in need of experimental proof and such a method of infection has hardly any practical significance. As a matter of fact, for penetration of the infection the most minute break in the continuity of tissue, often imperceptible to the naked eye, is sufficient. In the oral cavity of cattle and sheep which eat rough fodders such defects in the membrane can be a most ordinary phenomenon.

Various damaging and lacerations of the upper layers of skin --

abrasions, scratches, cracks, and others -- can also be entrance gates for infection. In consequence of the strongly manifested tropism of the virus for epithelial tissue, it finds there, in these defects of the skin, favorable conditions for its original fixation and multiplication.

The high sensitivity of the skin to the virus has repeatedly been verified by us in experiments on cattle. In intracutaneous infection of cattle we succeeded in causing sickness in 14 hours after infection.

Waldmann and Trautwein developed a method of artificial infection of cattle by means of transferring the virus to the mucous membrane surface of the upper lip which has first been scarified. Walter proved the possibility of infection of the guinea pig by means of rubbing the virus into the skin. In Southern Rhodesia intranasal infection is applied, by means of introducing pulverized virus, for the purpose of causing a light, unnoticeable case of the sickness.

The basis of all these methods of artificial infection is also the high sensitivity of the skin to the virus.

Ozerov assumes that for infection in young cattle during the period of replacement of teeth the unhealed openings in the membrane on the gums can be entrance gates. In one instance he observed foot-and-mouth-disease sickness first on a farm in a calf about 2 years in age, which exhibited, aside from aphthae and erosions of foot-and-mouth disease origin, small ulcers, which had not yet healed, on the gums around recently exposed protrusions. In this case they evidently

served as entrance gates for the infection.

In another instance Ozerov established the fact of infection of a pedigreed bull by a cow which had been sent from a settlement contaminated with foot-and-mouth disease to be served by this bull. Before breeding, the cow was examined, certified healthy, and accepted for breeding, and in only two days clinical signs of foot-and-mouth disease were observed on her. Ozerov considers that infection of the pedigreed bull in this instance occurred during breeding. In Orlov's opinion, for adult swine also the post-natal period tends to be a favorable time for infection by foot-and-mouth disease.

With respect to the possibility of infection by means of air which has been exhaled and contains "particles" of the virus in suspended state, there are conflicting opinions. Buchmann and Murray hold that aerogenic infection has not been proved in foot-and-mouth disease.

In his experiments with hedgehogs Lawarow, on the other hand, proved that they can exhale the virus and, at the same time, swallow it together with inhaled air. This also explains the extremely rapid spread of foot-and-mouth disease among hedgehogs under experimental conditions. Guinea pigs, according to the author's assumption, do not give off the virus together with exhaled air, and therefore foot-and-mouth disease among them does not spread naturally in the way that it spreads among hedgehogs.

Edwards holds possible the infection of animals by means of transference of minute infectious substances, particularly during the period of so-called rupture of the aphthae. In his opinion a minute quantity of infectious material is dangerous for one type of animal

susceptible to foot-and-mouth disease, through transfer of the virus, and not dangerous for another. Foreman assumes that wind can spread the virus, with dust, over large distances. This report is in need, for the time being, of serious proof.

EXPERIMENTAL INFECTION

In artificial infection of animals, the site and method of introducing the virus have very great significance in connection with adaptation of the virus. Loeffler, and also Lebailly, considered the most effective method of infection to be intravenous injection of the virus. Other investigators (Waldmann and Trautwein, Lignieres, Vallee and Carre, Housse, and others), on the other hand, consider this method less reliable and not deserving of serious attention, insofar as intravenous injection of the virus does not always cause generalization of the infection.

The subcutaneous method of injection gives more favorable results as far as infection is concerned (Loeffler, Roux, Vallee and Carre, Nocard, and others). In intramuscular infection under the same conditions more virus is always necessary than with ordinary methods of infection. It is possible first to sensitize the organism of the animal with normal serum, after which the animal develops foot-and-mouth disease, but the disease in this case usually runs its course in milder form (the English commission). Intracutaneous infection with the virus is the most reliable and effective of all existing methods of artificial infection. This has been proved by numerous investigations carried out with cattle, swine, guinea pigs, and other animals (Skomorokhov, Vallee and Carre, Waldmann and

Trautwein, and others). Galloway caused infection with the virus by means of pulverization of the mucous membrane of the nasal cavity.

In experiments with pulverization of the cornea, and also the conjunctiva, on the other hand, negative results were obtained. Infection can be achieved only through repeated pulverization. Janssen injected the virus into the marrow of guinea pigs. They developed foot-and-mouth disease with signs of generalization, as the result of which a firm immunity developed. In 24 hours following infection it was no longer possible to prove the presence of virus in the marrow of infected guinea pigs. Van Vaveren, on the other hand, with intracerebral injection of the virus into the guinea pig, suckling pig, calf, rabbit, and white mice obtained negative results. He injected the virus into the marrow, repeating this up to 3 or 4 times, and in no case was able to prove its adaptation.

It has been possible to prove the presence of the virus in the marrow of white mice for 3 days, with intracerebral injection; it disappears considerably earlier from other organs (the spleen and liver). In the latter case it was not possible to prove its presence 24 hours following infection. Thus, all attempts to cause the "marrow form" of foot-and-mouth disease -- foot-and-mouth-disease encephalitis -- through intracerebral infection ended in failure. Van Vaveren produced intratesticular infection of the guinea pig and rabbit with filtered virus. The guinea pig, as a result of this method of infection, developed sickness with subsequent appearance in the usual "favorite" places of aphthae, but on the site of injection of the virus multiplication of it did not take place. The rabbit exhibited no specific

phenomena as a result of such infection, after repeated passages as well as after the first.

In artificial infection, particularly subcutaneous, there is great significance not only in the virulence of the virus, the site and method of its injection, but also in the amount of virus used for infection. Susumi, Kurango, Tatsuo, and Nogami consider that a minimum of 2 cubic centimeters of virus is necessary for experimental infection of cattle. For artifical infection Cosco and Aguzzi recommend the use of virulent blood which has been defibrinated in preliminary (with the aid of glass beads), the quantity of virus injected, according to their report, not having great significance. Roux, Vallee and Carre, and Nocard used virulent serum, a minimum of 1 cubic centimeter, for artificial infection. For subcutaneous infection they used a minimum of 1 to 2 cubic centimeters.

In artificial infection of cattle by means of introducing the virus on a surface of mucous membrane of the upper lip which has first been scarified, more or less precise dosage is not of essential importance. Nevertheless, strong concentrations of the virus should not be used, particularly with the application of this method under the conditions of the practical struggle against foot-and-mouth disease.

EXPERIMENTAL LABORATORY ANIMALS

The guinea pig is considered to be one of the best experimental animals for work with the foot-and-mouth-disease virus under laboratory conditions. In contact with sick animals guinea pigs, as is known, do not develop foot-and-mouth disease. With artificial infection, on the other hand, they develop the disease in a fashion similar to

cattle, with the appearance of primary aphthae on the site of infection, subsequent generalization of the infection, and the appearance of secondary aphthae on all extremities and in the buccal cavity. The infection of guinea pigs is accomplished by means of cutaneous or intracutaneous injection of the virus on the plantar surface of one of the rear extremities, the other extremity remaining as a control.

The generally accepted method of infection of guinea pigs consists in preliminary scarification by means of slight incisions in the surface layer of the epithelium of the sole of the foot and the transference to these places of the virus, with the aid of a glass Pasteur pipette. At the site of introduction of the virus a so-called primary aphtha appears in 24 to 48 hours. On the following day, seldom later, secondary aphthae appear in the buccal cavity and on all remaining extremities. The virus attains maximum virulence in 18 to 24 hours after infection, following which its activity considerably decreases. Therefore, it is recommended that gathering of the virus for subsequent passages be made from primary aphthae which have developed in the first 18 - 24 - 36 hours. The aphthae on the extremities of guinea pigs do not rupture, as in animals spontaneously susceptible to foot-and-mouth disease, but gradually decrease in size, dry up, and disappear, and afterwards the exfoliated epithelium itself vanishes with the passage of time.

Under laboratory conditions for work with the virus it is recommended that guinea pigs with white paws be selected, so that it will be easier to observe the appearance and development of aphthae. It must also be considered that not all guinea pigs are uniformly

suitable for experimental work. The most constant results are given by guinea pigs of medium age weighing from 350 to 450 grams (Waldmann and Trautwein, the English and American commissions on foot-and-mouth disease, and others).

For titration of anti-foot-and-mouth-disease serum in guinea pigs it is recommended that pigs of medium weight, ranging from 300 to 350 grams, be used. Certain investigators (Lignieres and others) admit the use of guinea pigs of heavy weight for the same purposes. We consider guinea pigs weighing no less than 300 and no more than 500 grams the most suitable for experimental purposes. It is necessary to be particularly cautious in using young guinea pigs for experimental purposes. Certain investigators (Gins and Weber, and others), in passages of the virus in young guinea pigs, observed a weakening of it almost to full loss of virulence. Galea, on the basis of his experiments, was convinced that the susceptibility of guinea pigs is not strictly constant. It also depends on age.

The breed of guinea pig evidently is not of essential significance, although the so-called "rosette" (Japanese) guinea pigs are proving more sensitive to foot-and-mouth disease, according to our observations.

The guinea pig as an experimental animal for the study of foot-and-mouth disease entered into practical laboratory investigations only in 1920, after Waldmann and Pape had developed a technique of artificial infection of guinea pigs by means of introducing the virus on a scarified surface of the pads of the rear extremities. Gins and Weber in place of scarification of the pads suggested a method

of infection by means of "tunneling" with the aid of a hypodermic needle.

The very fact of the possibility of using guinea pigs for experimental study of foot-and-mouth disease had enormous consequences for the solution of a whole series of leading questions of theoretical as well as practical character. However, for the production of experiments connected with the study of immunity preference must be yielded unconditionally to cattle as the type of animal most sensitive to foot-and-mouth disease. The guinea pig possesses relatively high resistance to foot-and-mouth disease, and this phenomenon must invariably be considered in the evaluation of the results of experiments carried out with that type of animal.

The hedgehog, according to the assertion of certain investigators, can be another very useful experimental animal in the study of foot-and-mouth disease. This animal, as shown by the experiments of the English Commission on Foot-and-Mouth Disease (Minett, Chrites, Edwards), is characterized by an extremely high susceptibility to foot-and-mouth disease. It develops the disease under conditions of natural infection through direct contact with sick animals, as well as with artificial infection. The English investigators infected them intracutaneously on the soles of the extremities, and also intramuscularly. Following the appearance of primary aphthae on the site of infection there came a generalization of the infection, as a result of which aphthae appeared on all remaining extremities, in the oral cavity, and on the snout. The virus, taken in the form of the aphthae of hedgehogs, caused infection of guinea pigs in dilution of 1:1,000,000. The serum of convalescent hedgehogs usually had sufficiently high titre (0.1 -- 1.0 cubic centimeters). The

incubation period in hedgehogs with foot-and-mouth disease infection lasts for 3 to 5 days. In the experiments of the English Commission on Foot-and-Mouth Disease it was possible without particular difficulty to cause infection by means of feeding with milk containing the virus. The sickness in the hedgehog runs its course in severe form, and on the 4th to the 7th day usually ends in death.

Among other animals, it is possible to infect rabbits, white and wild rats, marmots, field mice, cats, and dogs, but for experimental purposes these are of little suitability and are almost unused.