EUROPEAN COMMISSION FOR THE CONTROL OF FOOT-AND-MOUTH DISEASE

REPORT OF THE MEETING

OF THE

RESEARCH GROUP OF THE STANDING TECHNICAL COMMITTEE

at the

Institut Français de la Fièvre Aphteuse

Lyons, France
28 September - 1 October 1965

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

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EUROPEAN COMMISSION FOR THE CONTROL OF FOOT-AND-MOUTH DISEASE

Meeting of the Research Group of the Standing Technical Committee held at the Institut Français de la Fièvre Aphteuse, Lyons, France on 28 September - 1 October 1965

A meeting of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease was held at the Institut Français de la Fièvre Aphteuse, Lyons, France on 28 September - 1 October 1965.

The members of the Group who attended were: Prof. R. Willems (Belgium), Dr. B. Fogedby (Denmark), Prof. B. Ubertini (Italy) accompanied by Prof. L. Nardelli, Dr. J.G. van Bekkum (Netherlands) and Dr. J.B. Brooksby (U.K.) accompanied by Dr. R.H. Fagg.

Dr. G.M. Boldrini, Secretary of the European Commission for the Control of Foot-and-Mouth Disease, Sir Thomas Dalling, Consultant and Miss D. Guarino were present at the Meeting.

Members of the Institut Français de la Fièvre Aphteuse at the Meeting were: Dr. V.C. Mackowiak, Director, Dr. H.G. Petermann, Dr. J. Fontaine, Dr. J. Terré and Dr. H. Gilbert.

Invited to the meeting were: Dr. G. Moosbrugger, (Secretary-General) OIE Permanent Commission on Foot-and-Mouth Disease, Dr. B.F. Cox, Chief, European Mission for Research and Animal Diseases (U.S.A.) and Dr. C. Bernal, Pan American Center for Foot-and-Mouth Disease, Rio de Janeiro, Brazil, as well as Prof. F. Lucam, Director, Laboratoire de Virologie Animale, Lyon accompanied by Dr. G. Dannacher and Dr. M. Fedida, Dr. A. Lucas, Directeur du Laboratoire Central de Recherches Vétérinaires, Alfort accompanied by Dr. L. Dhennin and Dr. G. Gayot, Dr. J. Asso of the Institut National de la Recherche Agronomique, Thiverval, France, Prof. Dr. G. Eissner, Institut für Viruskrankheiten der Tiere, Tübingen, Germany, Dr. J. Cardassis, Foot-and-Mouth Disease Institute, Aghia Paraskevi, Greece, Dr. L. Ravaioli, Istituto Superiore di Sanità, Italy.

Dr. V.C. Mackowiak, Director of the Institut Français de la Fièvre Aphteuse was elected Chairman of the Meeting.

The main points for discussion were (1) Innocuity control of inactivated foot-and-mouth disease vaccines for cattle; inactivating agents of foot-and-mouth disease virus; (2) Control of efficiency of foot-and-mouth disease vaccines for cattle; (3) Dosage of inactivated vaccines in small ruminants; (4) Influence of vaccination of the mother against foot-and-mouth disease on the level of neutralizing antibody in her young.

On the second day a visit was paid to the various sections of the Institut Français de la Fièvre Aphteuse, where demonstrations and explanations were given of diagnostic techniques, the methods of production of foot-and-mouth disease vaccines as well as testing for innocuity and potency.

Control of Innocuity of Foot-and-Mouth Disease Vaccine for use in Cattle

Papers were read by Dr. Brooksby, Dr. Fogedby and Dr. Eissner.

Discussion.

Dr. Dhennin gave an account of the technique adopted for over 10 years at the Central Control and Research Laboratory, Alfort, before some recent changes were made. It consisted in the intradermolingual injection of 5 ml vaccine at 20 points followed 4 days later if no reaction had occurred, by the subcutaneous injection of three times the producer's recommended field dose, in the dewlap of each of 3 susceptible cattle. Susceptible cattle were used and observations were continued for 8 days. It was explained later in the discussion that the object of the subcutaneous injection was not to detect specific infectivity of the vaccine, but to show it contained no agent which could cause local or general disturbances. This method ensures 95 percent safety with a maximum risk of 6 percent with tests giving negative infectivity results. There has also to be taken into account the 24 susceptible cattle which receive subcutaneous injections of the vaccine for tests of efficacy. This technique has given excellent results which have been confirmed by the use of tested vaccine on many millions of cattle in France. Exceptionally few animals in the field had developed foot-and-mouth disease following vaccination on one or two farms, but the same batch of vaccine even in volumes of 250 - 300 ml and to which cortisone was added had, in later tests, failed to provoke the disease in young cattle.

There were 25 cases of sudden death immediately after the injection of vaccine in 1964, probably anaphylactic in nature, in individual animals on different farms. The cattle were between 3 and 7 years old.

The only vaccines in which true virulence was shown were experimental vaccines which were not adsorbed on aluminium hydroxide but on other substances such as embryonic extracts, milk extracts and whole milk. With such vaccines showing virulence, lesions did not appear until 18-20 days after the animals received the vaccine.

Dr. Willems expressed the strong view of the need to use cattle for innocuity tests of vaccine. Experience in Belgium over many years had proved its value. He emphasised that he was referring to vaccine prepared with filtered virus and inactivated by heat and formol when he said that this method using a very limited number of susceptible cattle enabled conclusions on innocuity readily to be made. The position might not be similar with vaccine prepared with unfiltered virus and he recalled testing such a vaccine received from a foreign colleague, when virulence was detected only after 10 days' observation of the vaccinated susceptible cattle.

In regard to other tests, for example, the use of baby mice, if such a test is more sensitive than the cattle test and both tests are taken into account in considering innocuity of a vaccine, it could occur that a vaccine quite safe for cattle was not considered acceptable. Again using cell cultures, if the vaccine contains some partly inactivated virus particles, there is always a chance under certain conditions, that virucidial agents particularly interferon, may be developed in the culture, resulting in masking the virulence of the vaccine.

Dr. Moosbrugger also stressed that the vaccine under discussion was the Schmidt-Waldmann-Vallée vaccine which experience has shown to be rendered innocuous for cattle by inactivation carried out for 14-20 hours. With this vaccine properly prepared, negative tests in a limited number of cattle as described give full assurance of its innocuity for cattle.

Dr. Lucam had experienced excellent results using the cattle test technique with many batches of vaccine both in France and abroad. It was only on about three or four occasions that "classical" vaccine, so tested, had shown virulence: in these instances, the lesions had covered the entire surface of the tongue and there was generalisation in 48-96 hours. He also had experience, however, of the occurrence of lesions, sometimes at the points of injection in the tongue, sometimes covering the whole surface of the tongue and sometimes in the form of generalisation, at 20-25 days after injection of the vaccine.

Dr. Moosbrugger spoke of dissociation which could occur with "safe" vaccines in which the complex provided by the proper preparation of the vaccine could be destroyed and virulent virus particles released.

Dr. Willems raised the question of the period between completion of inactivation by heat and the beginning of tests in cattle for innocuity. He leaves 15 days. He recalled an occurrence some years ago when to meet demands for vaccine during an epizootic, some vaccines were tested 2-3 days after their preparation: their virulence at that time was very evident by the development of typical local and generalised lesions in the test cattle. Similar tests carried out later with these same vaccines gave negative results.

Dr. van Bekkum spoke on continuation of inactivation of stored vaccine, particularly when the pH is above 8: at pH9 inactivation continues for 4-5 days. Thus, vaccine which may show some infectivity on initial test may later prove safe for use in the field.

Dr. Terré described the procedure at I.F.F.A. Monovalent vaccines are tested for both innocuity and efficacy before being used to prepare trivalent vaccines. Each monovalent vaccine is tested for innocuity the same week in which it is prepared and so the time elapsing between preparation and test

may vary from less than an hour to one or two days. Three susceptible cattle are used for each test. It is only rarely that a batch of vaccine shows virulence and it is not on all occasions that the disease appears in all three test cattle. When it does occur, however, it develops rapidly and severely, tongue and generalisation lesions appearing in 48-72 hours. He said later that virulence was shown in about 1 percent of batches. The occurrence could not be predicted: it may occur in successive batches or there may be very long intervals. It was stated by Dr. Mackowiak that the incidence of such vaccines is extremely small and that when tested a week later they show no evidence of virulence. Dr. Fontaine added that a monovalent vaccine which is shown to have virulence is not used in the preparation of trivalent vaccine.

Dr. Moosbrugger, continuing the discussion on the period between preparation and innocuity testing, spoke of the possibility of the change of temperature from 25°C in the mixing tank to 4°C as a factor in recovery of virulence by virus in a vaccine. He explained also that his procedure is to make innocuity tests on the monovalent vaccines at the completion of their inactivation and then, later, on the mixtures.

Dr. Terré explained that at I.F.F.A., the vaccine is cooled down in the mixing tank to 4°C and it is then that samples for tests are taken. He referred also to the steps taken to ensure that the cattle used in the tests are fully susceptible. Cattle used for monovalent type A vaccine tests weresubsequently challenged with virulent virus types O or C or a mixture of these two: none of the animals have ever been found to resist such challenge. The cattle used in tests come mostly from Finistère where there is no foot-and-mouth disease and where vaccination is not practised. Should some vaccination have been carried out surreptitiously, trivalent vaccine would have been used but the test animals would show protection against the virulent viruses injected after the innocuity test of the vaccine under examination.

Dr. Ubertini expressed the view that the transmission of foot-and-mouth disease should not be the only consideration when testing vaccines for innocuity: there is also the question of anaphylactic-like reactions. In Italy, probably about 20 percent of cattle show some degree of such reaction soon after vaccination. Vaccine in Italy is prepared by the classical technical technique, using chloroform. The reactions occur with vaccine in which antibiotics are or are not present: chemical tests have not disclosed the presence of histamine. Affected cattle may have been vaccinated only once or on several occasions. It is probably only the most serious cases that are reported by veterinarians.

Dr. Bernal mentioned attempts to carry out innocuity tests in pigs: inoculation of the vaccine inactivated by chloroform and formol, with saponin added but not adsorbed on aluminium hydroxide caused the disease in 24-48 hours.

Dr. Moosbrugger spoke of experiences in the vaccination of pigs. In outbreaks, pigs as well as cattle are vaccinated in Switzerland. There has never been any trouble with adult pigs, but young piglets proved highly susceptible to vaccine which was tolerated perfectly by adult pigs and cattle. Piglets under 3 weeks of age are no longer vaccinated.

Dr. Eissner said that, in Germany, pigs over 4 weeks old were vaccinated without any difficulty on several occasions.

Dr. van Bekkum spoke of the variability of the sensitivity of pigs to the virus of foot-and-mouth disease, mentioning particularly virus type C. Exposure to the virus by contact or by intramuscular injection of large doses may not cause any disease. In some cases, when contact is not too prolonged, pigs become immunised without having shown any reaction whatsoever.

Dr. Ubertini said that because of the expense involved and the variability of their susceptibility, pigs were not used for innocuity tests of vaccine in Italy.

Dr. Ubertini raised the subject of the use of baby mice for innocuity tests, explaining the difficulty of finding fully susceptible cattle in some countries, e.g. Italy, and the need to use imported cattle whose history, feeding, etc. were not known.

Dr. Lucam related his experiences with baby mice. In most batches used in innocuity tests, some died and each dead mouse had to be examined, using complement fixation, sometimes after a series of passages in mice, to demonstrate the presence or absence of foot-and-mouth disease virus. This was time-consuming and did not fit in with the commercial production of vaccine.

Dr. Brooksby said that he had tried to use mice in the determination of inactivation graphs but decided to use them as little as possible. He also said that the relative sensitivity of mice and cattle had to be considered. On this, there was some disagreement but he believed cattle to be the more sensitive, a view which seemed to be supported by the result of some experiments at Plum Island, U.S.A.

Dr. Cardassis spoke of some attempts to use cell cultures in innocuity tests at his Institute: toxic reactions from both the formol and the aluminium hydroxide were found and the pH also was of importance. This method is no longer used.

Dr. Mackowiak, in summing up this item of the discussion reminded the meeting that its purpose was not to make any conclusions, but simply to discuss the various subjects. He recalled the quotation used by Dr. Brooksby in his paper presented at this meeting "if at the moment of each test the interpretation of the result should be taken with reserve, the accumulation of tests permitted in retrospect a precise

enough conclusion of the average value of the material produced." (Quotation from a publication by Dr. Lucam and others in 1957.) It seems that innocuity tests, for the time being, should be carried out in cattle. There may, however, be envisaged other techniques, such as the use of baby mice, tissue culture and even perhaps piglets, all of which are in the experimental stage and have to be confirmed. According to the experiences in France, the present tests are sufficient to disclose traces of virus. If there should be evidence of any virulence at the first control, there are still the controls at the second or third stage of testing. This ensures that the control is quite sufficient despite all the statistical restrictions which might be imposed: virus has never been demonstrated at the second stage of testing.

The vaccine should be inoculated intradermolingually and possibly, some days later, subcutaneously, the latter being to detect non-specific reactions from the vaccine.

Control of efficacy of inactivated Foot-and-Mouth Disease Vaccine for use in Cattle

Papers were presented by Dr. Lucam, Dr. Dannacher and Dr. Fedida.

Dr. Mackowiak said that several points arose concerning the introduction of fficial, general control in France of commercial vaccines. The amount of protection, calculated in cattle, for Class I vaccines is taken as 85 percent: the methods are quantative, with three grades, using guineapigs and serum-neutralisation for vaccines considered as good, and the K index for vaccines considered as doubtful, by the other two methods. The choice of strain of the virus for challenge should be discussed.

Papers were presented also by Dr. Ravaioli and Dr. Cardassis.

Discussion.

Dr. Willems made a distinction between control of efficacy and control of activity. He agreed completely that in official tests for vaccines for use in a country in which foot-and-mouth disease is being dealt with, that producers should be required to provide vaccine which will conform to the tests which are fixed, each acceptable vaccine protecting against the strain of the virus present in the country. On the other hand, if France should purchase vaccine from say Belgium, Switzerland or indeed any country and if that vaccine were controlled with the strain of virus used for control purposes in France, it could well be that the vaccine was not acceptable although it was perfectly active where it was prepared. In other words, as Dr. Mackowiak made clear, Dr. Willems held the view that there are two controls: one by the producer who has the right to control the activity of his vaccine against the strain used in its preparation and the other, the official control, for showing the efficacy of the vaccine against the strain of virus in whichever country the vaccine is to be used.

Dr. Moosbrugger supported Dr. Willems and said this was the old problem of whether there should be used fixed strains of virus or strains found in the field.

It is quite evident that in countries where foot-and-mouth disease is present, the vaccine must protect against the current strains; but the position is different in a country free from the disease. He spoke on the difficulty or even the impossibility of forecasting which strains of the virus might invade a country at any time. He also pointed out the possibility that nothing might be known about the immunising power of an invading strain and also that the invading strain could break the immunity in animals which had been vaccinated against other strains of the same type of the virus.

Dr. Lucam still thought that the term "control of activity" was sufficiently explanatory. He dealt with the choice of the strain for control purposes. In France, in 1957 or 1958, the Commission for Sera and Vaccines, established officially by the Ministry of Agriculture, following a report on the early vaccine control methods used, stated that it was not logical to control vaccine with the strain of virus used for its preparation because a vaccine should give immediate protection. This view had been followed. Should a new strain appear in a country, some time would be required to carry out the necessary examinations to establish its identity and relationship to other strains of the same virus type, before it could be used for vaccine control purposes. Work would also be necessary to show whether it could be successfully used for vaccine production. The chances also are that it would not be so very different from the strains already used for the production of the available vaccine. In any event, the whole position would be laid before the above-mentioned Commission.

Dr. Mackowiak said there are three kinds of countries concerned: countries in which the controller and the producer are, in principle, the same official body and where both agree on the choice of the strains; countries like France and Germany where the producer is a private organisation and the controller is an official body; countries like Italy in which the set-up is mixed.

He explained, as an example, of how the appearance of strain Middle-East A had been dealt with. He would not meet the request to provide vaccine until field tests of its efficacy were carried out. Field tests of several vaccines presented by different European governments proved that the vaccines were ineffective. It was only when vaccine prepared with the Middle-East A strain was used that the excellent results now recorded were obtained.

Dr. Eissner said that in Germany the challenge strain is always the strain used for production of the vaccine and is provided by the producers and Dr. Ravaioli said that in Italy, the position is as in Germany.

Dr. Lucas gave some further information on the choice of strains by the above-mentioned Commission, on which there are representatives of the producers as well as the controllers. Meetings can be arranged whenever necessary and agreement can be reached on the strains to be used both in the preparation of the vaccine and in its control. Strains may be changed when considered necessary.

Dr. Fontaine spoke from the point of view of producers and took as an example the Spanish strain O of virus which appeared recently at the frontier. The questions put by the Ministry were whether the current vaccine would protect against this strain and whether an efficient vaccine containing two different O variants could be prepared. Experiments carried out in collaboration with the Research Laboratory, Alfort, showed that trivalent vaccine containing two variants of the same strain gave equivalent protection against each of the variants. There were also some experiments carried out in guineapigs at I.F.F.A. which showed that with half doses of vaccine prepared with two very different O variants, protection against each variant was equal to or even greater than that from a full dose of each.

Dr. Mackowiak explained that monovalent vaccines were stored and whenever a new strain of virus appears, a new monovalent vaccine is prepared with it and trivalent vaccines are prepared with the monovalent vaccines required.

Dr. Cardoesio thought that when a new strain or type of the virus appears in a country, the producing institutes should continue to produce the current vaccines, in case their stocks become exhausted. The production of vaccine against such a new strain should be regarded as a special undertaking or the vaccine may be purchased from elsewhere.

Dr. Mackowiak, in summing up this part of the discussion, said that it seemed that in most countries the vaccine is controlled for efficacy by the use of the production strain of the vaccine. This strain, however, should be as close to the strain causing the disease in the country. There should be close collaboration between the producer and controller so that the strain could be quickly changed following all its necessary examinations.

There was always the possibility of studying a strain before it invaded a country. This would, however, require international collaboration. He gave as an example the help given by the French Ministry in establishing a laboratory in Teheran for studying strains of the virus which could invade Europe: the position is that if Middle-East A strain appeared in France to-morrow, there is already a vaccine for its control.

Inactivating Agents

Dr. Fagg presented his paper.

Dr. Petermann gave an account of some experiments with 0.05 percent beta propiolactone as the inactivating agent for O, A and C type viruses cultivated by the Frenkel technique. Inactivation, carried out at 25°C and pH 7.4 was shown by tests of diluted and concentrated treated virus on pig kidney cell cultures, to have been reached in 4 hours. Aluminium hydroxide-adsorbed vaccines prepared with these inactivated viruses were compared for efficacy with formol-inactivated, aluminium hydroxide-adsorbed vaccines: the tests were carried out in cattle, by serum-neutralisation with sera of vaccinated cattle and of vaccinated chicks, and by estimation of the 50 percent guineapig vaccinating dose. The results indicated that the beta-propiolactone-inactivated vaccines were at least as efficient as the classical vaccines. The suggestion is that the viral particles are less severely acted upon by the beta-propiolactone than by formol. Complement fixation tests, which give indirect indication of antigenicity associated with the 22 mμ virus particles showed that virus inactivated with beta-propiolactone was of the same order as that of the initial untreated virus, while virus inactivated with formol was inferior. It would therefore seem that beta-propiolactone acts more mildly on the 22 mμ virus particles and so virus inactivated by beta-propiolactone provides a better immunogenic vaccine.

Techniques of the Control of Efficacy

Dr. Bernal asked how many batches of vaccine had been controlled during recent years by Dr. Lucam's method and how many had been refused.

Dr. Fedida, in reply, said that between 1960 and 1965, the number of batches controlled had increased ten-fold and while in 1960, only 50 percent of the batches were authorised

for use, the percentage rose to 97 in 1964 with 3 percent refused or authorised as Class II vaccines. It was also pointed out that relatively few vaccines were controlled in 1960 when compulsory submission of samples was not in force. To-day, control is carried out on practically all vaccine produced, with nearly 100 percent acceptances.

Dr. van Bekkum raised the question of the interval of time between injection of the vaccine and the challenge test. He noted that this varied in different countries, e.g. 3 weeks in France and Greece and 4 weeks in Italy. After 10 years during which the interval in the Netherlands was 2 weeks, a change was made in 1964 to 3 weeks: in tests on 500 cattle with the three types of the virus, no basic difference was found in the antibody titres at the two intervals.

Dr. Brooksby said that in 1955, the interval was raised from 14 to 21 days because of the increase of antibody titre in many animals after 14 days: in some few animals the maximum titre was not found before 28 days. Because groups of animals were used, the small number requiring 28 days did not have much influence on the overall result and so the interval was fixed at 21 days.

Dr. Moosbrugger agreed on the 21 day-interval which is adopted in Switzerland, but pointed out the need to have vaccine which will induce immunity as rapidly as possible because, in Switzerland where ring vaccination is adopted, quarantine measures cannot be enforced for more than about 15 days.

Dr. Lucam supported a 3 week-interval. By means of graphs, he showed that with vaccines with very high log K (3.5) and log K2, immunity develops rapidly; vaccines with lower values (log K1) would be eliminated if 15 days' interval was taken although they would be usable.

Dr. Fontaine gave an account of an experiment in which titration of antibodies and DV_{50} confirmed the results shown by Dr. Lucam. He showed that a curve obtained with serum-neutralisation figures was similar to that using the K index. The duration of immunity was shown to be related to the antibody titre and with a rising titre, the higher it is at 3 weeks, the longer is the duration of the immunity. It was found that the antibody titre corresponding to the DV_{50} was about 0.7.

Dr. Willems brought up the question of the cattle in which the control was carried out, from the results of which the vaccines were classified into Class I (K index 1.2 or above), Class II (K index between 0.6 and 1.2), and refused (K index below 0.6). The control is made on animals which have not been previously vaccinated. He spoke of cattle in countries where annual systematic vaccination is carried out and asked whether there might not be some wastage of the Class II (acceptable) vaccine (only 5-10 percent being permitted in any one year), which might be of value in stimulating the immunity in these previously vaccinated animals.

Dr. Mackowiak referred to reference sera for use in each serum-neutralisation test. While individual laboratories may use their own reference sera, the question was whether there could be introduced common reference sera which could be used by all laboratories, so that results could be readily compared.

Dr. Dannacher supported the idea of acceptable standard reference sera for general use.

Dr. Cardassis wondered which strains of virus would be used in the preparation of such standard sera.

Dr. Brooksby thought it would be extremely useful to have standard reference sera prepared in cattle from specific strains of the virus. He said, however, to avoid the inclusion of any extraneous substances, it would be necessary to use specifically vaccinated cattle and not animals which had recovered from the disease.

Dr. Mackowiak thought the question of standard reference sera including strains of the virus might be brought forward at the next meeting. Referring to control in guineapigs, he wondered whether it is really a C index that is obtained or whether it is simply a guineapig test with a single dose of the virus. He made the point that following the occurrence of primary lesions, the amount of virus required to produce generalisation is not known.

Dr. Fedida, in reply, referred to some published work on post-vaccinal work in cattle in which comparisons were made, in vaccinated and non-vaccinated animals, of primary and secondary lesions following the use of different dilutions of virus. There must first be primary lesions and the greater the intensity of the primary lesions, the more extensive are the secondary lesions. There is thus a very close relationship between the occurrence of the primary and the secondary lesions.

Dr. Mackowiak, in summing up the discussion on the control of efficacy said that it is quite evident that official, generalised control, as far as possible, carried out apart from the producers, has influence on the homogenicity and quality of vaccines; that the challenge virus should be the same as that used in the production of the vaccine, provided the vaccine protects against the current disease; that so far as techniques are concerned, while it was not affirmed precisely that they should be quantative, it would appear that this is desirable because a more exact answer is obtained, although qualitative is still being used, mainly for economic reasons.

Dr. Mackowiak now presented the paper on "the Dose-effect Law and the Correlation between 50 percent vaccinating doses in guineapigs and cattle", explaining that this is an account of work by the IFFA team of workers, some of which has recently been published and some about to be published.

Dr. Terré then presented the paper by his colleagues and himself on "Quantative Control of Foot-and-Mouth Disease Trivalent Vaccine in Cattle by the Simultaneous Inoculation of the Three Viruses".

In the discussion of these two papers, in reply to Dr. Ubertini, Dr. Mackowiak said that normally, allowing for losses, there was 0.26 - 0.3 grammes of epithelium per dose of monovalent vaccine prepared by the Frenkel technique.

Dr. Willems mentioned some work he carried out some years ago on the recognition of viruses by complement fixation from lesions in the mouth and feet of Irish cattle injected with a mixture of O, A and C virulent viruses. All three were never recovered from the same lesion, but sometimes only one type was demonstrated and sometimes a mixture.

Dr. Lucam asked if with the present knowledge, using only this guineapig method, it would be possible for an official laboratory to refuse a vaccine.

Dr. Mackowiak pointed out that the method he described referred only to monovalent vaccines; the work reported was with known strains and a known method of production; he does not use this method to reject a vaccine. There is still further studies to be made and probably in a year or two it may be possible to introduce the method in official control work but not yet.

Dr. Gayot then presented the paper on "Deterioration of the Value of Foot-and-Mouth Disease Antigen with Time, evaluated in In-Bred Mice". Dr. Mackowiak pointed out that this contribution contained two interesting points: the use of mice in the control of vaccines and the problem of the conservation of vaccine.

Dr. Terré spoke on the result of vaccinating cattle with a type O monovalent vaccine stored for 4 months. When the vaccine was first used, the DV_{50} was 0.05 ml: after storage for 4 months, it was 0.08 ml which is not significantly different.

Dr. Brooksby spoke of tests with African strains of virus stored in his laboratory up to one year. No deterioration was found after storage for 4 months, but after a year, the activity had decreased about two-fold. Much will depend, however, on the batch of vaccine concerned.

Dr. Fedida found that in his preliminary experiments there did not seem to be much reduction in the quality of vaccine stored for up to 8-10 months as shown by the protection index, and there was some slight difference with the three valences of the vaccine. The impression was that the C valence loses its activity more rapidly than do the others.

Dr. Willems recalled an experiment he had already reported a considerable time ago at an O.I.E. meeting in Madrid. Waldmann type O vaccine stored at 4°C was used and at six-monthly intervals one of a group of six Irish cattle was vaccinated and challenged with 10,000 minimum infecting doses of virus. No generalisation occurred in any of the animals vaccinated with the vaccine stored for 6 months, one year, one year and a half, two years, two years and a half and three years. The animal vaccinated with the vaccine stored for three and a half years showed that the value of the vaccine had declined. The pH of the vaccine at the beginning was 7.6 and it gradually was reduced to 7.35.

Dr. Mackowiak, in summing up this part of the discussion, said that it was evident that some vaccines withstand conservation for a longer time than others and that conditions, strains and preparation techniques have always to be taken into consideration.

Vaccination of Sheep

Dr. Fontaine, after presenting his paper, gave some more recent information on the duration of immunity and on the value of viraemia estimations in the challenge of immunity: he illustrated his remarks by graphs showing dose-effects, percentages of protection and 50 percent vaccinating doses at 3, 4, 5 and 6 months after vaccination. The results indicate that during the first few months when there is optimal immunisation, the dose of the vaccine is of primary importance, while later the degree of immunisation in individual protected sheep is less related to the dose. The findings also suggest that after 5-6 months, in sheep vaccinated with 1/4 or 1/8 of the cattle dose, there is about 80 percent protection. It appears that it may be concluded that sheep injected with a correct dose of vaccine have an acceptable immunity for 5-6 months.

It was found also that vaccinated sheep showing no viraemia always have a much higher antibody titre: this is additional confirmation of the value of viraemia as an indication of infection. Evidence that viraemia i.e. the presence of virus in the blood, is also indicative of generalisation was shown by experiments with virus type O in guineapigs, in which the dose-effect curves for generalisation and viraemia were parallel.

Experiments showed also that the DV_{50} measured by viraemia was greater than the 50 percent vaccinating dose measured by the development of foot-and-mouth disease lesions.

Dr. Gilbert, Chief of the IFFA mission in Iran for the preparation of vaccine against exotic viruses described some of his work on sheep in the Middle-East. There were outbreaks, all caused by virus type A, in the regions of Mesched on the Irano-Russian border of Kermamsha.. in the south of Iran, and to the west of Teheran. The finding of the Middle-East strain of the A virus interrupted work in these regions, but research with strains SAT1 and Middle-East A viruses was carried out in sheep. It was found that the sheep derived from European breeds were more susceptible than the local Astrakan breed.

In all, 15 passages with SAT1 virus and 7 with Middle-East A virus were made in sheep, beginning with virus obtained from cattle. The injections were made intradermolingually and the results were very similar to those recorded by Dr. Fontaine. After 24-27 hours, antigen could be collected at the site of the injection. There was some irregularity in the occurrence of lesions of generalisation: with SAT1 virus, for example, they were found in the rumen and the feet in only 80 percent of the sheep. On the other hand, viraemia was present at all passages from the first.

Foot-and-mouth disease in sheep is of much importance in the Middle-East: because often affected sheep show few if any clinical evidence of the disease and most of the flocks belong to nomadic tribes. Sheep may play an important part in the spread of the disease.

Dr. Gilbert also put forward a suggestion that probably sheep could be used with advantage in the control of foot-and-mouth disease vaccines in the Middle-East where susceptible cattle are difficult to procure and are expensive. Advantage might be taken of the viraemia in sheep and it should be relatively easy to establish flocks of susceptible sheep on experimental farms for laboratory work.

Dr. Lucam raised the question of the present practicability of compulsory vaccination of the total sheep population in a country with an extensive sheep population, e.g. in one of the countries in South America.

In reply, Dr. Fontaine explained that research was still in progress with trivalent vaccine, on determining the 50 per-cent vaccinating dose for each of the valences and on further aspects of the duration of immunity. Probably in about a year Dr. Lucam's question could be dealt with. To-day we know that sheep can be properly vaccinated against foot-and-mouth disease. This latter point, which is new, was emphasised by Dr. Mackowiak.

Dr. Cardassis pointed out that in his country, sheep are regularly vaccinated in the buffer zone and in areas in which there are outbreaks. Compulsory vaccination is also carried out in sheep before the seasonal pastoral movements.

Dr. Moosbrugger spoke on the importance of foot-and-mouth

disease in small ruminants. He mentioned an outbreak of the disease in a region in Switzerland some years ago which was not brought under control until it was discovered that goats were the reservoirs of the virus, although showing no clinical symptoms. This only became apparent when lesions were found in the rumen of slaughtered goats. He also referred to an outbreak among sheep in Switzerland originating in a place where dipping against sheep scab was being carried out. The disease spread throughout all the flocks within 3 days, but vaccination carried out during a period of 12 days proved highly effective. Flocks of sheep in Switzerland on the Italian border are vaccinated regularly each year. Dr. Mossbrugger felt that a decision to enforce large-scale vaccination of sheep is a matter for the government concerned but veterinary officials can give advice.

Dr. Dhennin referred to the compulsory vaccination of sheep at annual movements to and from pastures which has been in operation in France for many years. He pointed out, however, that in order to prevent abscess formation with anaerobic infection, subcutaneous injection of vaccine should be strictly observed. This point was referred to later by Dr. Fontaine who said that with the reduction of the size of the dose of vaccine and therefore of the aluminium hydroxide content, there is now less liklihood of abscess formation.

Dr. Brooksby recalled the experiences of vaccinating sheep in south-west Africa with live attenuated vaccine, when some 400 animals, mostly under 3 months of age in which the vaccine was used against instructions, developed muscular lesions. Inactivated vaccine was then used on all the sheep which had not been vaccinated within a month of the beginning of the outbreak and the results were reported as excellent. He also spoke of the high antibody titres in convalescent sheep.

Dr. Lucam raised the question of using vaccines of somewhat lower quality than those in Dr. Fontaine's experiments, particularly in countries with many millions of sheep. It was explained by Dr. Mackowiak that further work on sheep must be carried out to decide on norms, so that more information can become available on the vaccination of sheep, concerning which the work now in progress may be regarded as only a beginning.

Dr. van Bekkum presented the paper on "The Influence of Foot-and-Mouth Vaccination of the Mother on the Level of Neutralising Antibody in her Offspring".

There was no discussion on this paper.

CONCLUSIONS

Innocuity Control of Inactivated Foot-and-Mouth Disease Vaccine for Cattle.

The problem should be given different consideration according to whether a vaccine to be controlled is produced commercially by a current technique or is an experimental vaccine.

In the first case, using experience which has been acquired, useful conclusions can be arrived at using even a restricted number of animals for the control work.

In the second case, a much greater number of animals has to be used in order to obtain a statistically reliable response.

In the present atate of our knowledge, the innocuity test should be carried out in cattle. The use of unweaned mice, piglets or cell cultures to detect the possible presence of virulent particles should, for the present, be considered as techniques still in an experimental stage.

The injection of the vaccine for control purposes should be carried out only intradermolingually. It is recommended that, after observation over several days, a further large dose of the vaccine should be injected subcutaneously in order to demonstrate possible non-specific pathogenicity.

From the observations reported, it may be concluded that by the control of innocuity carried out in two stages, viz. by the producer of the vaccine and by the official controller, all batches of vaccine having a residual virulence will be excluded, thanks to the technique most often used, i.e. the intradermolingual injection of the vaccine in cattle.

Attenuation of possible virulent particles occurs even during storage of vaccines in the cold room. It would be desirable to learn the exact time when the innocuity control of vaccine should be carried out after the beginning of the period when attenuation of virulent suspensions is occurring.

It seems that if a vaccine contains virulent particles, evidence of their presence is usually shown soon (48 - 72 hours following the intradermolingual injection of the vaccine) by local lesions and generalisation.

Instances have been reported where the appearance of foot-and-mouth disease in innoculated animals was very late (12, 15 or 20 days). This occurred in all the cases with experimental vaccines prepared according to techniques which differed from the classical methods.

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Control of Efficiency (Potency) of Foot-and-Mouth Disease Vaccines for Cattle

Cattle should always be regarded as the animals of reference and all techniques used should be based on the percentage of protection in cattle.

There is general application of the K index method in France for the official control of the vaccines. It is therefore highly desirable that the methods should be the subject of experimentation in other countries and that the results obtained should be brought to the knowledge of international workers who are competent in foot-and-mouth disease.

The use of the method of serum-neutralisation in cattle or in other species of animals (particularly the guineapig) should, in the present state of our knowledge, be used only as methods for distinguishing vaccines of high potency.

The results of the experiments which were given, the different experiments now in progress and the accumulation of the results will perhaps enable the possibilities offered by these methods to be increased in the future.

The choice of the strain of the challenge virus is of primary importance. This strain should, in fact, have the same characters as the strain which threatens the livestock of a country. Close collaboration between the controller and the producer is therefore necessary in the choice of the strain, while its introduction into the composition of the vaccines may be found indispensable.

The control carried out in the two stages, i.e. by the producer and the controller, gives an additional guarantee for eliminating all batches of vaccine which are judged to have insufficient activity.

All the controls are based, in general, on the degree of immediate immunity. Experiments now in progress will perhaps enable information on the duration of immunity to be obtained from the results of the controls.

It would be desirable to have a standard reference serum in order to be able to compare the serum-neutralisation results obtained in the different laboratories.

Study of the Vaccination of Sheep, using inactivated Foot-and-Mouth Disease Vaccine.

From the reports presented, it is confirmed that a very good immunity against foot-and-mouth disease in sheep can be obtained.

It is not possible to control foot-and-mouth disease vaccine in

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sheep by direct test in sheep because of the irregularity of the response of sheep to virulent challenge.

Indirect methods based on the titration of antibodies and particularly the study of the viraemia seems, nevertheless, to enable the degree of the immunity to be measured.

Influence of Vaccination of the Mother against Foot-and-Mouth Disease on the Level of Neutralizing Antibody in her Young

From the work presented, it is concluded that the titres of antibodies varies within very wide limits in accordance with the ways in which the vaccination is carried out in the mother and with the age of the animals.

The Problems of Innocuity Tests for Inactivated Foot-and-Mouth Disease Vaccines

by

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The division of innocuity testing from studies of inactivation of virus is arbitrary, for confidence in the safety test depends to a considerable extent on knowledge of the inactivation process which it is designed to control. As Gard (1960) has said in relation to the testing of poliovirus vaccines, safety must be built into the production method itself and Salk and Gori (1960), discussing the significance of safety tests on poliovirus vaccine, emphasize that information on the rate of inactivation of the particular batches of virus is essential as a guide to the proper conduct of the process. If the inactivation process has proceeded according to previous experience, there is greater confidence in the ultimate safety test which is used as a criterion of the suitability of the batch for issue.

In this paper I shall try to confine myself to the discussion of the safety test without overlapping too much into the sphere of virus inactivation techniques, but it is tempting at the outset to emphasize that in foot-and-mouth disease vaccines prepared by the conventional methods the inactivation techniques are not such as would lend themselves to confidence in the outcome. The formalin-aluminium hydroxide-virus complex cannot be precisely defined during or even after the process of inactivation and in the past much argument has ranged over the ultimate state of the vaccine. Such complexity, however, need not prevent us looking at the criteria which should be applied to safety tests of vaccines, no matter how they are derived.

The aspects under which this subject can be considered are the following:-

1. The statistical requirements which must be met in the design of the test to give indicated probabilities of the vaccine being non-infective.

2. The mechanics of the test, in which are grouped (a) the choice of species in which tests can be carried out and (b) the route of inoculation of the material.

1. Statistical Requirements

The classical paper in this field is that of Henderson (1952b) who, it must be remembered, was writing before the great development of safety testing of poliovirus vaccines began. Henderson's paper indicated many of the difficulties in the way of proper safety testing of vaccines and there is little that can be said to develop further the purely statistical considerations of the percentages of samples likely to be infective if a given number of observations have been made and have all been negative. I think it is true to say that the figures which Henderson brought forward surprised most biological scientists. It will be noted that these statistical considerations imply that a sample of material is taken from a mixture which contains a small but unknown proportion of particles capable of initiating infection. This model

system may not represent the true position; a vaccine may contain aggregates of inactivated and live virus which are capable of breaking up, or portions of inactivated virus material may recombine to make infective particles. At best one suspects an uneasy equilibrium between the virus, inactivated or not, and the adsorbant, with possible traces of the original inactivant still present. For most practical purposes, however, in considering sampling of bulks for testing and volumes which can be inoculated, the model will stand but it is important to be aware of the possible complexities of the system which can account for anomalies such as those described by Dimopoullos et al. (1959), in the recovery of infectivity in diluted samples when compared with the non-infectivity of undiluted material. This point will be discussed later.

The types of question which can be asked in relation to the statistics of innocuity testing are:—

i) How many samples of vaccine, of what volume, should be taken from the bulk sample which is to be tested?

ii) Is it desirable to test a proportion of individual bottles after the bulk sample has been divided up?

iii) How do tests on large volumes compare with repeated tests on the sum total volume?

Unfortunately, the answer to each of these questions is coloured more by practicability and by the processes involved than by statistical desirability. There is obviously no virtue in increasing the volume of samples unless the entire sample is to be innocuity tested. There is equally no object in taking large numbers of small samples from a pooled bulk if the pooled bulk is maintained in a reasonable state of mixing throughout the sampling procedure. Similarly, there is no object in collecting individual samples from individual bottles of the final product unless it is believed that some reactivation can occur during the process of bottling. Again, if the material being bottled is maintained in a state of homogeneity during the process, there would be no case for the sampling of individual containers. On the other hand, in control of bacteriological sterility the sampling of containers is obviously of great value, and may influence our approach to the selection of samples for innocuity tests.

2. Mechanics of the Test

(a) Choice of species

It must be generally agreed that the test should be carried out in the system most likely to detect small amounts of virulent material. At the time when Henderson wrote in 1952, it was clear that cattle were the animals of choice since the susceptibility of the baby mouse had only just been discovered and no steps had been taken to test this species in comparison with cattle on the question of innocuity of vaccines. The fact that the mouse was soon shown to be equal in sensitivity to cattle in the detection of end-point dilutions of virus led one to believe that this species could be substituted for cattle in innocuity tests. When end-point determinations were carried out on time of inactivation by formalin, however, it was found that samples which were innocuous when tested in mice still had considerable residual infectivity

when inoculated into cattle. In inactivation with 0.05% formalin, Brown et al. (1963) found that the end-point in mouse tests was approximately 72 hours at 26°C at pH 7.6 but in the experiments in which vaccines were tested for innocuity by intradermal inoculation of cattle tongues infective virus was detected in suspensions which had been incubated with the same concentration of formalin up to 144 hours. A similar extension of period on a different time scale was obtained with inactivation with acetylethyleneimine. Similar results have been obtained using tissue cultures and a method of detection of active virus but my colleague, Mr. Fagg, who will be giving a paper later at this meeting, will be describing experiments in which the blind passage of material inoculated into tissue cultures has enabled him to detect the presence of active virus. Comparative experiments on the determination of the end-point following formalin inactivation in cattle and in tissue cultures have yet to be conducted but it would seem that the use of blind passage might be of a sensitivity nearly approaching that of cattle. This would be of considerable advantage for the routine testing of foot-and-mouth disease vaccines. Until such work is taken further the cattle test must be accepted as the base line for the consideration of innocuity tests of vaccines.

(b) Route of inoculation

The greatest controversy has ranged over the relative merits of inoculation of a large dose of vaccine into cattle by the subcutaneous route or inoculation of a relatively small dose intradermally in the tongue. This is no new question for it was discussed by Henderson (1952b) and an experiment was devised by him to demonstrate the way in which a large dose of vaccine given subcutaneously interfered with the detection of a small number of infective doses of live virulent virus given in the same inoculum. Only 2 of 8 cattle inoculated with the mixture reacted, whereas 4 out of 4 inoculated with the same amount of virus without vaccine by the subcutaneous route reacted. When the vaccine-virus mixture was given at 21 sites on the tongue of each of 4 animals, 4 sites were found to be positive, which was approximately the calculated number based on the dilution of the virus in the inoculum.

To attempt to gain the best of both worlds it has been suggested that the simultaneous inoculation of vaccine intradermally in the tongue along with a large dose subcutaneously would avoid the interfering effect in the case of the tongue inoculation and give the advantage (if advantage there be) in the inoculation of a large dose of material subcutaneously. Such a procedure would overcome the problem of the rapid development of immunity following the subcutaneous dose since the tongue lesions might be expected to appear within 48-72 hours of the tongue inoculation, i.e. before any antibody response would be detectable. This method presents no real advantage over the inoculation by the intradermal tongue route alone and may possibly lead to interference even with a rapidly developing tongue lesion. As far as I am aware, no critical experiment has been carried out on this system except the one in Henderson's paper in which the subcutaneous dose of vaccine did not interfere with the development of tongue lesions. Henderson felt that the giving of a large dose of virus subcutaneously would preclude later sensitivity testing on the animal to determine whether it had in fact an immunity before the experiment began. This would be a useful control in countries where the susceptibility of animals is in question but some observations made in this laboratory would suggest that, with a potent vaccine

given in the tongue for innocuity testing, some immune response may be obtained, and the susceptibility of the animals may in any case be in doubt if the test is deferred until 14 to 21 days after the primary test.

The decision, then, on whether to inoculate vaccine only intradermally in the tongue or subcutaneously as well depends essentially on whether one is prepared to trust the intradermal tongue inoculation as being the most sensitive method for the detection of virus. Henderson (1952a) compared the sensitivity of cattle inoculated subcutaneously and intradermally on the tongue with a number of strains and found that they varied in their relationship. In the extreme case 250,000 times as much virus was required to infect by subcutaneous route as by intradermal but there were strains in which the ratio was as low as 1:4 (one strain) and 1:40 (one strain). Most strains, however, fell in the range from several hundred to several thousand times for the advantage of the intradermal tongue route. Clearly, for the majority of strains, therefore, the advantage in testing lies with the intradermal tongue route and for those strains where the two routes are of equal or nearly equal sensitivity there is little advantage in the subcutaneous route. In preparing successive batches of vaccines from different strains one cannot know without preliminary test into which category the unknown strain falls. If the strain is a routine vaccine strain it may of course be possible to determine this property beforehand and modify the innocuity test accordingly but for random strains it would seem better to make the innocuity test by intradermal route and so avoid the previous factor of interference by the subcutaneous route.

So far I have been discussing the tests of foot-and-mouth disease vaccines prepared from cattle tongue epithelial material. For such material there is no doubt that the tests on cattle tongue are more sensitive than tests in any other way so far described but if, in the future, vaccines are prepared from virus passaged in, for example, cells derived from pigs or from a cell line which might be derived from hamsters, the question of innocuity tests might require to be re-examined. It is probable that virus vaccines derived from cattle tissues (for example, calf kidney materials) might very well be adequately tested in cattle but the question of adaptation of other cells, making the virus less adapted to cattle, raises an interesting problem. With experimental batches of vaccine prepared from BHK cells we have not noticed any greater sensitivity of the BHK cells for detection of active virus than inoculation of cattle but we are fully aware of the possibilities and will continue to do comparative tests. It is of interest that the work of Dimopoullos et al. (1959) indicated that residual virus could be detected more readily in severely heat-treated material by cattle inoculation than by tissue culture inoculation, although the two methods appear to be of roughly equal sensitivity in respect of materials which have not been so severely handled. The same authors have found that unweaned mice failed to detect such virus at all and guinea pigs were also of no value. Whatever the result of further tests in these directions, however, I think we can conclude that we would be very reluctant to use a vaccine which, for example, was infective in cell cultures but not in cattle as, sooner or later, one might expect infection to arise through the clinical use of such a product. Another question which arises is that a vaccine which has been found to infect cattle intradermally in the tongue might be considered for subcutaneous inoculation on the basis that it is unlikely to set up infection by this route. Such an approach, I think, has been very generally ruled out by most producers of vaccine, largely on the basis of the fact that although such a vaccine might be safe in very many cases inevitably the outside chance of infecting an animal with the material would arise.

The realization that the single innocuity test on a batch of vaccine was open to such statistical criticism as was put forward by Henderson has led workers in the field of both poliovirus and foot-and-mouth disease vaccine preparation to consider whether a series of negative tests on successive batches of vaccine justifies greater confidence in the results of the test. Lucam et al. (1957) reported the innocuity tests on 271 batches of vaccine, for which 542 cattle had been used. These tests had been entirely negative and the authors concluded that, "if at the moment of each test the interpretation of the result should be taken with reserve, the accumulation of tests permitted in retrospect a precise enough conclusion of the average value of the material produced". A similar statement in relation to poliovirus vaccines was made by Salk and Gori (1961). They concluded that "while the size of the sample is of great importance for increasing confidence in the significance of a negative test on a particular batch, the significance of a negative test on a sample of any size is greatly enhanced if it is one of a long series of negatives rather than one interspersed among positives". The thinking in both cases is clearly on the lines that a series of negative tests gives confidence in the techniques of preparation and the ability of the producer to replicate the technique and so arrive at a safe product. This may be a satisfactory approach for the well-established vaccine producing institute but would seem to underline the necessity for as large-scale innocuity tests as possible in the early stages of production by an institute without experience in this field and even within the experienced institute if a new strain is incorporated in the vaccine. Increased confidence as a result of repeated tests on successive batches should not be allowed to blind the producer's eye to the fact that on the individual test the limits are those laid down in Henderson's paper and I am sure that we should continue to make our innocuity tests as carefully as possible with the information we have until further work results in new methods being devised and tested.

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CONTROL OF EFFICACY OF INACTIVATED FOOT-AND-MOUTH DISEASE VACCINES.

GENERAL CONSIDERATIONS

by

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Preliminary Remarks

The term "control of efficacy" has been used because, up to the present time, it has been in common use and also because it is the term in the item in the agenda of this meeting. We prefer the view of those who propose to replace it by "control of activity". In fact, a well prepared and perfectly useful vaccine may not be efficacious in the circumstances under which it is used.

Control of efficacy is normally independent of the control of innocuity and in principle should be undertaken only when the latter has been finally completed. This implies the use of definitely susceptible animals for each of these tests.

This will always be the case when an amount of vaccine above the normal dose is used in innocuity tests. Sometimes, however, the two tests are carried out in the same group of animals, when cattle are used, mainly for economic reasons.

For example: subcutaneous injection of a normal dose of vaccine and observation for at least 15 days; then, if no evidence of foot-and-mouth disease is found, experimental injection to demonstrate the resistance conferred by the vaccine.

Or: injection of a quantity of the vaccine at one or several points on the tongue and subcutaneous injection of a normal dose: then, observation for at least two weeks followed by experimental infection. While these procedures may be justified to some extent when cattle are used, they should not be used with small laboratory animals whose use in large numbers do not incur any particular difficulties or great expense.

Choice of Animals.

It may be considered to be logical to carry out controls of efficacy in the species of animal in which the vaccines should afford protection against foot-and-mouth disease.

It is for this reason that experimenters first used cattle because they were the particular animals to be immunised. At the present time many vaccines are still being tested only in cattle or, on the other hand, the tests may be completed by procedures based on the use of other animals e.g. guinea pigs. Extension of vaccination to small

ruminants and pigs led vaccine-producers to use also these species in control work. While, up to the present time, it is not possible to do without animals for control work, an obvious point claims the attention of experimenters: animals to be used, no matter what their species have, generally speaking, somewhat poor biological reactivity because of the inequalities in their behaviour to different viruses and to their immunological responses. This drawback is found particularly when small numbers of animals are used and applies specially to cattle.

It is quite difficult to establish strict statistics under these conditions whereby significant differences are obtained and experiments can be accurately reproduced.

While some researchers have already done something in the study of several factors capable of influencing the behaviour of the animals during experiments, it does not seem that this important question has been examined very extensively or very deeply whereby strict and definite conclusions can be drawn.

Among the factors which have to be taken into consideration, are particularly the following:-

1) Breed: Without any doubt, differences of sensitivity to the virus have been noted, for example in Irish and Moroccan cattle, as well as in Montbeliard and Breton cattle by inoculation of virus SAT1.

2) Age: This is an important factor as much from the point of view of receptivity as of the ability to react to different antigens. An extreme case is the difference in behaviour of calves and adult cattle.

3) Sex: This should perhaps be taken into consideration although earlier tests did not seem to show up any difference in animals of different sex, but of the same size.

4) General Condition: The behaviour of animals in poor condition is often quite different from that of animals in good health. While bodily condition does not seem to influence receptivity to the foot-and-mouth disease virus, diseases, according to Parat, are unfavourable to the development of vesicles. The capacity for immunisation is certainly better in healthy than in unhealthy animals.

5) The Time of the Year: (?)

6) Feeding: The work of Cottereau-Gilbert and Keusch on this point should be looked at in particular.

7) Temperature and Humidity [illegible] place where the animals are kept. The same vaccine tested at the same time in two groups of cattle as similar as possible, one lot kept in a stable with a low temperature and one in a stable at 20°C, may give quite different results. Shach, using inoculated guineapigs, showed "that cold delayed the appearance

of foot-and-mouth disease or even suppressed it, while heat had the opposite effect".

8) Atmospheric Pressure: The work of Primault in Switzerland on this subject should be borne in mind. According to this author, cattle may perhaps resist a low concentration of virus when they have only slight receptivity. On the other hand, increased receptivity may be demonstrated by a low barometric pressure in the ambient surroundings.

9) Pigmentation of the tongue mucosa: It would be well to show whether this has any definite influence: it may be problematical.

10) Thickness of the skin: Parat showed that there was a relationship between the amount of virus collected from an animal injected intradermolingually and the thickness of its skin. The facility of forming and developing vesicles therefore varies with the animal: the result is that it may not be the same in animals having theoretically similar degrees of immunity but whose skins differ in thickness.

11) The injection of hormones and antihormones in animals for slaughter. This factor entails a difference in behaviour (+ or -) between treated and non-treated animals, when injected with virus.

12) Weight. This is especially important for guineapigs and mice.

13) Pregnancy (?)

14) Production: Particularly milk production.

15) Natural or acquired immunity. Natural immunity of different degrees is found in at least 0.5% of cattle. This factor is far from negligible with the small number of animals generally used.

Systematic study should be made to understand these different possibilities.

It is doubtful, however, whether experimenters scattered throughout many countries, despite previous agreement, will adopt the practice of control methods in almost identical cattle.

With some uniformness of the conditions required in the choice and use of control cattle, there would be an appreciable measure of reduction in the inequalities which are too often seen. Concerning small laboratory animals it would be tedious again to stress the need to use sufficient numbers in order to reduce and even eliminate the differences in their behaviour following identical treatment.

Statistical study of the results recorded in observations of any kind of biological phenomena is of the greatest importance in directing researchers into the right path, sometimes very winding, to lead to scientific truth. This mathematical enquiry which must be entrusted to specialists, applies to all producers and controllers who have the heavy task of providing vaccines of indisputable activity, for veterinary practitioners.

Administration of the Vaccine.

Although the subcutaneous route is invariably used, the dose of the vaccine may, of course, vary. The appreciation of the value of a vaccine is often based on the resistance conferred by the dose normally used. The use of varied doses, reduced doses being more important, tends to give a more precise appreciation of the quality of the vaccine. This is notable with the technique proposed by Henderson. While the results obtained are more exact (and this undoubtedly is the case), a larger number of cattle, however, has to be used to obtain them.

In spite of this disadvantage, the advantages from the use of this method with fractionated doses are such that they should receive our most serious attention.

Time required for the development of immunity.

The time depends on both the animal and the nature of the vaccine. In fact, it is evident because of the influence of the factors mentioned above that the development of immunity is not identical in all experimental animals and some vaccines appear to act more rapidly than others. The addition of saponin seems particularly to increase defense reactions. Usually, a period of 15 days is given as the time between vaccination and challenge. Would it not be preferable to fix the time at 3 weeks, which would allow for the production of better serological reactions?

Challenge - method of infection.

Different techniques are used.

(a) The vaccinated animals are simply placed in contact with clinically affected animals during the acute stage of the disease and they are kept in contact for at least 15 days.

This is apparently a more severe test than those mentioned below. All the vaccinated animals should resist infection entirely (no temperature, no lesions in the mouth, no other symptoms).

(b) A pad soaked with virus is rubbed quite forcibly on the tongue and the mucosa of the mouth.

It can readily be appreciated that it is difficult to treat all the animals identically with this method so that similar amounts of the virus are introduced into the superficial layer of cells of the mucosa of the mouth. While it is difficult to state the importance which this method has on the future development of the infection, it can, nevertheless, be said definitely that it lacks precision. However, it gives results in practice and some vaccines are still being controlled in this way. Well-immunised animals should resist the infection entirely. While a few local losions arising from the energetic treatment may not be taken into consideration, in no case must generalisation occur.

(c) By injection of the challenge virus, exact titration of the amount of virus used can be ascertained.

The infective power of the challenge virus has to be carried out as precisely as possible before the challenge is done.

When this has been ascertained, an emulsion e.g. 0.2 ml containing 10^4 DL50 is prepared. This quantity is usually injected intradermolingually and, with rare exceptions, causes generalisation (foot lesions) in the control cattle.

The Challenge Virus.

Not only should the challenge virus be the same type as that used in the preparation of the vaccine - it should be the same sub-type.

It is best therefore to use the same virus as that used for the preparation of the vaccine.

While with some strains of virus, the vaccine confers an immunity somewhat wider than that expected from its apparent antigenic quality, there are others whose specificity is very narrow: in these latter vaccines, control by a strain of virus of slightly different antigenic structure runs the risk of not making proper conclusions of the activity of the vaccine. Therefore, in practice, control should be carried out with the strain of virus used for the preparation of the vaccine: further tests with other strains and particularly with sub-types would probably show up exactly all the possibilities of a vaccine. There are many examples of difficulties in connection with vaccination in the field which arise from lack of sufficient similarity between the vaccine and the strain of the virus causing the epizootic. The particular plasticity of the virus of foot-and-mouth disease, as shown by the already recognised sub-types, is not the least of the difficulties to be overcome in the field of immunisation against foot-and-mouth disease.

The Techniques.

The author of this note does not propose to go into detail or to compare the respective values of the different techniques which several research workers have already proposed. There is already an appreciable diversity among them and each of them would merit a special note on all its characteristics and on the information which could be given about it. The first group consists of three techniques based on the use of highly susceptible cattle; (contact; rubbing the virus on the tongue and mouth; injection). The most widely prevalent or at least the one which has been mostly used is that proposed by a committee of experts in Berne in 1947 and which we had occasion to give in detail in 1953.

It is based on the study of possible generalised lesions in vaccinated cattle which have been experimentally infected. Its objection is, and its critics agree, that too small a number of animals is used and general immunity is not taken into consideration. However, after having used this technique in the control of over 800 vaccines,

mostly trivalent, with the necessary use of over 9,000 cattle, it may be stated that it is perfectly allowable under the following conditions:

(a) to use only completely susceptible animals;

(b) to apply severe and unchangeable criteria.

There must be no lesion of generalisation on any of the 3 vaccinated cattle, while the control animals must show secondary foot vesicles. The use of vaccine satisfying these conditions will ensure local and general immunity of an absolutely satisfactory degree in the field. However, and here is a shortcoming, the method only allows a vaccine to be classified as good or not good. It does not allow of a comparison between good vaccines and gives no information whereby producers can change the formula of preparation for improvement of the products. The second method which is much more precise shows great progress because it is quantitative. Proposed by W.M. Henderson and L.A. Galloway, it is based on the 50% protective dose, preventing the occurrence of secondary lesions following the intradermolingual injection of 10^4 DL50. Unfortunately, its use is very expensive, for a considerable number of cattle is required. Nevertheless, the fact that it enables a very exact appreciation of the value of vaccines to be made in animals in which they are to be used, seems to justify its use on each occasion that a decision is required or to confirm that a substantial modification in the method of preparation of vaccines is well founded. We think it should be the reference test on which there should be based the justification of the value of new techniques to replace others, with all the guarantees of exactness, facility and reproductibility. It is in this spirit and with this in mind that F. Lucam and M. Fedida proposed the third technique of this first group: as the authors say "it consists in calculating an index of foot-and-mouth disease immunity, or briefly, an index of protection by comparative titration of the virus of foot-and-mouth disease in control and in vaccinated cattle". It is based on the observation of primary and secondary lesions on a considerable number of inoculated cattle.

Lucam and Fedida state that the primary tongue lesion completely conditions the secondary foot lesions. After having studied several hundred vaccinated animals, they conclude that foot-and-mouth disease immunity may be expressed in terms of the local immunity quite as well as in terms of the general immunity. This led them to propose an index of protection, K, which is calculated by the formula $K = \frac{T}{V}$ where T is the titre of the challenge virus in control animals and V is the titre of the same virus in vaccinated animals. This technique is easily applied and requires fewer animals than does the titration according to the Henderson and Galloway technique. We have already expressed the wish that the value of the K index should be tested by different workers and we regret that, with the exception of a critical study presented in 1960 by Leunen and Strobbe, no views, so far as we are aware, have been expressed on the subject.

We repeat the view that this important contribution to research on a foot-and-mouth disease immunity test should be a study of research workers in specialised institutes.

The second group of techniques comprises those based on the use of other animals, e.g. guineapigs. Observations made on guineapigs have already been recorded by several workers, e.g. Rosenbuch and Cordarelli, Scholein Rivenson, Ubertini and Nardelli, Mackowiak et al., Olechnowitz-Heining and Ludwig, etc.

Also today, our attention is directed to some important work of the IFFA team on this interesting method of control.

Mice have also been successfully used, it seems, by Cunha-Uhlmann and Traub and by Dhennin et al. The authors stress the fact that the tests should be carried out on a sufficiently large number of animals which should conform to certain conditions concerning breed, weight, health and uniform feeding and kept under fixed conditions. Undoubtedly, the use of small laboratory animals in control work has real advantages. There is a fact, however, which must be stressed: following vaccination, these small laboratory animals should be tested with viruses which have previously been adapted to their species. In adapting virus by a variable number of passages, there is a risk of its structure being somewhat modified. This modification may be only minimal and without serious effect on the titration of the activity of a vaccine: still, it is important to ensure the avoidance of deviations which would lead to inexactitude of the method.

The third group comprises techniques for study of specific antibodies in the serum of vaccinated animals (cattle, guineapigs, mice, chickens), by serum-neutralisation, serum-precipitation, complement fixation, serum-protection. Many authors have worked on these subjects and reference may also be made to the interesting report on the control of foot-and-mouth disease vaccines presented by Mackowiak and his colleagues at the Biological Standardisation Congress in 1959, where a long list of publications on the subject is given. It is becoming more and more evident that there is a parallelism between the antibody titre, as shown particularly by serum-neutralisation, and the degree of protection conferred by vaccination.

In the fourth place, we note with interest that some research workers combine different techniques: in this connection, there is the combination of tests proposed by F. Lucam, M. Fedida and G. Dannacher. This consists successively in establishing an index of protection C, for the guineapig, followed possibly by calculation of a sero-neutralisation index, S, and even by an index, K, in cattle. The details are given in a report presented at this meeting.

Finally, it may be recalled that K. Hibohm in 1949 worked on the determination of an antigen in a vaccine by elution of the virus and titration using complement fixation.

What conclusions can be drawn from this brief survey of the many control methods? All of them have advantages as well as disadvantages of various degrees mainly because, almost without exception, they are based on animal reactions of different kinds.

Nevertheless, a great deal of progress has been made. It seems necessary to endeavour to recognise all the reactions of the vaccinated subject (general reaction, local lesion, generalised lesion, antibodies, viraemia) following upon a vaccination and the behaviour of the animal when subjected to challenge with a virus.

A wise choice among the proposed techniques should allow combinations of enough factors for evaluation to be made.

OFFICIAL CONTROL OF FOOT-AND-MOUTH DISEASE VACCINES IN FRANCE

by

F. Lucam, M. Fedida and G. Dannacher

The control of foot-and-mouth disease vaccines in France is laid down officially by Ministerial Order of 8 June 1965, which specifies, in its Annex, details of the organization of the control and gives the essential requirements of the methods which are to be used.

This report will, therefore, comprise of three parts. In the first, we shall give the full text of the Order and its Annex. In the second, we shall comment on several particular points, which are of importance and should be stressed. Finally, in the third part, we shall indicate the practical scope of these texts.

The Official Texts

Ministry of Agriculture

Official control of foot-and-mouth disease vaccines

Minister of Agriculture
Articles 611-617 of the Code of Public Health;
Decree No. 52-166 of 14 February 1952, modified by
Decree No. 55-301 of 15 March 1955, by the one
Article of the law No. 58-147 of 17 February 1958,
by Decree No. 60-663 of 4 July 1960;
Decree No. 53-449 of 13 May 1953, modified by
Decree No. 60-663 of 4 July 1960;
Order of 31 July 1953, modified and completed by
Orders of 21 July 1955, 1 July 1957, 17 August 1961
and 19 November 1962;
Views given by the Veterinary Committee on Sera and
Vaccines at its Meeting on 8 April 1965.

Order

Article I

In applying Article 611 of the Code of Public Health, the preparation, importation, detention, sale or the free transfer of foot-and-mouth disease vaccines are authorised by the Minister of Agriculture only in the case where these products have been sampled for official control and have been found satisfactory according to the conditions prescribed in the Annex of the present Order.

Article 2

The official methods for control are those of Protection K Index in cattle, Protection Index 2 in guineapigs and sero-neutralisation Index S in tissue culture.

Article 3

The central control and research laboratory of the Veterinary Service, Alfort, and the Animal Virology Laboratory, Lyons, are designated for the control work mentioned above.

Article 4

The Director General of Production and Markets of the Ministry of Agriculture is designated for the execution of the present order, which will be published in the Official Journal of the French Republic.

Paris, 8 June 1965
For the Minister and by delegation
Director of the Cabinet
Jean Vaudeville

Annex of the Order

Section I - Organization of the Control

A. General arrangements

1. All foot-and-mouth disease vaccine, produced in France or imported, shall be presented for official control.

2. This control may be a control of authorisation, a control of importation or a usual control.

a) Control of authorisation is one concerning the first consignment of the vaccine produced by a Laboratory which has presented a request to the Minister of Agriculture for a first authorisation to produce this vaccine or a request for renewal of this authorisation.

b) Control of importation is one concerning all vaccine prepared abroad for which a request for authorisation for its importation has been lodged by a French Laboratory.

c) Usual control is that concerning every consignment of vaccine produced in France or imported and to be given with or without payment to veterinarians or to the State.

3. All the controls, no matter the type, shall be carried out on the final product, i.e. the vaccine in containers ready to be sent out. Each container shall have a permanent label, so that the batch from which it comes may be identified, in particular.

4. All the controls, no matter the type, shall be carried out on samples taken from batches of a minimum of 50,000 doses of trivalent vaccine for cattle.

B. Controls of authorisation and importation

1. The organization of the controls of authorisation and importation shall continue in accordance with the regulation now in force.

2. The method of control is that of the Protection K Index in cattle.

3. According to the results of the tests applied, the vaccines shall be "authorised" or "prohibited".

C. Present Control

1. Declaration: When a batch of vaccine has been distributed into containers ready for issue, the producing laboratory shall make the declaration to this effect:

a) to the Departmental Director of the Veterinary Services where the laboratory is situated and who is in charge of taking the samples;

b) to the Director of the Animal Virology Laboratory, Lyons, who distributes the samples between the official laboratories designated for the control.

2. Taking samples: The Departmental Director of the Veterinary Services or his representative shall take, with as little delay as possible, samples under the following conditions:

a) he shall select at random four containers containing a total of at least 100 doses of trivalent cattle vaccine: he will place the official seal on each of them and shall apply his signature to the labels on them;

b) he shall reserve two for the control work: the other two shall remain at the laboratory of the producer or importer who shall be required to keep them under the conditions which he normally applies during the period of validity of the vaccine;

c) he shall cause to be stored by the producing or importing laboratory all the other containers in the same batch in safes which he shall seal. On each safe, he shall apply a label similar to that on the containers and shall place on it an official seal with his signature.

3. Despatch and distribution of the samples for control: On receipt of the declaration that a batch of vaccine has been distributed into containers, the Director of the Animal Virology Laboratory, Lyons, shall inform the Departmental Director of the Veterinary Services in charge of taking the samples and the producing or importing laboratory, of the destination of the two containers kept for control work. This destination is either the Central Research Laboratory, Alfort, or the Animal Virology Laboratory, Lyons.

The Departmental Director of the Veterinary Services shall send at the same time as the two containers, but under separate cover, a notification containing the following information, of which some items shall be provided by the producing or importing laboratory:

i) name of the producing or importing laboratory;
ii) number of the batch of the vaccine;
iii) date of authorisation of the vaccine;
iv) size of the batch as shown by the number of trivalent cattle doses;
v) methods and results of tests carried out by the producing or importing laboratory;
vi) date of distribution of the vaccine in containers which he has received;
vii) date of taking the samples;
viii) date of despatch of the samples.

Following receipt of the samples and the note, the Director of the official Laboratory designated to carry out the tests shall inform the producing or importing laboratory whether the control work will be put in hand at once or will be deferred.

D. Classification of the vaccines after the control

The following table shows the modifications in the old classification.

Old classification	New classification	
"Authorized" vaccine	"Accepted" vaccine:	class I
"Acceptable" vaccine	"Accepted" vaccine:	class II
"Refused" vaccine	"Refused" vaccine	

E. Releasing the batches of vaccine

1. When the control work of a batch is carried out at once, the producing or importing Laboratory, shall be free to issue the batch in question only if the results of the tests are favourable and only up to a maximum period of 50 days from the day when the samples were taken.

2. When the control work of a batch is deferred, the Director of the Laboratory designated with the official control shall propose to the Minister of Agriculture, release of this batch without delay, while still in accordance with the arrangements laid down in Chapter 6 (Depreciations) without taking into account the time of the year and without the necessity of the producing or importing laboratory to make a request to him to this effect.

3. The Director of the official control Laboratory shall inform the producing or importing laboratory and the Departmental Director of the Veterinary Services who took the samples, of the results of the control tests of each batch of vaccine and, if they are satisfactory, the date of the release of the batch.

4. The release shall be carried out by unsealing the safes in which the containers were placed and sealed up, the unsealing being done under the supervision of the Departmental Director of the Veterinary Services.

5. No "refused" batch of vaccine shall be released. With the exception of repeat control work under the conditions mentioned in Chapter 7, the complete batch shall be destroyed by the producing or importing laboratory within 8 days after being notified of the refusal. This will be carried out in the presence of the Departmental Director of the Veterinary Services. He shall send an official report of the destruction to the Minister of Agriculture and in it, shall be stated: -

a) the name of the producing or importing laboratory;

b) the number of the batch of the vaccine;

c) the size of the batch, i.e. the number of trivalent cattle doses;

d) the method adopted for the destruction;

e) the date of the destruction.

He shall send a copy of the official note to the official laboratory charged with the control work.

6. For all producing and importing laboratories, the proportion of "accepted - class II" vaccine shall not exceed 5 percent of the annual production, the year considered as beginning on 1 May. Any excess of this amount of "accepted - Class II" shall be classed as "refused".

The application of this arrangement is made for series of 20 batches as follows: -

A single batch "accepted - Class II" is permitted in the first series; if all the batches in the first series are "accepted - Class I", then two batches "accepted - Class II" are permitted in the second series; if all the batches in the two first series are "accepted - class I" then three batches "accepted - Class II" are permitted in the third series; and so on for the full annual production.

F. Derogations

Between 1 January and 15 April of each year, and by derogation to the arrangements of Chapter 5, batches of vaccine might be released before the results of the control tests are known, under the following conditions: -

i) the producing or importing laboratory shall make a separate request anticipating release, for each batch, to the Director of the official laboratory which has received the samples. In the request there shall be stated the conclusions which the producing or importing laboratory consider sufficient for control purposes which it has carried out, viz. -

a) the margin of safety required;

b) the probable degree of protection by the vaccine;

ii) the Minister of Agriculture may permit release on the proposition of the Director of the Control Laboratory if: -

a) the margin of safety is as great as that applied in the official control work;

b) the probable activity of the batch of the vaccine is greater than the minimum required;

c) in the last 20 batches officially controlled from the same laboratory (producing or importing), no batch was "refused" and not more than one batch was "accepted - class II";

iii) the arrangements in para (c) of the preceding chapter shall incur rejection of all requests for anticipated release if at least 20 batches of vaccine shall not have been officially controlled since 1 May of each year for each producing or importing laboratory.

This shall also hold good for: -

a) a new producing or importing laboratory;

b) a new vaccine produced by a laboratory already authorized to produce other types of foot-and-mouth disease vaccine;

iv) if, after the anticipated release has been agreed, the official control shows later that the batch of vaccine which was the subject of the request, is harmful or inactive, all new requests for anticipated release shall be rejected for the remainder of the production year.

This should also apply if a batch shall be classified as "refused", in accordance with the conditions of Chapter 5 paragraph 6.

G. Repetition of Control

Repetition of control tests for activity might be granted at the request of a producing or importing laboratory, only one repetition for each batch of vaccine being permitted.

In its request to the official laboratory which carried out the first control work, the producing or importing laboratory shall be required to agree to reimburse the State with the sum of 4,000 francs for each new control in the case of the batch being classified a second time as "refused".

The repetition is carried out in collaboration between the two official laboratories, and in the laboratory where the first control work was done, in the presence of a representative of the producing or importing laboratory, if the latter so wish.

Each request for repetition of control necessitates decision for release of the batch concerned for a second period of 50 days from the despatching of the new samples.

The sample used for the new control work shall be one of the two containers officially sealed and retained by the producing or importing laboratory. This container shall be sent by the Departmental Director of the Veterinary Services who shall verify its integrity, as well as that of the safe in which the batch of the vaccine concerned is contained.

If the results of the second control are different from those of the first control, two possibilities are envisaged: -

a) the difference might not be explained by the discovery of a chance incident in the course of either of the controls: in this case, the final result shall be the average of the results of the two controls;

b) the difference might be explained by the discovery of such a chance incident: here, the final result shall be that of the control which was not affected by the incident.

If, as the final result of the second control, the batch is classified as "refused", the batch shall be destroyed within 8 days of the notification of the refusal, under the conditions in Chapter E, para 5.

Section II - Methods of Control

A. Control of innocuity

Principle

No matter how it is used, the vaccine should not be able to set up foot-and-mouth disease. Also, it should not contain any substances which could set up any other important pathological signs in its normal application.

Material

The test is carried out in cattle fully susceptible to the virus of foot-and-mouth disease and which have the following characteristics: - 2 - 7 years old; have never been affected with foot-and-mouth disease nor vaccinated against the disease; do not possess any neutralizing antibodies in their sera against virus types O, A or C, at a significant titre. Cattle are considered as having a significant titre of those antibodies if the method of sero-neutralization Index S in cell cultures, described later, has a value for S higher than $10^{0.3}$.

Method

a) Test in cattle

The vaccine is injected intradermolingually at 20 placed on the tongue, 0.1 ml at each place. At least three cattle are used, thus there is a minimum of 60 places of injection. The animals are kept

under observation for four days. If, after this period of observation, no lesions have appeared, the vaccine is injected again in the same animals, following indications of the producing or importing laboratory, but at a triple dose of each which is recommended. The animals are then kept under observation for a further three days.

b) Test of the vaccine

The vaccine is subjected to different tests laid down by regulations in force concerning biological products.

Results

The vaccine is considered safe if: -

a) the cattle show no foot-and-mouth disease lesions or any other important pathological sign;

b) the vaccine does not contain any substance capable of being toxic in the course of its normal use.

In all other cases, the vaccine is considered as harmful and is "refused".

B. Control of Activity

Bases and norms

The activity of foot-and-mouth disease vaccine is expressed in terms of the percentage of cattle which this vaccine can protect against the appearance of secondary foot lesions following the most severe challenge with virulent virus.

The degree of protection required for minimal activity is between 70 and 85 percent for all foot-and-mouth disease vaccines.

The most severe test is that consisting of inoculating intradermo-lingually the amount of challenge virus necessary to set up in all completely susceptible cattle, secondary lesions in all the four feet.

Only one strain of challenge virus is used for the control work for each vaccine valence. The choice of the strain is fixed in terms of the needs of the general prophylaxis of foot-and-mouth disease, by the Minister of Agriculture on the advice of a Commission set up by the Chief of the Veterinary Service, composed principally of the Directors of the two official control laboratories as well as representatives of all the producing and importing laboratories.

Within the framework of the present controls, vaccines are classified in the three following categories: -

a) "Accepted - class I" vaccines

These are highly active vaccines, with above 85 percent protection.

b) "Accepted - class II" vaccines

These have less activity, with protection between 70 and 85 percent.

c) "Refused" vaccines

These are inactive vaccines, with less than 70 percent protection.

Within the framework of authorization and importation controls, the vaccines are declared to be: -

a) "authorized"

if they have at least between 85 and 95 percent protective value;

b) "prohibited"

if their protective value is less than 85 percent.

Methods

The Protection K Index method is associated with the Protection C Index method in guineapigs and the sero-neutralization Index S method in cell cultures.

The Protection K Index method in Cattle

Principle

The Protection K Index method is defined by $K = \frac{T}{V}$ in which T is the titre of the foot-and-mouth disease virus calculated for control cattle, and V is the titre of the same virus in vaccinated cattle.

It represents a number of infective units which when inoculated into the mucosa of the tongue of vaccinated cattle causes primary lesions in 50 percent of the animals.

Index K varies with the degree of post-vaccination immunity, characterized by the amount of protection against secondary foot lesions.

It follows that by the determination of K for a given vaccine, the probable degree of protection that the vaccine can confer on cattle can be known.

Material

A. Cattle: They should respond to the conditions laid down in the chapter "Control of Immunity".

B. Virus: The strains of the challenge virus types O, A and C, are produced "in vivo" by intradermolingual inoculations in cattle. The titre should be at least equal to 10^7 DI_{50} to g for cattle.

Method

For each vaccine valence, 4 vaccinated and 4 control animals are used.

The inoculations of the virulent material and the reading of the titrations are much facilitated by the use of tranquillisers in association with anaesthesing of the tongue.

A. Vaccinations: These are carried out according to the methods recommended by the producing or importing laboratory.

B. Titrations: These are carried out three weeks after the vaccination and are made at the same time with the same suspensions of virulent material in the control and the vaccinated cattle.

The suspensions in use at present are between 10^{-4} and 10^{-7} for the control cattle and from 10^{-3} to 10^{-6} for the vaccinated animals.

a) Titrations in the control animals:

This is the classical method on which there is no need to make any modifications: the results are read at the end of 24 hours.

b) Titrations of the vaccinated animals:

The same method is used, but the readings are not carried out until the end of 40 hours and the lesions should be returned as "abortive vesicles".

Results

Calculations: To determine K, it is sufficient to make the quotient T by V

Example : say $T = 10^{7.5}$ and $V = 10^{5.2}$, then

$$K = \frac{T}{V} = \frac{10^{7.5}}{10^{5.2}} = 10^{2.3}$$

Remarks: When the value of K is less than 1, log K becoming negative, the vaccines have very low activity, round about nullity. In such cases, in fact, the vaccinated animals are quite as susceptible as the control cattle. Reading of their titres at 40 hours shows that the lesions may then appear with the highest dilutions, which does not occur in the control animals at the 24th hour.

Taking into account the errors in reading due to the merging together of the lesions, there may be a V titre greater than the T so that K becomes less than 1, and its value decreasing to about $10^{-1.5}$.

Interpretation

Within the framework of the present control tests, when the Protection K Index for each vaccine valence is: -

a) lower than $10^{0.6}$, the vaccine is "refused", its protection value for cattle being probably less than 70 percent;

b) equal to or above $10^{0.6}$ but less than $10^{1.2}$, the vaccine is "accepted - Class II", its protective value being between 70 and 85 percent;

c) equal to or above $10^{1.2}$, the vaccine is "accepted - Class I", its protective value being above 85 percent.

Within the framework of the controls of authorization and importation, when the Protection K Index is, for each vaccine valence:

a) below $10^{1.2}$, the vaccine is "prohibited";

b) equal to or above $10^{1.2}$, the vaccine is "authorized".

Protection C Index Method in Guineapigs

Principle

The Protection C Index is defined by the relation $C = \frac{T}{V}$, in which T is the titre of the foot-and-mouth disease virus in control guineapigs and V, that of the same virus calculated in vaccinated guineapigs.

It represents the number of infective units which, when injected in the plantar pad of vaccinated guineapigs causes the appearance of generalized foot lesions in 50 percent of them.

Index C varies, within certain limits, with the degree of immunity set up in cattle by the same vaccine.

It follows that the determination of C for a given vaccine indicates, within these limits, the probable degree of protection that this vaccine will confer in cattle.

Material

Guineapigs: guineapigs without coloured feet for preference, weighing 500 grammes to the nearest 50 grammes.

Virus: the challenge strains of virus, types O, A and C are adapted to the guineapig. The strain is said to be adapted when, following the injection of 0.5 ml of a virulent suspension at 10^{-3} into the foot pad, the last one secondary foot lesion develops in a minimum of 90 percent of animals.

The adaptation should occur with a limited number of passages, 5 - 7 at most.

Method

For each vaccine valence, 28 vaccinated and 28 control guineapigs are used.

Vaccinations: are made subcutaneously, 1/20 of the cattle dose recommended by the producing or importing laboratory being used.

Titrations: are carried out three weeks after the vaccinations, on both vaccinated and control guineapigs at the time and with the same virulent suspensions. The current virulent suspensions in use are 10^{-3} - 10^{-6} for the control animals and 10^{-2} - 10^{-5} for the vaccinated animals.

a) Titrations in the control guineapigs:

The titre is expressed by the DG_{50} or the Generalization Dose to 50 percent, i.e. the quantity of virus necessary to give rise to generalized foot lesions in 50 percent of the guineapigs.

The 28 guineapigs are divided into four groups, each of 7 animals, all of which receive intradermally into the plantar pads 0.5 ml of the abovementioned virulent suspension. Readings are made at the 72nd hour. A guineapig is considered to show generalization when it shows one secondary foot lesion. The DG_{50} is calculated by the method of cumulative totals.

b) Titrations in the vaccinated guineapigs:

This is exactly similar as in the controls.

Results

These are only acceptable if the virulence titres in the controls are at least equal to 10^5 DG_{50} to g.

For determination of C, the quotient $\frac{T}{V}$ is used.

Example: $T = 10^{5.8}$; $V = 10^{3.2}$ then

$$C = \frac{T}{V} = \frac{10^{5.8}}{10^{3.2}} = 10^{2.6}$$

Interpretation

Within the framework of the present control tests, if the Protective C Index of a type of virus is equal to or above 10^2, the vaccine is "accepted - class I". The probable protective value of this vaccine for cattle is, in fact, equal at least to 85 percent. Below this value, it is not possible to draw any conclusions and recourse must be had to the method of Protection K Index in cattle. Within the framework of the authorization and importation controls, recourse must always be had to the K Index method, irrespective of the value of C.

Method of Sero-neutralization S Index in cell cultures

Principle

The sero-neutralization S index in cell cultures is the average amount of serum antibodies in a group of vaccinated cattle. It is expressed by the average dilution of sera of vaccinated cattle which protects 50 percent of the tubes of culture against cytopathogenic action of a virulent suspension of known strength which destroys all normal cell cultures.

Index S varies within certain limits in accordance with the degree of post-vaccinal immunity. It follows that the determination of S for a given vaccine, within these limits, indicates the probable degree of protection which this vaccine can confer in cattle.

Material

Cattle: At least 4 cattle, conforming to the conditions mentioned in the chapter "Control of Innocuity" are vaccinated according to the methods recommended by the producing Institute.

Serum: The sera of each bovine animal are obtained three weeks after the vaccination.

Cell cultures: These are first explant cultures of monolayer cells of calf kidney. They are made in tubes and are used when 4 - 7 days old. When they are used, the medium is replaced by new medium but without normal calf or foal serum which the medium normally contains.

Virus: The strains of challenge virus O, A and C, are adapted to monolayer cell culture. Their titres should be at least 10^7 $DICT_{50}$ to the ml (10^7 50 percent infective doses for cell culture).

Method

This is fixed amount of virus and varying amounts of serum.

Virulent suspensions: These are made in the medium used for cell cultivation, but without normal serum which the medium normally contains: the suspensions contain 500 $DICT_{50}$.

Serum dilution: Each serum after inactivation by heating at 56°C for 30 minutes is diluted in the same medium as the virulent suspensions. The dilutions are in geometrical progression of 2 rising from 2^{-1} to 2^{-7}.

Virus-serum reaction: To 1 ml of each serum dilution, 1 ml of virulent suspension is added. After shaking, the mixtures are placed in the incubator at 37°C for one hour and are then shaken again.

Final reaction

a) Preparation

Cell cultures + serum-virus mixture

On removal from the incubator, the serum-virus mixture is distributed into 5 tubes in 0.2 ml amounts: each tube receives about 50 $DICT_{50}$.

Serum control

0.1 ml of the serum being tested for neutralization value is placed in each of 5 tubes.

Virus control

0.1 ml virulent suspension, say also $DICT_{50}$ is added to each of 5 tubes of cell culture.

The virulent suspension itself is titrated in cell cultures according to the classical methods in order to confirm that the quantity of virus used in the tests is always certainly equal to 50 $DICT_{50}$.

b) Incubator

All the series of tubes are placed in rotating drums in the incubator at 37°C for 72 hours.

Results

A note is made of the number of tubes in each series in which the cellular structure is destroyed.

The results are calculable and interpretable only -

a) if no destruction has occurred in the culture control and the serum control tubes;

b) if there is complete destruction in all the virus-control tubes;

c) if the titre of the virulent suspension is shown to have been 50 $DICT_{50}$ in the tests.

Calculations

For each serum, using the series of tubes containing the virus-serum mixture and the method of cumulative totals, the dilution corresponding to the destruction in 50 percent of the tubes is calculated. This being expressed as the log of 2, it is more convenient to express it as the log of 10 which for practical purposes is done by multiplying the log 2 by 0.3.

Example: if the amount is $2^{-5.6}$, it may be written as $10^{-5.6 \times 0.3} = 10^{-1.68}$ or by convention, $10^{1.68}$.

The sero-neutralization S Index, being the average dilution of the four sera studied, is calculated by taking the arithmetical means of the logs of the individual dilutions:

Example: for the four sera, the individual findings were 10^2, $10^{1.5}$, $10^{1.5}$ and $10^{1.8}$.

S Index then is $10^{\frac{1.2 + 1.5 + 1.5 + 1.8}{4}} = 10^{1.5}$

Interpretation

a) Within the framework of the present control tests, if the S for a given type of virus is equal to or above $10^{1.5}$, the vaccine is "accepted - class I". The probable degree of protection of this vaccine for cattle is, in fact, at least equal to 85 percent. If S is below this value it is not possible to make any conclusions, and the K method in cattle should be used.

b) Within the framework of authorization and importation controls, recourse is had always to the K Index method, irrespective of the value of S.

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COMMENTS ON THE OFFICIAL TEXTS

I. ORGANIZATION OF THE CONTROL

A. General arrangements

These arrangements, without any ambiguity, establish the general application of control of all batches of vaccine produced or imported in France.

The question may be asked on whether such extensive tests are indeed useful. The simple reply is that the logical purpose of the tests is to eliminate as completely as possible unsatisfactory vaccines.

Now, what has happened during many past years? Only a small amount of the produced vaccines were controlled and, naturally, a certain number of them were rejected as unsatisfactory. It may therefore be logically assumed that there were also some unsatisfactory vaccines among those not tested. As the batches which were not tested were more numerous than those tested, the conclusion must be that there were more unsatisfactory batches passed than eliminated.

This is why it was not only useful but quite necessary that control tests be applied to the greatest number of batches produced and if possible to all of them.

Batches of vaccine should comprise a minimum volume of 50,000 trivalent cattle doses. There are two reasons for this. The first is to avoid too great a number of batches to be controlled which might interfere with the principle of generalization of the control. The second is that, whereby it is relatively easy for a laboratory with a small amount of equipment to prepare a few ml of excellent vaccine, it is much more difficult under such conditions to prepare batches on a large scale. This large-scale preparation of vaccine is the only way to ensure regular supplies of vaccine in a country where vaccination is compulsory for all the cattle. This then is an indirect way of ensuring the technical capacities of such or such a possible producer of vaccine.

B. Authorization and Importation Controls

There are already texts of regulations for these kinds of control and therefore the present order only recalls their existence. However, there are added two clarifications which were not previously made, viz., the method of control which is best to use and the classification of the vaccines, to which we will make reference later.

C. Present Control

For this type of control there were no legal arrangements before 8 June 1965: this is why many details are given about its organization.

We will deal with three of them: classification of vaccines; the limitation of "accepted - class II" batches to 5 percent of the annual output by each producer; repetition of control tests.

The first two only concern the results obtained by the tests. They will be discussed later.

Regarding repetition of the tests, the principle is admitted but with certain reservations in order to limit the frequency of its occurrence. Thus, this repetition shall not be requested by a producer unless the first tests have been unfavourable and only one repeat test shall be carried out with a batch of vaccine. Again, if the batch is classified as "refused" following the second test, the producer shall have to reimburse the State to the sum of 4,000 francs on each occasion.

The results of the second test may differ from those of the first test for two reasons: there may be a mistake in the technique or some incidence occurring during the first or second test (wrong choice of challenge strain, bad storage of the vaccine, etc.), or the difference corresponding to the margin of error which affects the method of control testing used.

This is why the final result of the repetition of the tests shall be, in the first case, that which is not affected by any incident and in the second case, the average of the results of the two control tests.

II. METHODS OF CONTROL

A. Control of innocuity

It is to be noted that double tests are made in cattle. In the first place, the vaccine is injected intradermolingually and the animals are kept under observation for four days. This first test will show the probable virulence of the vaccine by the occurrence of characteristic lesions at one or several points of the inoculation.

In the second place, if the result of the first test is negative, the vaccine shall be again injected in the same animals under the usual conditions of its administration, but three times the dose being used. The object of this second test is to show any probable pathogenic action of the vaccine which could arise from any toxic substances contained in it.

B. Control of Activity (Potency)

a) Bases and norms

The efficacy of the vaccine is expressed by the percentage of cattle which this vaccine is capable of protecting against the appearance of secondary foot lesions, following very severe challenge.

The degree of protection corresponding to the minimal activity required is between 70 and 85 percent.

It should be remembered that the choice of these norms originated in the interpretation of the results of the method of control called "Protection K Index in cattle".

This method, in fact, was used during a time when the disease was very prevalent in France and where, therefore, it was possible to compare the value of post-vaccination immunity as found in the vaccination campaigns during the controls. From this, it was possible to note that the frequency

of immunity breaks did not begin to cease almost entirely until the time after the vaccines in use were shown to have minimal values of log 2 between 0.6 and 1.2.

These values became "ipso facto" the norms for the minimal efficacy of the foot-and-mouth disease vaccines. This way of expressing the efficacy of the vaccines caused some surprise. In fact, in saying that a vaccine is sufficiently efficacious when it has a value of log K 0.6 - 1.2, it means that this vaccine protects 50 percent of the animals against all occurrences of primary or secondary lesions following intradermolingual injection of 4 - 16 DI_{50}.

In many laboratories, the efficacy of the vaccines is expressed as the percentage of cattle protected against generalized foot lesions only and this, following the intradermolingual injection of a very large amount of virus amounting to several thousand cattle DI_{50}.

The two systems seemed to be so different from one another that one has the right to ask if there is really any agreement between them.

The reply to this question was the relation which was established between the variations in K and the corresponding variations in the degrees of protection against the foot secondary lesions during the 8 - 10 days' observation when they could occur. The first results obtained in 1961 have since been completed. They are now shown in Table I and in the graph in Table II.

Table I

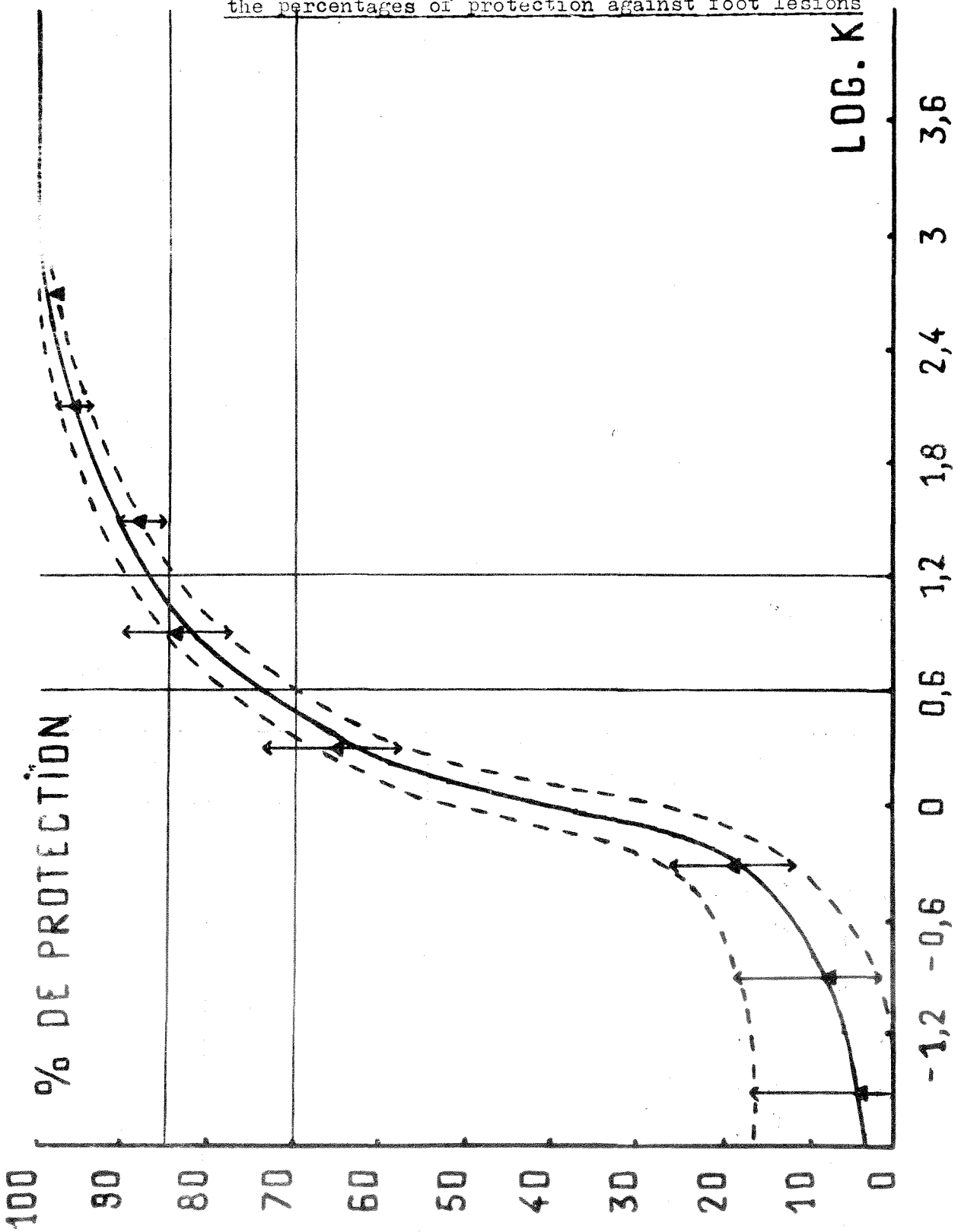
Agreement between the values of K and the percentage of protection against foot lesions

(An animal is considered as protected if no foot lesions occur)

Log K	No. of cattle tested	No. of cattle protected	Percentage of protection
$K < -1,2$	28	1	4 %
$-1.2 \leq K < -0.6$	52	4	8 %
$-0.6 \leq K < 0$	112	22	19 %
$0 \leq K < 0.6$	136	89	65 %
$0.6 \leq K < 1.2$	154	129	84 %
$1.2 \leq K < 1.8$	160	141	88 %
$1.8 \leq K < 2.4$	301	289	96 %
$2.4 \leq K < 3$	389	381	98 %
$3 \leq K < 3.6$	520	520	100 %
$3.6 \leq K$	490	490	100 %

TABLE II

Relation between the values of log K and the percentages of protection against foot lesions



The graph in Table II corresponds well with the percentages which are shown in Table I. The two curves between the lines show in both the limits of the threshold of confidence to 95 percent. Only the lower curve interests us, in that it shows the minimal percentages of protection.

These two tables show the agreement which exists between the two systems of expression of efficiency of the vaccines and it can be seen at once that, for the values of log K between 0.6 and 1.2 which have been taken as norms of minimal efficacy, the degree of protection against foot lesions is 70 - 85 percent.

b) Strain of challenge virus

It is anticipated that for each vaccine valence, only one strain of virus shall be used, the choice being fixed by the required foot-and-mouth disease prophylaxis. This arrangement is different from the usual action taken up to the present whereby for challenge the strain used is that with which the vaccine is prepared. It is necessary to ensure, in fact, that the vaccine protects, above all, against the strains of the virus found in the field when it is used and not against strains which are perhaps no longer present.

c) Method of Protection K Index in cattle

The only point to which we will refer is the interpretation of the results, i.e. the classification of the vaccines within the framework of the present controls, the vaccines are divided into 3 classes: -

for log K $\geqslant$ 1.2, the vaccine is "accepted - class I";
for log K $<$ 0.6 - 1.2, the vaccine is "accepted - class II";
for log K $<$ 0.6, the vaccine is "refused".

The question might be asked why two classes have been established among the "accepted" vaccines with the limitation of production for class II to 5 percent, since field evidence of vaccination has shown that from the time log K has been between 0.6 and 1.2, the probability of immunity breaks have tended to become almost none, showing that the degree of protection is about 100 percent.

The reason is that a vaccine to be considered as truly efficacious, it must not only protect a sufficient number of animals soon after its administration but must also protect them for a sufficiently long period of time.

We must also remember that the results of the enquiries made in connection with the vaccination campaigns dealt with a period of observation no longer than five months following the vaccinations. There is no evidence that the results were still good over a longer period.

It seems practically certain that the duration of the immunity after vaccination will be in direct relationship to the degree of so-called "immediate" immunity, i.e. the immunity found three weeks after the vaccination. This duration will be precisely ascertained when a sufficient number of animals has been used in experiments. However, it can already be stated that with vaccines with log K of 0.6 to 1.2, it is extremely short. So, if too much vaccine of this quality was used, there would be

much risk of breaks in the prophylactic campaigns.

This is why we consider that a special class with limited production should be established.

Within the framework of the authorization and importation controls, the vaccines are divided into two classes: -

for log K $\geqslant$ 1.2, the vaccine is "authorized";
for log K $<$ 1.2, the vaccine is "prohibited".

Here, there are more severe present controls. The reason is very simple. It happens very often that with a "new type" vaccine of which the control tests are satisfactory, it is necessary to be assured that the producer has the technical capacities for regular production on a large scale. It seems logical to require that the sample he submits for control tests, is above, from its quality point of view, the vaccines which are accepted normally in the present control, but with reserve.

d) Method of sero-neutralization S in cell cultures

There are three points which deserve comment; the degree of virulence of the suspensions; the serum-virus reaction; the interpretation of the results.

1. Virulent suspensions

These should contain 500 $DICT_{50}$ per ml so that in the final test, the culture tubes receive 50 $DICT_{50}$, this amount appearing to be necessary and sufficient.

It appears that from one reaction to the other, all the control virus culture tubes should have the cell structure entirely and regularly destroyed, so that, therefore, the interpretation of the results can be made without any ambiguity. It seems sufficient to have the amount of antibody even slightly raised, like those which can affect vaccines of feeble efficacy.

2. Serum-virus reaction

The duration is fixed at 1 hour at 37°C. This is largely sufficient because, for a given serum, the neutralization of the virus reaches its maximum. It has also been confirmed that a virulent suspension without any added serum, kept at 37°C for an hour, retains its titre to about 0.1 or 0.2 log 10. Thus, the fall of the titre which is characteristic of sero-neutralization in the final reaction is due not to any other thing than the specific action of the serum.

3. Interpretation of the results

When log S is above or equal to 1.5, the vaccine can be considered as "accepted - class I". Below this value, it is not possible to draw any conclusion and recourse should be had to Index K in cattle. Thus the method is limited in its use, but these limitations are probably only the results of the conditions concerning its application.

This was, in fact, done during a time when the epizootic outbreaks of foot-and-mouth disease were dying down and the numbers had become very small. It was no longer possible, therefore, to compare directly the amounts of antibody after vaccination with the results obtained during the vaccination campaigns, using the same vaccines.

There was one possible solution: to take a sufficiently large number of vaccines whose efficacy was between the least and the greatest and compare for each of them, the amount of neutralizing antibody which was developed, with their efficacy expressed as by means of a quantitative method taken as the reference method. Thus, by taking Index K as the reference, the relation between log K and log S was established.

This relation is illustrated by the dispersion diagram of Table III which has 166 points, corresponding to 166 vaccine valences, each point being determined in the abscissa by log K and in the ordinate by log S.

Table III

Relation between log K and Log S

(See page 54)

Each point of the right of regression AB of the diagram gives the theoretical average of the different values of log S for a value determined for log K. It can be seen at once that the vaccines having values of log S equal or are above 1.5, also have values of log K equal or above 1.2 and which may therefore be considered as "accepted - class I".

On the other hand, the vaccines with values of log S below 1.5, have also values of log K distributed in a manner whereby in about 2/3 of the cases, they are "accepted - class I", in 1/6, "accepted - class II" and in 1/6 "refused".

Such a result has therefore no significance since it is not possible to place a vaccine in one class rather than in the other. For this reason, under these conditions, it is better to have recourse to Index K in cattle.

Being given the average quality of the vaccines now being produced, by the method of sero-neutralization S, more than 1/3 of the cases would not have to be passed over to the virulent test in cattle.

e) Method of Protection C Index in guineapigs

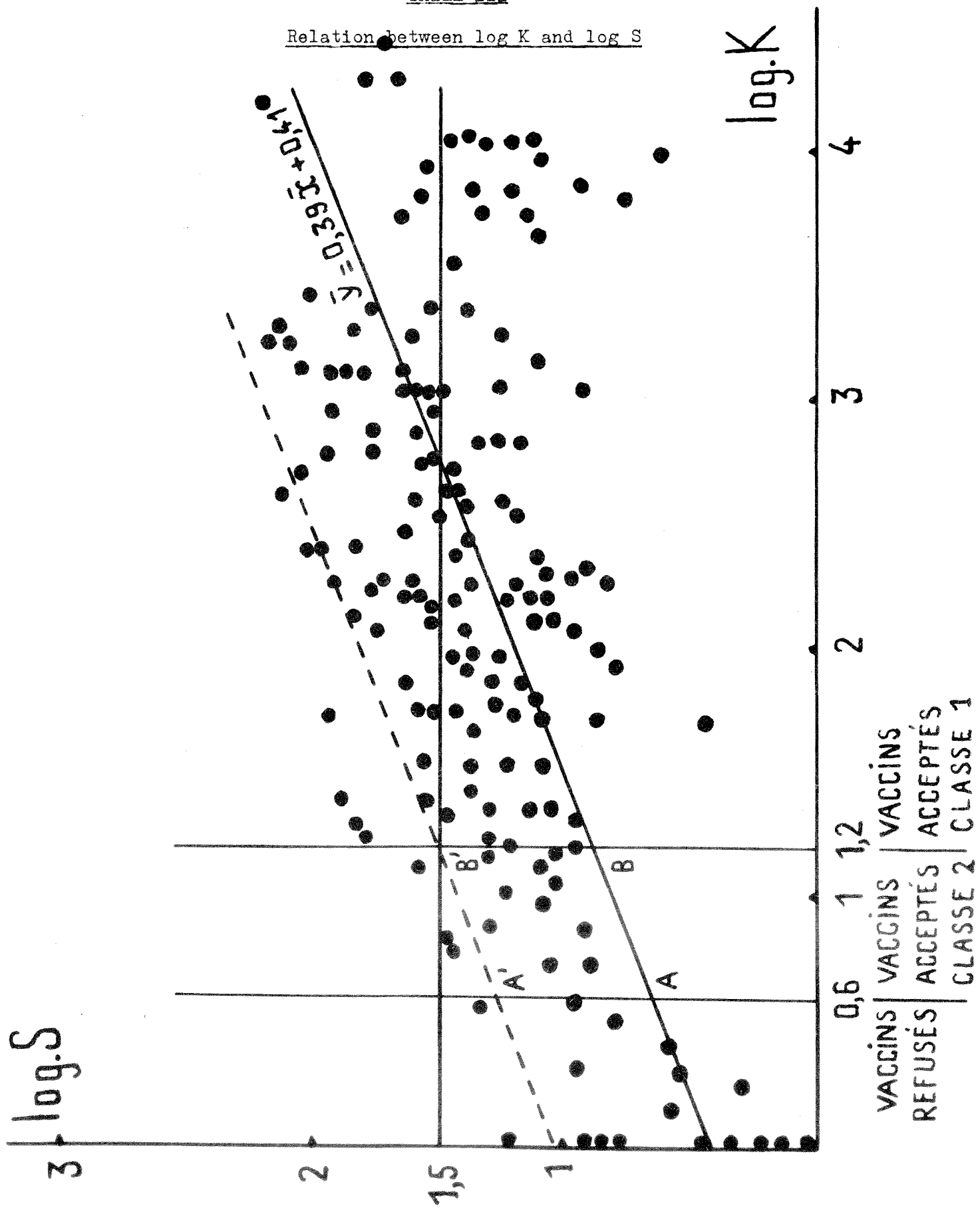
There are four points for consideration: the challenge virus, the dose of the vaccine, the titration, and the interpretation of the results.

1. Challenge virus

The virus has to be adapted to the guineapig, a maximum of 5 - 7 passages being the limitation of the passages. It is important to limit the number of passages to prevent possible modifications of the virus. It is possible, in fact, that after "M" passages its virulence for the guineapig might be different from that from further passages. One would risk, therefore, the finding of variations in the values of log C which

TABLE III

Relation between log K and log S



would no longer be in agreement only with those of the efficacies of the respective vaccines being tested.

2. Dose of vaccine for the guineapig

This is fixed at 1/20 of the cattle dose recommended by the producing or importing laboratory.

This volume should be neither too large nor too small. It should be able to be injected easily without any marked errors. The general or local reactions, caused mainly by adjuvants in the vaccines are quite small and do not affect the health of the vaccinated animals in any way.

Also, and this is doubtless the most interesting point, the volume sufficient to establish the titre of the virus in the vaccinated guineapigs will almost always also show the immunizing value of the vaccine.

3. Titrations

The challenge virus is titrated in guineapigs three weeks after their vaccination. This time of three weeks must be faithfully observed because experience shows that it corresponds with the maximal post-vaccination value and that, after this time, the immunity decreases very rapidly.

The titres are expressed by the inverse of the 50 percent generalizing dose, i.e. the amount of the virus required to cause generalization (foot lesions) in 50 percent of the guineapigs, generalization being accepted if a single secondary foot lesion appears.

This way of expression is more exact than that which consisted in taking into consideration the 50 percent infective dose. In fact, experience shows that, to be calculable, it was necessary with this method, to use dilutions of virus up to 10^{-9} and this gives rise to possible errors which are by no means negligible. Again, the difference in titre between control and vaccinated animals was not more than 10^{2} which represents a small enough margin. On the other hand, using the DC_{50}, experience shows that all titrations are practically possible with dilutions not more than 10^{-6} and that the difference in titres may reach 10^{-4}.

The reading of the titrations should be carried out at the 72nd hour in both the control and the vaccinated guineapigs, for here again, experience shows that after this time, the number of animals developing secondary lesions does not increase.

4. Interpretation of the results

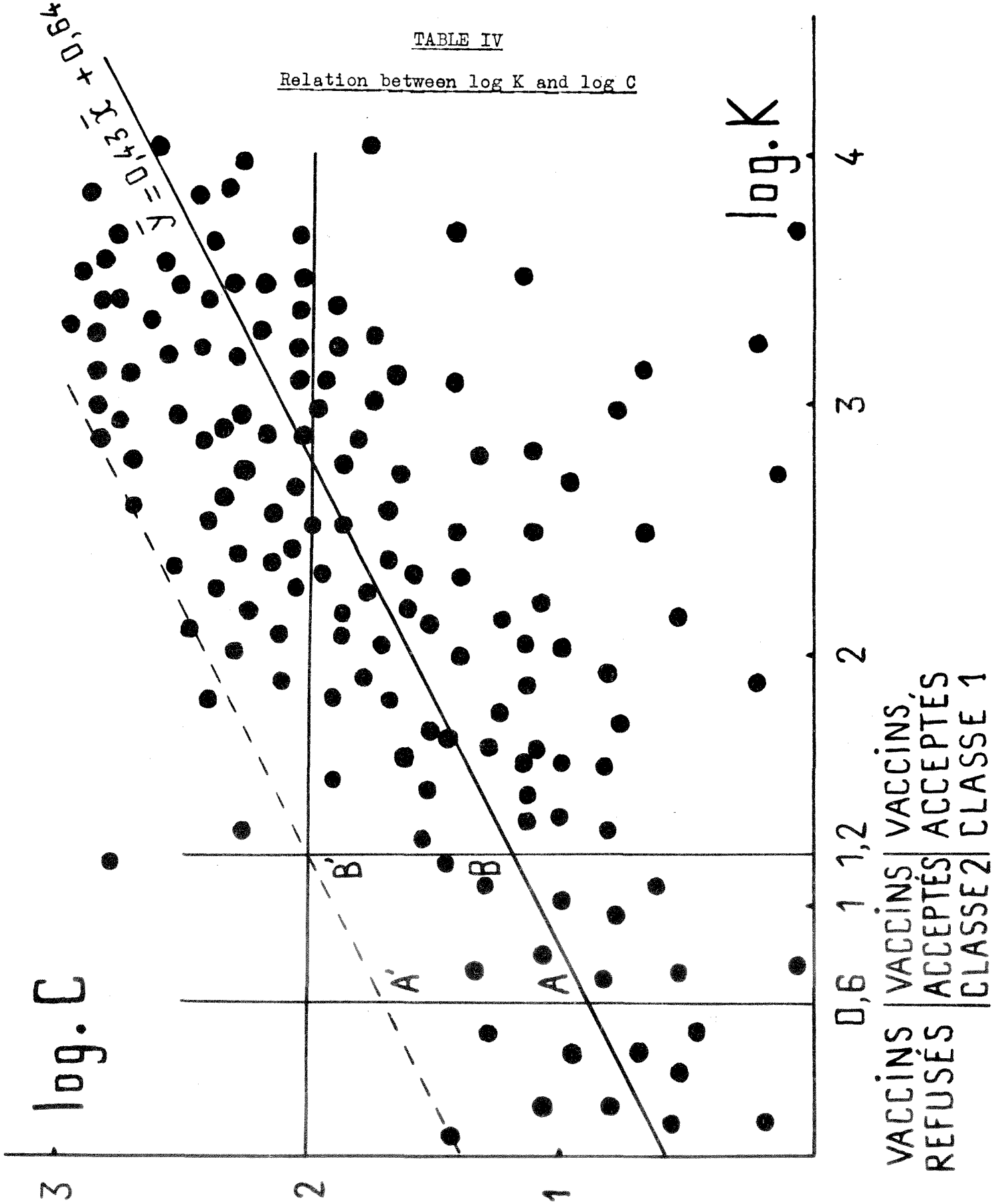
When log C is above or equal to 2, the vaccine can be considered as "accepted - class I". Below this value, it is not possible to draw any conclusions and recourse should be had to the K Index in cattle.

Here again, the use of the method is limited as is the sero-neutralization S method, the same reasons applying to both. There is no need to discuss the reasons or make comments for they are similar to those given in the case of the sero-neutralization S Index.

The minimal values of C, however, which should be retained, being different from those of S. The diagram in Table IV shows how they are established.

TABLE IV

Relation between log K and log C



The diagram in Table IV comprises 147 points corresponding to 147 vaccine valence controls, each point being determined in the abscissa by log K and in the ordinate by log S. The right AB is the right of regression of which each point gives the theoretical average of the different values of log C for a value determined by log K. The right A'B' marks for the average values of log C, the upper limit of the threshold of confidence (95 percent).

It is seen at once that the vaccines with values of log C equal to or above 2, have also values for K equal to or above 1.2 and so, may be considered as "accepted - class I".

On the other hand, with those having values of log C less than 2, there is no reason to place them in one class rather than another. This is why, here again, it is better to have recourse to K Index in cattle.

It should be noted, however, that despite this limitation, with the method of Protection Index C in guineapigs, about 2/3 of the cases may be passed by cattle.

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PRACTICAL APPLICATION OF THE OFFICIAL TEXTS

While the official texts establish generalization of the control, the question is whether, with the methods they indicate, this generalization is possible.

It should be emphasized in the first place that, concerning the methods, the decree only consecrates a state of affairs which already exists. In fact, the practical use of Index K began at the beginning of 1960: that of Index S in the middle of 1962; that of Index C at the beginning of 1964. Using these methods of control, the number of batches controlled were 40 in 1960, 69 in 1961, 99 in 1962, 166 in 1963 and 190 in 1964. It is expected that the number in 1965 will considerably exceed 200.

But, since 1961-62, when general vaccination of cattle began to become effective, the annual production of vaccine has reached a little over 300 batches, corresponding to nearly 20,000,000 doses of trivalent cattle vaccine.

Now, taking into account the present average quality of the vaccines produced in France, which has improved considerably in recent years, and because of which nearly 80 percent of the batches are released by the Index C and Index S method, the estimate of the number of animals required in the control of these 300 batches will be about 30,000 guinea-pigs and 1,600 cattle.

There is no difficulty in obtaining such numbers each year. There is also every reason to hope that from next year, the integral application of the Decree of 8 June 1965 will be effective and so, official control of foot-and-mouth disease vaccines will have its full significance.

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Some Studies on Different Inactivating Agents in the Preparation of Foot-and-Mouth Disease Vaccines

by

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INTRODUCTION

Studies on the inactivation of foot-and-mouth disease virus in the routine production of vaccines combining formalin and aluminium hydroxide lead to the conclusion that the processes involved are complex and probably incapable of precise definition. To attempt to simplify this situation we have been interested in studying agents which would regularly inactivate the virus in the absence of aluminium hydroxide under well defined conditions. Two of these are Beta-propiolactone (BPL) and acetylethyleneimine (AEI) and the present paper contains the results of inactivation studies with these agents and with formalin.

METHODS

Virus, of type SAT1 Strain SA.13/61 or type A Strain A-119, was derived either from tongue epithelial cultures or from BHK cell cultures. The source of virus did not appear to influence the inactivation results though the relative sensitivity of the assay system used varied, as discussed below.

In order to control the conditions of inactivation as precisely as possible, inactivation of virus samples was carried out in magnetically stirred glass vessels maintained in a water bath at the required temperature (Smith and Burrows, 1963 - Lab. Practice). The virus suspension was mixed with an equal quantity of a solution buffered at the pH of inactivation and brought to the required temperature before the inactivant was added. In inactivations where the virus was pre-adsorbed on to aluminium hydroxido, an equal quantity of this was added to the mixture about 15 minutes before the inactivant to allow maximum adsorption to take place. pH was controlled by the buffer used and not by gassing. For pH 7.6 the buffer was M/25 phosphate, for pH 9.0 0.05 molar glycine prepared as follows:-

Sodium hydroxide	3.19 g.
Sodium chloride	6.5 g.
Glycine	9.5 g.
Distilled water	1,035.0 ml.

The virus substrate was in most cases lactalbumin hydrolysate/Tyrode containing 0.2 per cent sodium bicarbonate instead of the usual 0.1 per cent (Morrow and Buckley; 1964). When two or more inactivants were compared under similar conditions of temperature and pH, this was done using the same batch of buffer in the same water bath at the same time. Comparative inactivations were done using 0.05 per cent acetylethyleneimine, beta-propiolactone and formaldehyde, the percentage being calculated on a volume-for-volume basis. The conditions of inactivation chosen were 37°C, 25°C and pH 7.6 and 9.0.

Samples taken at intervals during inactivation were prevented from inactivating further by the addition of one-tenth volume of 20 per cent sodium thiosulphate for AEI, by 0.04 per cent final concentration of sodium bisulphite for formalin and by rapid freezing in a mixture of solid carbon dioxide and alcohol for BPL, in the latter case samples being diluted before freezing and kept frozen until titrated. Samples were diluted in all cases, regardless of pH of inactivation, in M/25 phosphate buffer, pH 7.6, at log intervals.

Samples were titrated in 5-6 day old 'P' strain mice using 4 to 8 mice per dilution in the early stages of the inactivation cycle and 20-40 mice for undiluted material around the point of inactivation. The same dilutions were also titrated in 48 hour BHK monolayers, the virus being adsorbed for 30 minutes at 37°C, the plates then being overlaid with a mixture consisting of 74 per cent 1.3 x concentrated Eagle's medium, 1 per cent serum and a solution of 1 g. "Difco Bacto-Agar" in 25 ml. distilled water. The plaques obtained were read 48 hours later after staining for 30-60 minutes with a solution of 1:1000 neutral red chloride.

In some cases undiluted samples of inactivated material were put on to 48 hour BHK monolayers, adsorbed for 30 minutes, then Eagle's medium added, the culture being incubated for 48 hours. They were then examined for cytopathic effect. Negative cultures were seeded on to fresh monolayers and incubated for a further 48 hours. This was repeated if necessary. Cultures which showed no sign of viral growth after the third passage were presumed negative.

RESULTS

Inactivation

The comparative inactivation curves with 0.05 per cent AEI, BPL and formalin at 25°C and 37°C, pH 7.6 and 9.0, are shown in graphs 1 - 4, the assay system being mice. From these curves it will be seen that, as expected, inactivation is faster in all cases at the higher temperature of 37°C. At 25°C pH does not appear to influence the rate of inactivation by AEI or formalin, the slightly faster rate at pH 9.0 probably being due to pH inactivation of the virus rather than to the inactivant as such. It will be seen, however, that inactivation by BPL is greatly prolonged at pH 9.0 compared with pH 7.6. This is entirely due to a property of the glycine buffer, the buffer being strong enough at the molarity used to prevent rapid hydrolysis of the BPL, an essential requisite for viral inactivation. At 37°C, pH inactivation at the higher pH, 9.0, plays an important part in causing rapid inactivation of the virus but again the inactivation by BPL at this pH is prolonged compared with that at pH 7.6.

Since viral suspensions for inactivation often contain serum or extraneous protein material, inactivation of virus by the three inactivants was studied in the presence of 5 per cent normal bovine serum. Graph 5 shows that serum has no effect on the inactivation by AEI or formalin (formalin differing very slightly from the control) but does prolong the inactivation time by BPL. Alternatively, to obtain inactivation in the same period of time, a greater concentration of BPL might be used.

Although it is an object of this experimental work, of which the present paper forms part, to avoid the difficulties involved in the study of adsorption plus inactivation in the same system, it was of interest to determine how the addition of aluminium hydroxide along with the inactivating agent affected the reaction. If aluminium hydroxide is added at the same time as BPL, the pH falls at once below the range of virus stability (Graph 6). If the aluminium hydroxide is added during hydrolysis of BPL, the pH fall does not take place unless the addition is too rapid (Graph 7). After hydrolysis is complete, addition of aluminium hydroxide has no effect on pH. No such effect is observed with AEI and inactivation can be effected in AEI-$Al(OH)_3$ mixtures (Graph 9).

As mentioned above, samples have been assayed in mice and BHK cells. For the tongue epithelium passaged virus used, the BHK cells proved less sensitive than mice, giving generally lower titres and often not detecting virus when it could be detected in mice. This may be due to the presence of inactivated material interfering in some way with the growth of the virus in the tissue culture system. In later experiments using type A virus strain A-119 which had been passaged in suspended BHK culture, the method of serially passaging material in BHK monolayers proved to be the most sensitive method of detecting live virus (Table 1).

Potency

To determine whether the resultant inactivated virus retained its antigenicity, vaccines were prepared from virus of type SAT1, strain SA.13/61, of the 10th tongue epitehlial tissue culture inactivated under the following conditions:-

i)	AEI	25°C	pH 9.0	30 hours
ii)	AEI	37°C	pH 9.0	10 hours
iii)	BPL	25°C	pH 9.0	48 hours
iv)	Formalin	25°C	pH 9.0	48 hours

Vaccines were made by adding 50 per cent aluminium hydroxide to the material after inactivation except in the case of formalin, where the virus was pre-adsorbed on the aluminium hydroxide. All proved innocuous when inoculated intraperitoneally into 6 day old mice or by multiple tracking of the plantar pads of guinea-pigs. These vaccines were tested by inoculating 1 ml. subcutaneously into groups of 20 guinea-pigs of 500-700 g. weight. These pigs were challenged 21 days later by intradermal inoculation into one hind pad only of a 1:50 EK-filtrate of the 4th guinea-pig passage of the homologous vaccine virus. Lesions were scored 1-3 on all four pads and tongue and pigs assumed to be immune with a score of 4 or less, i.e. slight generalization to one pad other than the primary inoculation site. Results are shown in Table 2. From this it will be seen that virus inactivated by AEI and BPL not only retains its antigenicity but appears to produce a more efficient vaccine than the standard inactivation with formalin in the presence of aluminium hydroxide.

To investigate further the effect of inactivation procedure on the resultant potency, further vaccines were made with the same virus inactivated by AEI and BPL under various conditions. These were again tested by subcutaneous inoculation into guinea-pigs, either undiluted or diluted 1:5. The results of this experiment are shown in Table 3. This shows that the method of inactivation is as important as the inactivant in that the longer the inactivation period, within limits defined by the rate of pH and temperature inactivation, the better the resultant vaccine.

DISCUSSION

It has been shown by Brown at Pirbright and Fellowes at Plum Island that successful vaccines can be made from foot-and-mouth disease virus inactivated by AEI and BPL respectively. The present work confirms the results. Both these inactivating agents produce a vaccine which is certainly not inferior and may be superior to the standard formalin inactivated vaccine using virus cultured by the Frenkel technique in tongue eipthelial cells. It would also appear that the conditions of inactivation are as important as the inactivating agent in relation to potency of a vaccine. A BPL vaccine, inactivated over a 48 hour period at 25°C, pH 9.0, was more potent than an AEI vaccine inactivated in 24 hours under the same conditions, which in turn was more efficient than a vaccine rapidly inactivated by BPL in 60 minutes at 37°C, pH 7.6.

The choice of system for detecting non-infectivity is also of importance and laboratory methods must depend on the previous passage history of the strain used.

The present work suggests that AEI and BPL offer the possibility of preparation of foot-and-mouth disease vaccines of equal or greater potency than the formalin-aluminium hydroxide vaccine. The control of innocuity is less open to criticism in that it can be effected without the possible interference of the adsorbant, and the kinetics of inactivation make it more certain that the product is innocuous.

These results require confirmation in cattle and experiments are at present in progress.

Table 1

Detection of Residual Foot-and-Mouth Disease Virus, Strain A-119 (BHK Suspended Culture Material) after Inactivation by Chemical Agents

Inactivant	Mice (0.03 ml.)	Baby Hamster Kidney Cells (1 ml)			Pig Kidney Cells (1 ml)		
		1st passage	2nd passage	3rd passage	1st passage	2nd passage	3rd passage
AEI, 25°C, pH 9.0							
12 hours	2/4*	10/10**	-	-	10/10	-	-
16 hours	0/4	0/10	10/10	10/10	0/10	0/10	0/10
20 hours	0/20	0/10	0/10	9/10	0/10	0/10	0/10
22 hours	0/20	0/10	0/10	0/10	0/10	0/10	0/10
24 hours	0/20	0/10	0/10	0/10	0/10	0/10	0/10
BPL, 25°C, pH 9.0							
30 hours	0/8	0/10	0/10	10/10	0/10	0/10	0/10
36 hours	0/20	0/10	0/10	1/10	0/10	0/10	0/10
40 hours	0/20	0/10	0/10	10/10	0/10	0/10	0/10
42 hours	0/20	0/10	10/10	10/10	0/10	0/10	0/10
48 hours	0/20	0/10	0/10	0/10	0/10	0/10	0/10

* No. of mice positive/No. inoculated

** No. of monolayers positive/No. inoculated.

Table 2

Protection in Guinea-Pigs from Foot-and-Mouth Disease Vaccines

Inactivated by Various Chemical Agents

		Guinea-pigs protected
i)	0.05% Formalin, 25°C, pH 9.0, 48 hr. (pre-adsorbed)	4/20*
ii)	0.05% BPL, 25°C, pH 9.0, 48 hr.	12/19
iii)	0.05% AEI, 25°C, pH 9.0, 24 hr.	9/20
iv)	0.05% AEI, 37°C, pH 9.0, 12 hr.	11/20
v)	Controls - vaccine base only	0/10

* No. protected/No. inoculated.

Table 3

Guinea-Pig Potency Test of SA.13/61 Foot-and-Mouth Disease Virus grown in Tongue Epithelial Cell Culture, Inactivated by AEI or BPL under Varying Conditions

Method of Inactivation	Undiluted Vaccine	Vaccine diluted 1:5
AEI, 25°C, pH 9.0, 24 hr.	17/17*	9/16
AEI, 37°C, pH 7.6, 10 hr.	13/17	5/17
BPL, 25°C, pH 9.0, 48 hr.+	16/17	13/18
BPL, 37°C, pH 7.6, 1 hr.+	11/18	9/16

Virus titre:	Tongue epithelium passaged virus	$10^{6.25}$ mouse ID_{50}/ml.
Total complement-fixing titre:	T.E. virus	32**

* No. protected/No. challenged
** Reciprocal of dilution causing 30% reduction of haemolysis
\+ BPL allowed to complete hydrolysis at +4°C before use

QUANTITATIVE CONTROL OF FOOT-AND-MOUTH DISEASE VACCINE

STUDY OF THE DOSE-EFFECT LAW AND CORRELATION BETWEEN THE 50% VACCINATING DOSE IN GUINEAPIGS AND IN CATTLE *

by

C. Mackowiak, J. Fontaine, J. Terré, C. Stellmann,
M. Roumiantzeff, H.G. Petermann **

The control of the activity of a biological product and in particular that of foot-and-mouth disease vaccine which is the subject of this report, can be considered from two points of view:-

- that of the controller who has to accept or refuse the product,
- that of the producer who makes the product, always having improvement of its quality in mind.

The controller first establishes the acceptance threshold which corresponds to the minimal value required for a vaccine considered as utilisable. In deciding upon this threshold, account is taken of the average protection to be guaranteed under experimental conditions: 70, 80, 90 percent or more, it being understood that in practice this percentage will be higher.

Norms differ between countries, mainly according to the development of production techniques and the prophylactic requirements. Vaccines are classified in two large categories: "accepted" and "refused". It is evident, however, that in biological control, the answer is always given with a certain amount of probability, which is naturally related to the conditions and the system of control which is used. It is therefore necessary to have between these two categories a zone of insecurity which applies to indifferent or bad vaccines, concerning which it is difficult to take a definite decision. This zone should have greater importance attached to it than does a possible mistake by the control system used in order to prevent a product being accepted

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* DVC_{50}: Guineapigs vaccinating dose protecting 50% of guineapigs at challenge carried out ten days after vaccination.

DVB_{50}: Cattle vaccinating dose protecting 50% of cattle at challenge carried out 21 days after vaccination.

** Work done by the Institut Français de la Fièvre Aphteuse, Lyons
Director: Dr. Vre. C. Mackowiak.

at one test and then refused at another.

Briefly, the principal role of the controller concerns the establishment of the minimal value of a product, in order to know that it meets the requirements of the acceptance threshold.

The point of view of the producer, on the other hand, is quite different. He has to produce vaccines which are of course above the acceptance threshold, but he must also know as definitely as possible their activity value, even if it is very high. This is absolutely necessary if he wishes to compare two vaccines and improve the quality of his production. He must therefore undertake not only control but also titration of the vaccine.

We, as producers, experience difficulties all the time in distinguishing between two satisfactory lots of vaccine, one of which is better than the other, so that we may know if any variation of any factor in the technique has had some effect on the quality of the vaccine.

On this point, we have considered that there should be a unit of measurement of immunity and have chosen the 50% vaccinating dose (DV_{50}). The idea of using the DV_{50} in the control of foot-and-mouth diseas vaccine was introduced by Henderson and Galloway (1) and, adopting it, they were able to compare the immunogenic values of two vaccines of different origin. These authors also reported that, using 3 DV_{50}, about 80% protection was obtained in cattle, and with 10 DV_{50}, there was at least 95% protection (2). The progress made with production techniques is such that now the preparation of vaccines containing 10 DV_{50} and even more per dose can be envisaged. It would seem, therefore, that further improvement in their quality need not be considered.

However, there is another problem: all activity controls of foot-and-mouth disease vaccines are done by tests carried out 15, 21 or 30 days after vaccination: a static answer is obtained giving the value of the immediate immunity. It would be of very great interest, after titrating the vaccine, to be able to estimate the duration of immunity conferred by it with relation to its quality. By using the DV_{50} unit for control work, a dynamic character is obtained and the kinetics of the immunity can be forecast, since it can be ascertained how much DV_{50} is "lost" each month. In other words, the titre of a vaccine expressed as DV_{50} is not the same if the tests are carried out 21, 60 and 120 days after the vaccination, and it decreases with the time interval. This study is being made now and we will report the results in a later communication.

The production of the vaccine at our Institute is based essentially on monovalent vaccines which are then mixed for the preparation of trivalent vaccines. From the combination of several lots for the same valence, we have been able to obtain very homogeneous polyvalent productions for a long time. We know by experience that using good monovalent vaccines, good trivalent vaccines are produced systematically. It is therefore essential that we use a sure, exact, reproducible and easily applied technique in the titration of our monovalent productions. For this, we tried guineapigs in attempts to establish the DV_{50} (Mackowiak et al.(3) ; Roumiantzeff et al. (4)), but to ascertain the value of the response, it was necessary, with the same vaccines of each type, to establish the DV_{50} in cattle (Terre et al.(5)).

This article deals essentially with the study of the dose-effect law, the correlation between 50% vaccinating doses in cattle and in guineapigs, and thus, the possibilities and limitations of using guineapigs in the quantitative control of monovalent foot-and-mouth disease vaccines. It has meant studies over a long period, begun several years ago and in which, up to the present about 15,000 guineapigs and 1,000 cattle have been required. The work is not yet finished and some final experiments are in progress. We will deal successively with:-

1. Materials;
2. Methods;
3. 50% vaccinating doses in guineapigs;
4. 50% vaccinating doese in cattle;
5. Correlation between DV_{50} in guineapigs and in cattle.

After discussing the results which have been obtained we will point out the possible practical applications and will make conclusions.

I. Materials

1. Vaccine

Commercially produced monovalent vaccines are used, prepared by inactivating the virus by heat and formol and adding saponin and aluminium hydroxide. The virus is grown on living tongue epithelium. The average cattle dose is 1.6 ml. All the vaccines used in these experiments had already been tested in cattle for innocuity with satisfactory results.

2. Guineapigs

In our earlier experiments, we used guineapigs which came from different breeders and we found much irregularity in the results between the different groups.

Ubertini et al.(6), Lucam et al.(7) have already drawn attention to the importance of the selection of guineapigs for tests with vaccines and our observations completely confirm their findings.

After several tests, we were able to show that the guineapigs, in addition to being about 500 g. in weight, should be perfectly healthy, should come from the same recognized and supervised breeding establishment, should be the same strain and should not be subjected to changes of maintenance conditions during the experiment. For these various reasons we established a breeding unit of albino guineapigs (the strain was provided by the Pirbright Institute) which have given regular results in the experimental work.

3. Cattle

All the adult cattle come from the Department of Finistère which has been free from foot-and-mouth disease for many years and where the cattle are not required to undergo annual trivalent foot-and-mouth disease vaccination. On arrival at our quarantine farms, the cattle are bled and absence of foot-and-mouth disease antibodies in their serum is confirmed before they are used in the experiments. These cattle, as we have already shown, are highly susceptible to foot-and-mouth disease.

4. Virus

(a) Cattle

The strains of virus used for the challenge tests are those also used for the vaccine production and are contained in the vesicles of cattle, ground up, distributed in bottles in lots of 1 gramme, and kept at -70°C. All the viruses are titrated periodically in cattle by the Henderson method (8). The challenge strains "A" and "C" are the 4th passage in cattle; the "O" strain is the 7th passage.

(b) Guineapigs

The challenge virus used for guineapigs is that used for cattle but which has been adapted to the guineapig by 6-7 intradermoplantar passages. These stock virus consisting of plantar vesicles are titrated in guineapigs and preserved at -70°C.

II. Methods

1. Vaccination

(a) Cattle

The cattle are injected subcutaneously with different doses of vaccine. The volume of the inoculum is always 1.6 ml, buffer at the pH of the vaccine being used. The buffer consists of:

Na_2CO_3	-	0.530 g
$NaHCO_3$	-	16.800 g
NaCl	-	85,000 g
Distilled water	-	10,000 litres.

For the dilutions in geometrical progression, a four-fold dilution has been chosen so that the DV_{50} is always introduced.

The vaccine, the diluent and the dilutions of the vaccine are all kept at refrigerator temperature until just before the vaccination is carried out.

Four groups, each of 5 cattle are used: each animal receives, in a volume of 1.6 ml the following: -

Group I	-	1.6 ml vaccine
Group II	-	0.4 ml vaccine
Group III	-	0.1 ml vaccine
Group IV	-	0.025 ml vaccine.

The cattle are kept under observation on our farms for 22 days.

(b) Guineapigs

The guineapigs are vaccinated intramuscularly with vaccine diluted as for cattle, but the dilutions are two-fold.

Five groups, each of 8 guineapigs are used: each receives vaccine in a volume of 1.6 ml as follows: -

Group I	-	1.6 ml vaccine
Group II	-	0.8 ml vaccine
Group III	-	0.4 ml vaccine
Group IV	-	0.2 ml vaccine
Group V	-	0.1 ml vaccine.

The guineapigs are kept under observation for ten days.

2. Challenge tests

(a) Cattle

On the 22nd day after vaccination, all the cattle are brought into isolated stables at the Institute: blood samples are taken and then o.1 ml of the challenge virus is injected intradermolingually at two places, i.e. 10,000 DIB_{50} per animal, of the virus with which the vaccine was prepared. Only two controls are used in each challenge test because our experience over many years has shown susceptibility of our control animals to be over 98% (5).

(b) Guineapigs

On the 10th day after vaccination, each of the vaccinated guineapigs and 8 controls are injected intradermoplantarly with 0.1 ml suspension containing 10,000 DG_{50} of the virus with which the vaccine was prepared.

3. Reading of the results (lesions)

(a) Cattle

The animals are slaughtered five days after the challenge injection and lesions on the tongue, head and feet are noted.

An animal is considered as "not vaccinated" if one or several foot lesions are found. All the lesions are controlled by complement fixation.

(b) Guineapigs

The lesions are noted in the vaccinated guineapigs five and seven days after the challenge and in the controls, after three and five days. Two readings have to be made, particularly for the vaccinated animals because some slight secondary lesions which have undergone some healing may be missed.

4. Calculation method

Calculations are made by the Litchfield method with a probability of 95% (p = 0.95) (9).

For each vaccine, we have determined the following four parameters: -

DV_{50} : 50% vaccinating dose,
fD : precision on the DV_{50} at p = 0.95 (95%),
S : function of the slope. In the following we talk about "slope S" in order to lighten the text.
fS : precision on the slope S at p = 0.95.

III. Guineapig DV_{50}

After having studied the production of antibodies (3) we adopted in our research the DV_{50} which is more precise than the direct test. Its reproductibility and its value being controlled by statistical analysis, we tried to compare these results with those obtained in cattle and began a study of the parallel DV_{50} in cattle and guineapigs (4).

1. Reproductibility

To confirm the reproductibility of the method, we controlled several vaccines two or three times successively. The results in Figure No. 1 show that the true answers are always within the limits of theoretical calculation errors.

2. Statistical analysis

(a) Comparison of the "S slopes" of the laws representing the dose-response curves of the same valence.

The test of parallelism does not show any significant difference (at $p = 0.95$) between the slopes of the dose-response curves of vaccines of the same valence. It is therefore justifiable to compare the different DV_{50} results for one valence and to calculate the average slope (Sm) and the precise slope (fSm). The formulae used for obtaining the results shown in Figure No.2 have already been described (9).

(b) Estimation of the average slope (S_m') and its precision (fS_m') for the three valences.

After having shown that there was no significant difference between the slopes of the dose-response curves of the three strains of viruses studied, we calculated a "common average slope" (S_m') (Figure No. 2) and its precision (fS_m').

(c) Estimation of the degree of reliance ($p = 0.95$) of the DV_{50} for a titration.

The "average slope" calculated in 75 tests, in which about 3,000 guineapigs were used enabled us to trace the best theoretical slope of our foot-and-mouth disease guineapig vaccine system and to organize rationally our attempts to obtain a given precision for the DV_{50}.

Under our conditions so established (dose variation 2 in 2, 8 guineapigs per dose and 5 doses), we obtain one to three doses (8 - 24 guineapigs) entering into the calculations of fD, an fD between 1.55 and 2.1.

Experimentally, in 75 attempts, the average fD of 1.66 (Figure No.3) is the average degree of reliance in a case of -

$$\frac{DV_{50}}{1.66} < DV_{50} < DV_{50} \times 1.66$$

REPRODUCTIVITY ($p = 0.95$) of the DV_{50} in Guineapigs (calculated by the Litchfield method)

Vaccin®	Date of Controls	DV 50	Average of DV 50	fD	Theoretical limits of DV 50
Monovalent A n° 24	1.11.63 10.12.63	0,20 ml 0,13 ml	0,175 ml	 1,75	 0,07 < DV 50 < 0,21
Monovalent A n° 006	15. 9.64 20.10.64 10. 1.65	0,16 ml 0,19 ml 0,20 ml	0,183 ml	2 1,9 1,56	0,08 < DV 50 < 0,32 0,10 < < 0,36 0,13 < < 0,31
Monovalent O n° 53	17. 3.64 10. 4.64	0,54 ml 0,35 ml	0,42 ml	1,85 1,5	0,28 < DV 50 < 0,96 0,20 < < 0,50
Monovalent O n° 05	31. 1.64 4. 5.64	0,40 ml 0,20 ml	0,30 ml	1,7 1,6	0,235 < DV 50 < 0,68 0,125 < < 0,32
Monovalent C n° 019	20.11.64 27.11.64 15.12.64 16.12.64	0,20 ml 0,30 ml 0,16 ml 0,20 ml	0,22 ml	1,65 1,68 1,66 1,70	0,12 < DV 50 < 0,33 0,18 < < 0,55 0,10 < < 0,275 < 0,12 < < 0,34

Figure N° 1

Estimation of the average slope Sm of the Dose-response laws obtained in guineapigs for each valence and the Sm' obtained for the system

	For each Valence				For each of the three Valences		
	No. of tests	Average S	Average fS	Limits of average S	Sm'	fSm'	Limits of fSm'
Valence O	18	2,21	1,27	1,74 - 2,81			
Valence C	34	2,15	1 19	1,81 - 2,56	2,15	1,11	1,94 - 2,39
Valence A	23	2,10	1,18	1,78 - 2,48			

Figure N.° 2

Litchfield's charts indicate that under these conditions we can differentiate with this system, two vaccines, one on an average at least twice more active than the other ($p = 0.95$).

3. Preliminary confirmation of the agreement of the results of the DVC_{50} in guineapigs and the percentage of protection in cattle.

For this objective, we arranged the vaccines in three groups, according to the guineapig DV_{50}s:

DV_{50} - 0.025 at 0.1 ml

DV_{50} - 0.1 at 0.4 ml

DV_{50} - 0.4 at 1.6 ml.

We then confirmed the percentages of protection in cattle obtained with the vaccines of each group (see Figure No. 4).

Examination of these results shows that the percentage of protection in cattle increases as the value of the guineapig DVC_{50} decreases. This finding led us to carry out work to establish precisely the correlation between the results in cattle and in guineapigs.

So, we began the systematic study of a certain number of vaccines to establish the DV_{50} in cattle, in parallel with the DV_{50} in guineapigs.

IV. Cattle DV_{50} : Results and Statistical Analysis

1. Dose-effect law for O valence

The results of titration of the activity of five monovalent vaccines are shown in Figure No. 5. The parameters are given in Figure No. 6. (Here, it should be noted that the controls for the three types have always shown generalized lesions in all four feet (5)).

The test of parallelism showed no significant differences ($p = 0.95$) between the slopes of the five vaccines. Therefore, it is justifiable to:-

- Compare the different DV_{50} results and to reckon their relative value with their degree of reliance at $p = 0.95$, if they are significantly different; and calculate the average slope with $S_m = 2.15$ and its precision with $fS_m = 1.23$ ($p = 0.95$). The formulae used for obtaining these values have been described elsewhere (9).

2. Dose-effect law for A valence

The results of the titration of activity of five monovalent vaccines are shown in Figure No. 7 and the values of the parameters in Figure No. 8.

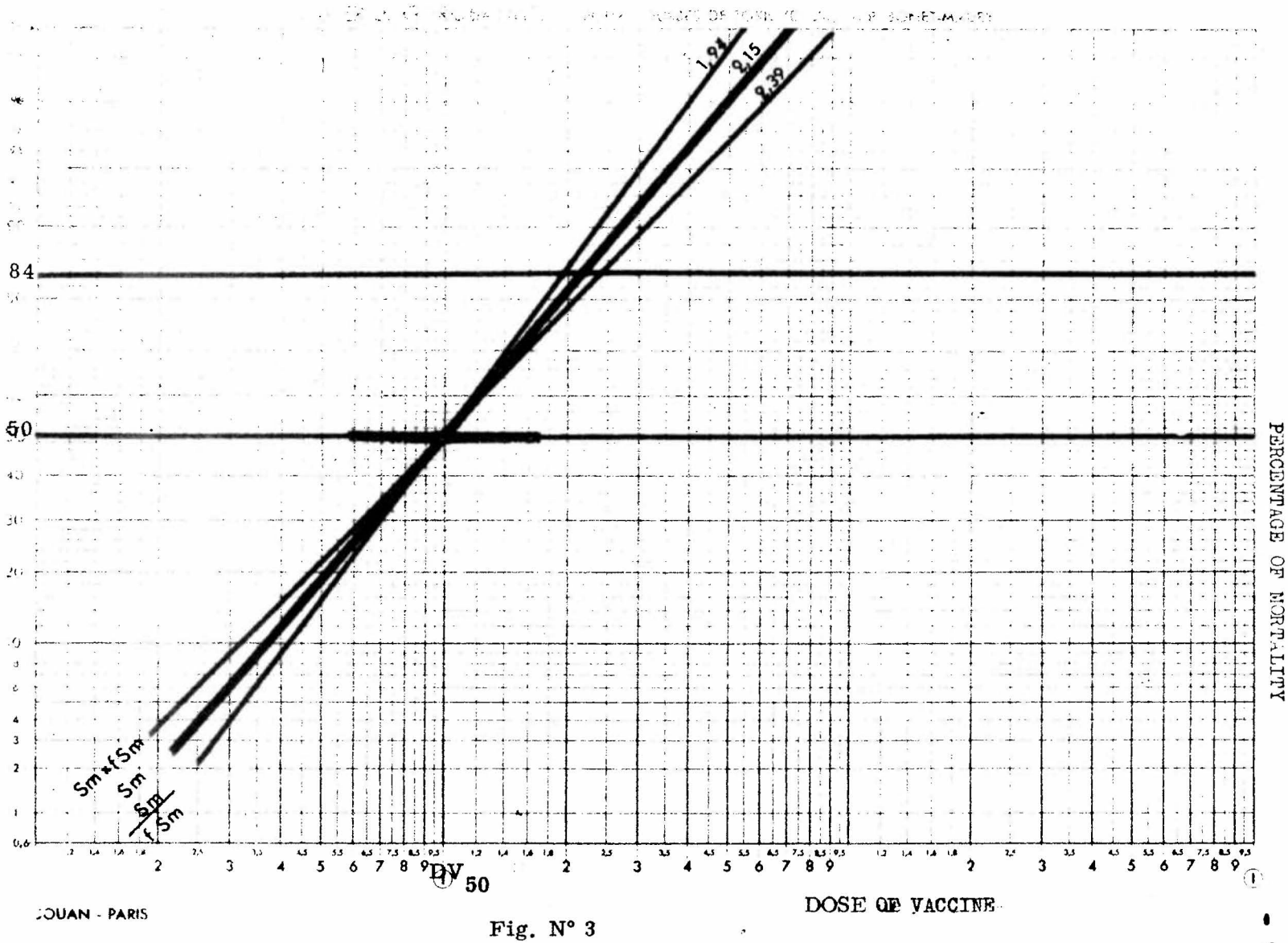

1,94
2,15
2,39
84
50
PERCENTAGE OF MORTALITY
DV 50
DOSE OF VACCINE
COUAN - PARIS

Fig. N° 3

As with the O vaccines, the test of parallelism did not show any significant differences ($p = 0.95$) between the slopes of these five vaccines. While these identical deductions have been made, in this case the average slope is 2.03 and its precision 1.21 ($p = 0.95$).

3. Dose-effect law for C valence

The results of the titrations of activity of five monovalent C vaccines and the values of the parameters are shown in Figures Nos. 9 and 10.

As with O and A valences, the same conclusions have been made. The average slope for this valence is 2.03 and its precision is 1.18 ($p = 0.95$).

4. Dose-effect law of the system

(a) Estimation of the average S_m' and its precision fS_m' for the three vaccine valences.

The test of parallelism did not show any significant differences ($p = 0.95$) between the average slopes of each vaccine valence. The average S_m' and its fS_m', characterizing the inactivated monovalent cattle vaccine corresponding to the three strains of virus studied. The result is:

$$S_m' = 2.06; \qquad fS_m' = 1.11$$

(b) Estimation of the degree of reliability ($p = 0.95$) in the average slope balanced for a titration.

The fS_m' precision of the average S_m', already described, corresponds to the precision of a balanced average, calculated from 15 titrations. The precision fS_i on the slope for one titration is $\sqrt{15}$ times greater (nearest log.). In Litchfield's system, log. $fS_1 = \sqrt{15}$ x log. fSm': thus, fS_i is 1.52. As already said, there is a 95 percent chance, since a titration under our conditions, for an S_i slope is between

$$\frac{2.06}{1.52} = 1.36 \quad \text{and} \quad 2.06 \times 1.52 = 3.14$$

All the S slopes of the 15 titrations which were the basis of these calculations are within the degree of reliability at $p = 0.95$ (Figures Nos. 6, 8 and 10).

(c) Estimation of the precision of the DV_{50} for a titration ($p = 0.95$).

The average of the 15 experimental values of the fD precision is 2.04. Under our titration conditions, the DV_{50} is known to be fD = 2 and, in decimal logarithms $\pm$ 0.3. In assimilating the population of experimental 15 fD to a Gaussian population, one can calculate 2 on an fD average $= \pm 0.32$ ($p = 0.95$).

Monovalent "O" vaccines: results of the 50% cattle vaccinating doses

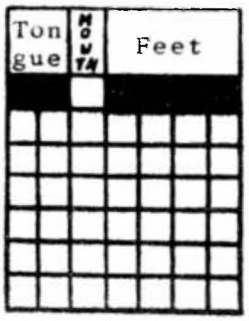
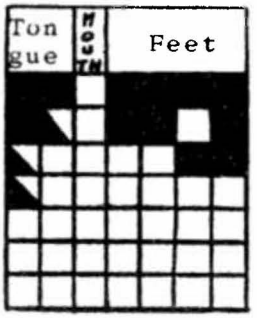
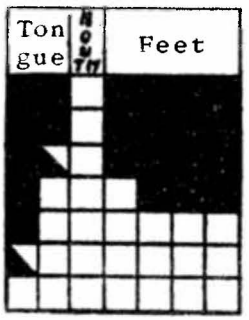
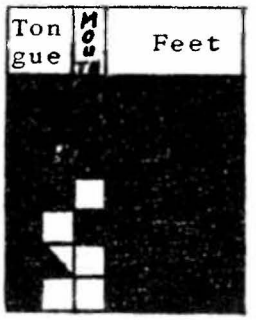
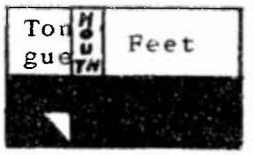
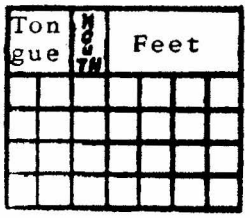
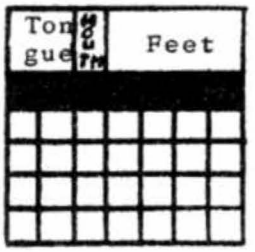
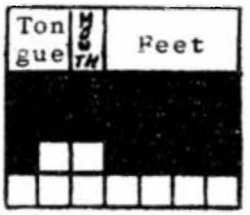
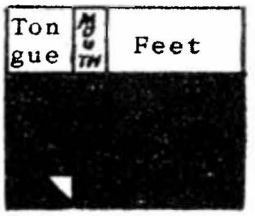
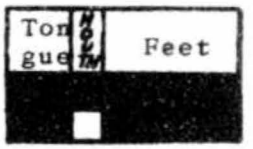
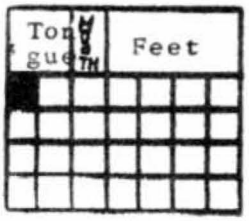
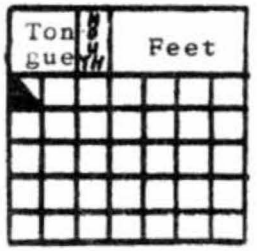
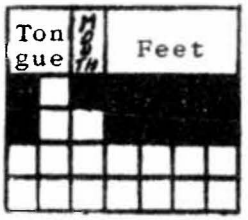
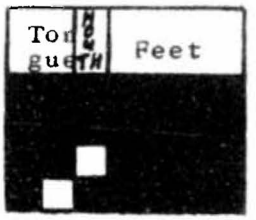
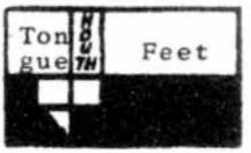
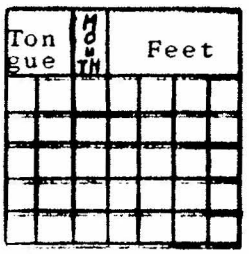
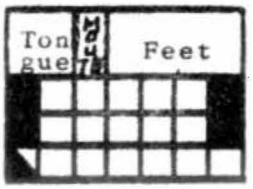
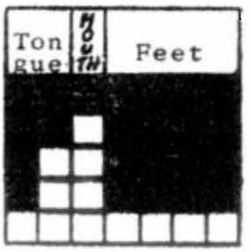
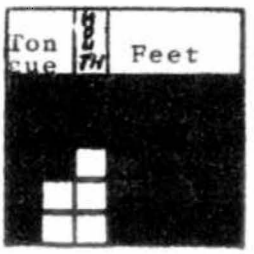
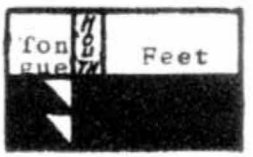
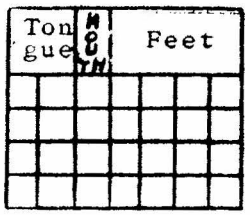
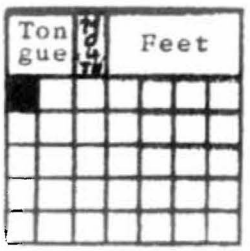
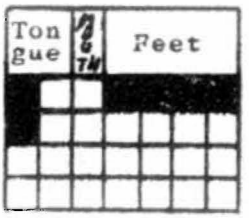
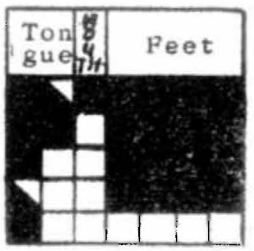
Batch N0	1 Dose	1/4 Dose	1/16 Dose	1/64 Dose	Controls
105					
118					
119					
131					
132					

Fig. N° 5

Values of the different parameters for monovalent "O" vaccines

VACCINE N°	DV_{50} (in ml)	fD	Theoretical limits of the DV_{50} (in ml)	S	fS
105	0,21	2,3	0,09 - 0,49	3,1	1,99
118	0,18	2,2	0,08 - 0,41	2,5	1,7
119	0,10	2	0,05 - 0,20	1,7	1,4
131	0,35	2,2	0,16 - 0,77	2,3	1,65
132	0,05	2,15	0,02 - 0,10	2,3	1,5

S : Function of slope

fD : Exactness of DV_{50}

fS : Exactness of the function of slope

Figure N° 6

Monovalent "A" vaccines: results of the 50% vaccinating doses

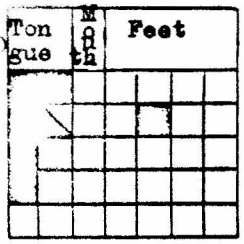
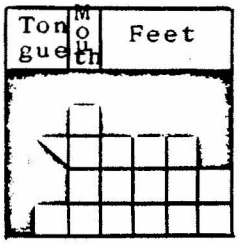
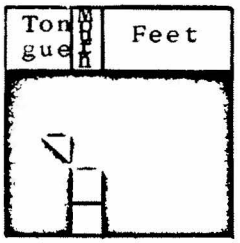
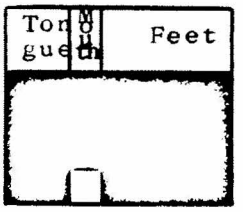
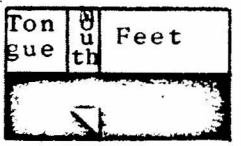
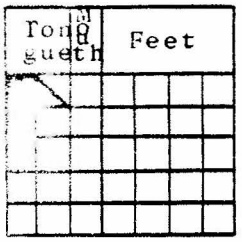
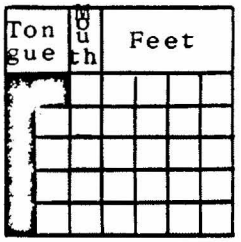
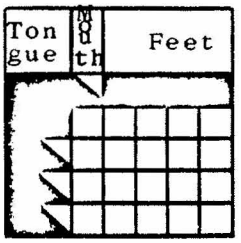
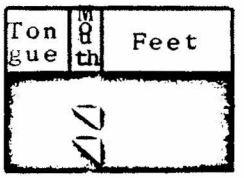
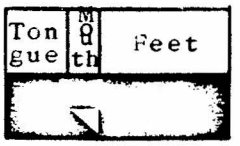
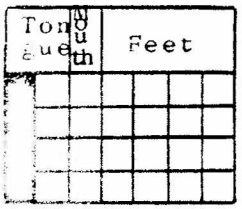
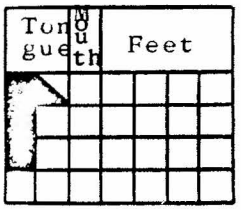
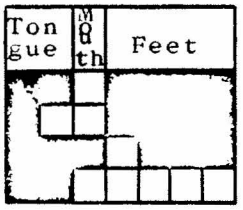
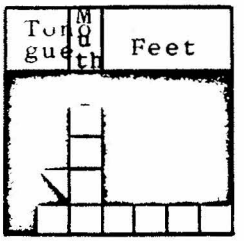
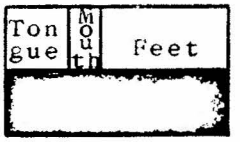
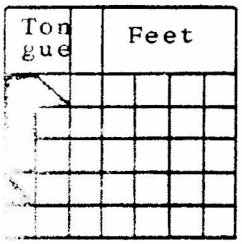
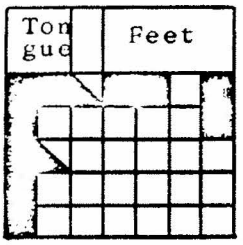
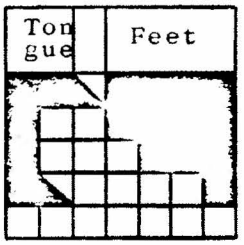
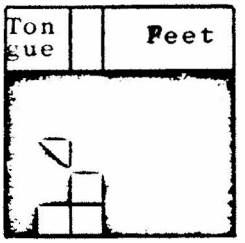
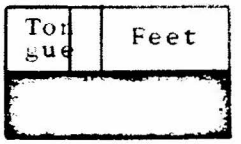
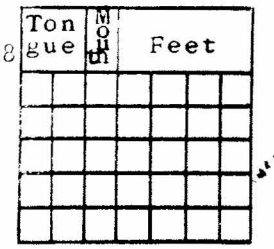
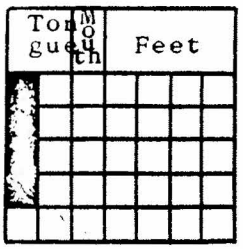
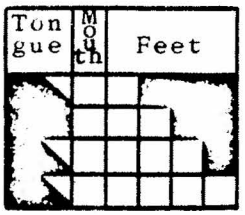
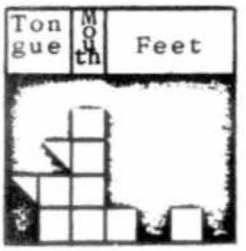
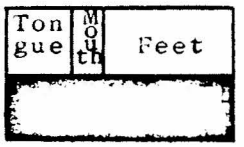
Batch No.	1 Dose	1/4 Dose	1/16 Dose	1/64 Dose	Controls
220					
224					
226					
227					
228					

Figure 7

Values of the different parameters for "A" vaccines

Vaccine No.	DV_{50} (in ml)	fD	Theoretical limits of the DV_{50} (in ml)	S	fS
220	0,62	2	0,31 - 1,24	2,3	1,5
224	0,07	1,7	0,04 - 0,12	1,6	1,9
226	0,09	2	0,04 - 0,18	2,2	1,5
227	0,25	2	0,12 - 0,5	2,25	1,5
228	0,12	2,2	0,05 - 0,26	1,75	1,4

Figure N° 8

Monovalent "C" vaccines: results of the 50% vaccinating doses

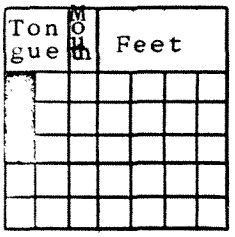
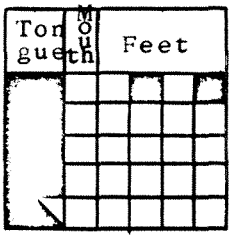
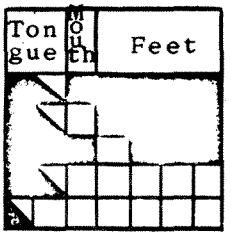
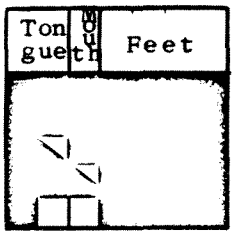
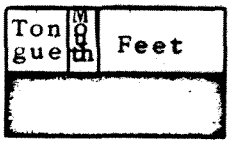
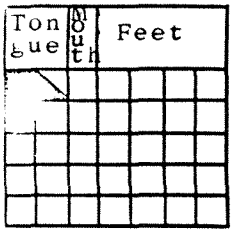
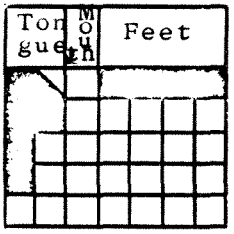
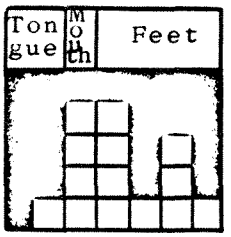
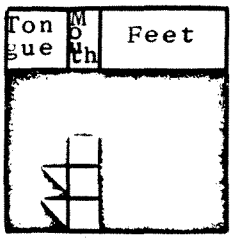
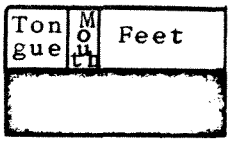
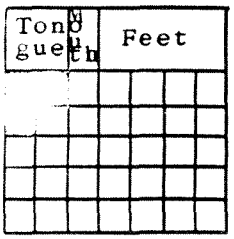
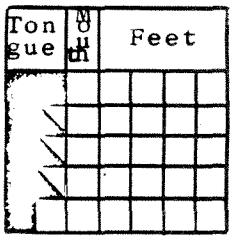
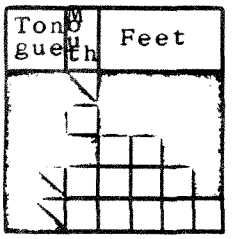
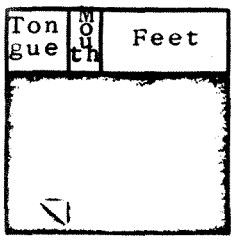
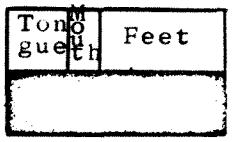
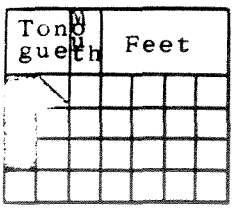
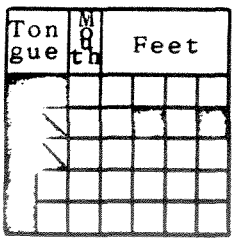
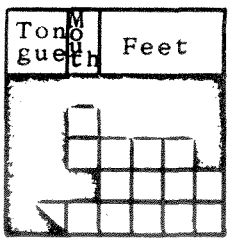
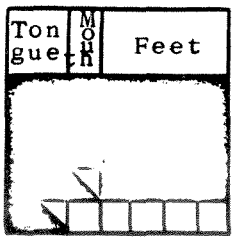
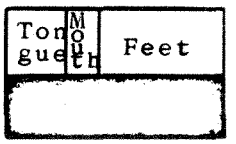
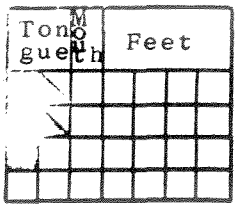
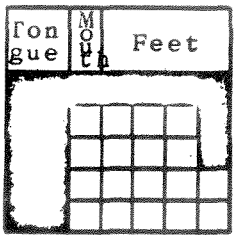
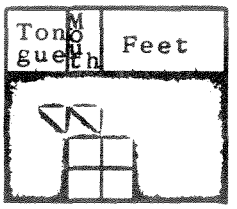
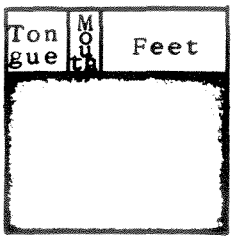
Batch No.	1 Dose	1/4 Dose	1/16 Dose	1/64 Dose	Controls
317					
319					
320					
322					
324					

Fig n° 9

Value of the different parameters for monovalent "C" vaccines

Vaccine No.	DV_{50} (in ml)	fD	Theoretical limits of DV_{50} (in ml)	S	fS
317	0,16	2	0,08 - 0,32	2,3	1,5
319	0,20	2,2	0,09 - 0,44	2,3	1,5
320	0,12	1,9	0,06 - 0,23	1,8	1,4
322	0,18	1,95	0,09 - 0,36	2,5	1,5
324	0,43	1,85	0,23 - 0,80	1,7	1,4

Figure N° 10

By and large, Litchfield's charts indicate that a difference can be made with this system between 2 vaccines, the average activity of one being at least 2.7 times that of the other (with a 95 percent precision).

5. Reproductivity of the method

Two "O" vaccines were titrated twice: the results are shown in Figure No. 11.

Vaccine No. 105 had a DV_{50} of 0.27 ml with an fD precision of 1.9, at the first titration. Four months later, the same vaccine had a DV_{50} of 0.21 ml with an fD precision of 2.3. The slopes being not significantly different, the test of relative power did not permit of differentiation of these two DV_{50} values.

Vaccine No. 119 had a DV_{50} of 0.10 ml with a precision of 2 at the first titration. Seven months later, the same vaccine had a DV_{50} of 0.10 with a precision of 1.9. From the same statistical tests, conclusions similar to those for vaccine No. 105 were drawn.

In addition to the reproductivity of the technique, it is interesting to note the good preservation at + 4°C of these two "O" vaccines for four and seven months.

6. Use of the DV_{50} system

(a) Comparison of two monovalent vaccines.

After calculating the parameters of each vaccine, it is confirmed that the slopes are not significantly different (test of parallelism at p = 0.95). The relative strengths and their precision for the two vaccines can then be calculated, if the DV_{50}s are significantly different (p = 0.95).

(b) Calculation of the average percentage protection of a vaccine.

The parameters of a vaccine being known it is confirmed that its S_i balanced slope is not significantly different from the average S_m' slope = 2.06. Then the number of DV_{50}s in the full dose of 1.6 ml of each vaccine is calculated. The figure so obtained, i.e. the average strength of the vaccine, is plotted on the abscissa from the DV_{50} point in Figure No. 12. With S_m' = 2.06, the average percentage of protection is read directly on the ordinate.

(c) Calculation of the minimum percentage protection of a vaccine.

The parameters of the vaccine being known, its S_i slope is confirmed as not significantly different from the average slope S_m' = 2.06. The minimum strength of the vaccine is then calculated which, in this case, is the number of "limits above DV_{50}, perhaps DV_{50} x fD" found in the full 1.6 ml dose. The minimum strength is plotted in the abscissa from the DV_{50} point in Figure No. 12. With S_m' x fS_i = 3.14, i.e. the upper limit of the S_m' slope, the percentage protection is read directly on the ordinate. The estimated percentage protection, under the conditions we have defined, has a 97.5 percent chance of being better. With this system, therefore, a minimum activity with a 97.5 percent chance of being exceeded is determined.

Reproductivity of the method

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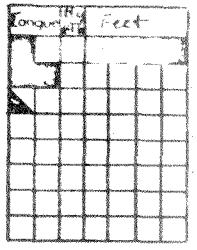
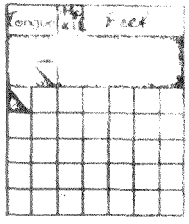
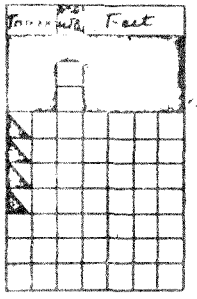
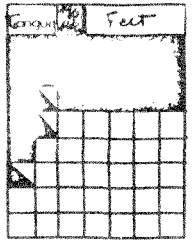
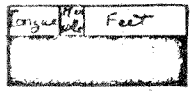
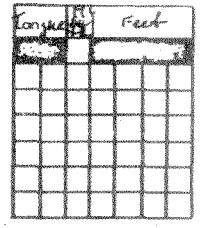
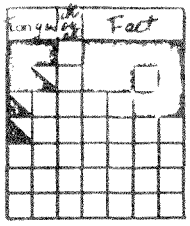
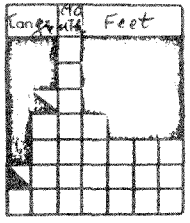
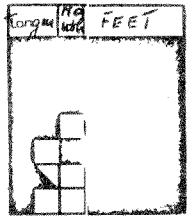
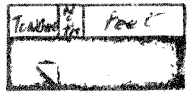
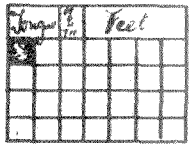
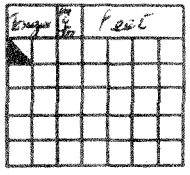
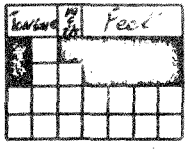
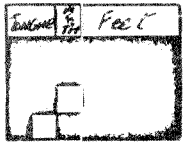
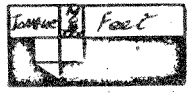
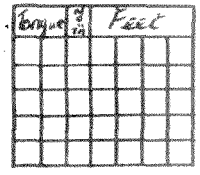
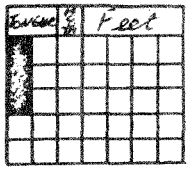
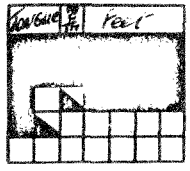
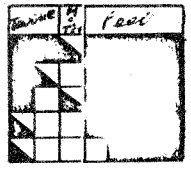
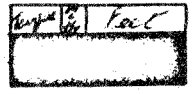
105 1 st Control							
2nd Control							
119 1 st Control							
2nd Control							

Fig. N° 11

PERCENTAGE OF PROTECTION

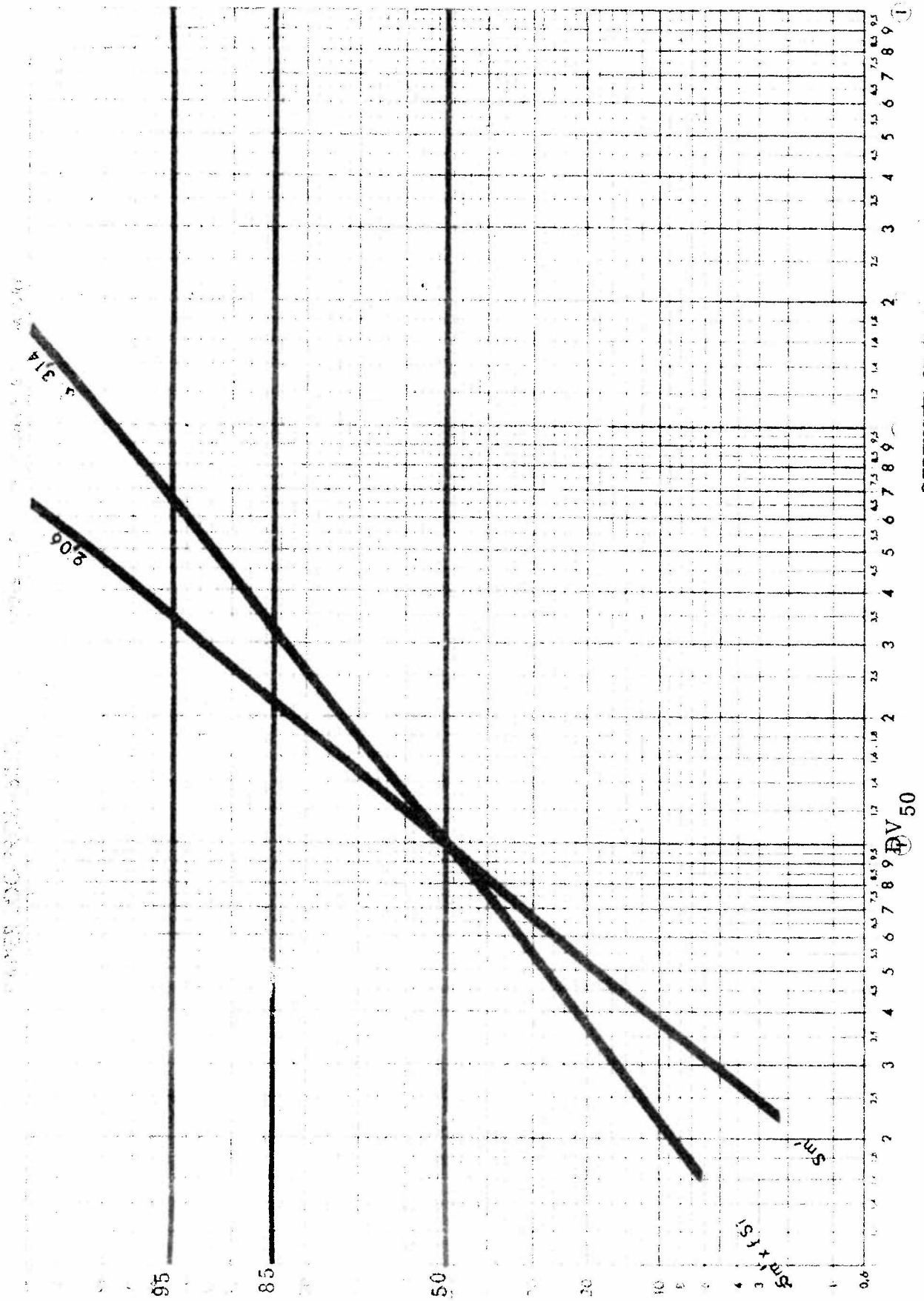


Fig. N° 12

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Following Figure No. 12, Figure No. 13 was made.

Figure No. 13

Percentage of protection in terms of the minimum strength of the vaccine

Minimum percentage protection of the vaccine (p = 0.975)	Number of DV_{50} x fD* per full dose: minimum strength of the vaccine
60%	1.4
65%	1.5
70%	1.85
75%	2.3
80%	2.7
85%	3.4
90%	4.5
92%	5
95%	6.7
97.6%	10

* DV_{50} x fD : Theoretical upper limit of DV_{50}.

In Figure No. 14 there is collected together the average percentages of protection (p = 0.95) and the minimum percentages of protection. There is better differentiation of the activity of the vaccines by considering the minimum rather than the average protection. This is particularly evident for good vaccines. This test of "minimum percentage of protection"leads to a gain of "strength" in the statistical sense of the word.

(d) Conclusion.

Two methods of use have to be retained:

- for the producer of monovalent vaccines: classification of these vaccines in order to produce very homogeneous trivalent mixtures;

- for the controller: determination of a threshold of activity, i.e. minimum percentage of protection.

The possibilities of using the DV_{50} system are described, the absence of all generalization (foot lesions) being taken as the criteria of protection. Other criteria are to be studied and may still further improve the system and enable work to be done quantatively on the characters related to the strains of the virus. The DV_{50} technique applied to trivalent vaccines is to be studied and will be the subject of a later communication.

Comparison between the average percentage of protection and the minimum percentage of protection

Vaccine No.	DV 50	fD	Upper limit DV 50 / DV 50 x fD	Average strength of vaccine	Minimum strength of vaccine	Average percentage of protection S = 2,06	Minimum percentage of protection (p = 0,975) S = 3,14
MONO O							
132	0,05	2,15	0,10	1,66 : 0,05 = 33,2	1,66 : 0,10 = 16,6	> 99,4 %	99,4 %
119	0,10	2	0,20	1,66 : 0,10 = 16,6	1,66 : 0,20 = 8,3	> 99,4	97
118	0,18	2,2	0,41	1,66 : 0,18 = 9,2	1,66 : 0,41 = 4	> 99,4	89
105	0,21	2,3	0,49	1,66 : 0,21 = 7,9	1,66 : 0,49 = 3,4	> 99,4	85
131	0,35	2,2	0,77	1,66 : 0,35 = 4,7	1,66 : 0,77 = 2,1	98,2	75
MONO A							
224	0,07	1,7	0,11	1,66 : 0,07 = 23,7	1,66 : 0,11 = 15	> 99,4 %	99,2 %
226	0,09	2	0,18	1,66 : 0,09 = 18,4	1,66 : 0,18 = 9,2	> 99,4	97,5
228	0,12	2,2	0,26	1,66 : 0,12 = 13,8	1,66 : 0,26 = 6,4	> 99,4	95
227	0,25	2	0,5	1,66 : 0,25 = 6,6	1,66 : 0,5 = 3,3	> 99,4	85
220	0,62	2	1,25	1,66 : 0,62 = 2,6	1,66 : 1,25 = 1,3	90	58
MONO C							
320	0,12	1,9	0,23	1,66 : 0,12 = 13,8	1,66 : 0,23 = 7,2	> 99,4 %	96 %
317	0,16	2	0,31	1,66 : 0,15 = 11,0	1,66 : 0,31 = 5,4	> 99,4	93
322	0,18	1,95	0,36	1,66 : 0,18 = 9,2	1,66 : 0,36 = 4,6	> 99,4	91
319	0,20	2,2	0,44	1,66 : 0,20 = 8,3	1,66 : 0,44 = 3,8	> 99,4	88
324	0,43	1,85	0,80	1,66 : 0,43 = 3,8	1,66 : 0,80 = 2	97	72

Figure No. 14

V. Correlation between the 50 percent Guineapig Vaccinating Dose (DVC_{50}) and the 50 percent Cattle Vaccinating Dose (DVB_{50}).

1. Comparison of the dose-effect laws linking the immunogenic power of foot-and-mouth disease vaccines in guineapigs and cattle.

Comparison of the slopes of the dose-effect laws established in cattle and guineapigs shows that there is no significant difference in the threshold of p = 0.95 (Figure No. 15). We are therefore justified in comparing the DV_{50} of these dose-effect laws to estimate the correlation between the DVB_{50} and the DVC_{50}.

2. Correlation between the DVC_{50} and the DVB_{50}.

For reasons of symmetry and mathematical coherence, we have changed the original data of the DV_{50}s expressed in ml in our calculations into decimal logarithms of the DV_{50} in 1/100 ml.

Example: If DV_{50} = 0.20 ml, then log (DV_{50} 1/100 ml) = 1.30.

On the other hand, we have calculated the average precisions of the DV_{50} (p = 0.95): -

	fD	Log. fD
DVC_{50}	1.66	± 0.22
DVB_{50}	2.0	± 0.3

In our correlation DVC_{50}/DVB_{50}, each pair of values will be arranged in a rectangle with the sides as follows: -

± 0.22 for the DVC_{50}
± 0.3 for the DVB_{50}

The calculations of the correlation is based only on the average values of the DVC_{50}s and the DVB_{50}s estimated for the same vaccine (Schwartz, 10).

(a) Calculation of the laws of correlation, giving the DVB_{50} in terms of the DVC_{50}.

We made separate calculations for O, A and C valences (Figures Nos. 16, 17, 18, 19, 20, 21). The correlation coefficients "r" are all significant, at least at the threshold of p = 0.95.

Figure No. 15

Comparison of the slopes of the Dose-effect Laws of the immunogenic power of foot-and-mouth disease vaccines in guineapigs and cattle

	S_m	fS_m	S2/S1	$f\frac{S2}{S1}$	Conclusions
Cattle (1)	2.06	1.11			Difference not significant
Guineapigs (2)	2.15	1.06	1.04	1.11	Parallel slopes

Correlation between the DVC_{50} and the DVB_{50} : "O" Valence

Vaccine No.	DVC_{50}		DVB_{50}	
	in ml	$X = \log.(\frac{1}{100}$ ml)	in ml	$Y = \log.(\frac{1}{100}$ ml)
1105 (1)	0,40	1,60	0,27	1,43
1105 (2)	0,25	1,40	0,21	1,32
1118	0,22	1,34	0,18	1,25
1119 (1)	0,11	1,04	0,10	1,00
1119 (2)	0,11	1,04	0,10	1,00
5131	0,22	1,34	0,35	1,54
5132 (x)	0,27	1,43	0,05	0,70
5134	0,27	1,43	0,16	1,20
515	0,20	1,30	0,13	1,11
1108	0,20	1,30	0,07	0,85
1109	0,40	1,60	0,50	1,70
1110	0,40	1,60	0,20	1,30
1111	0,45	1,65	0,20	1,30

(x) **Aberrant case : not used in the calculations (further work on it in progress)**
$r = 0,60$ **significant at** $p = 0,95$ - ddl = 10

Formula of the law of correction: $Y = (0,77\ X + 0,19) \pm 0,42$ à $p = 0,95$

Variance : $s^2y = 0,044$ $\sigma y = 0,21$

Figure N° 16

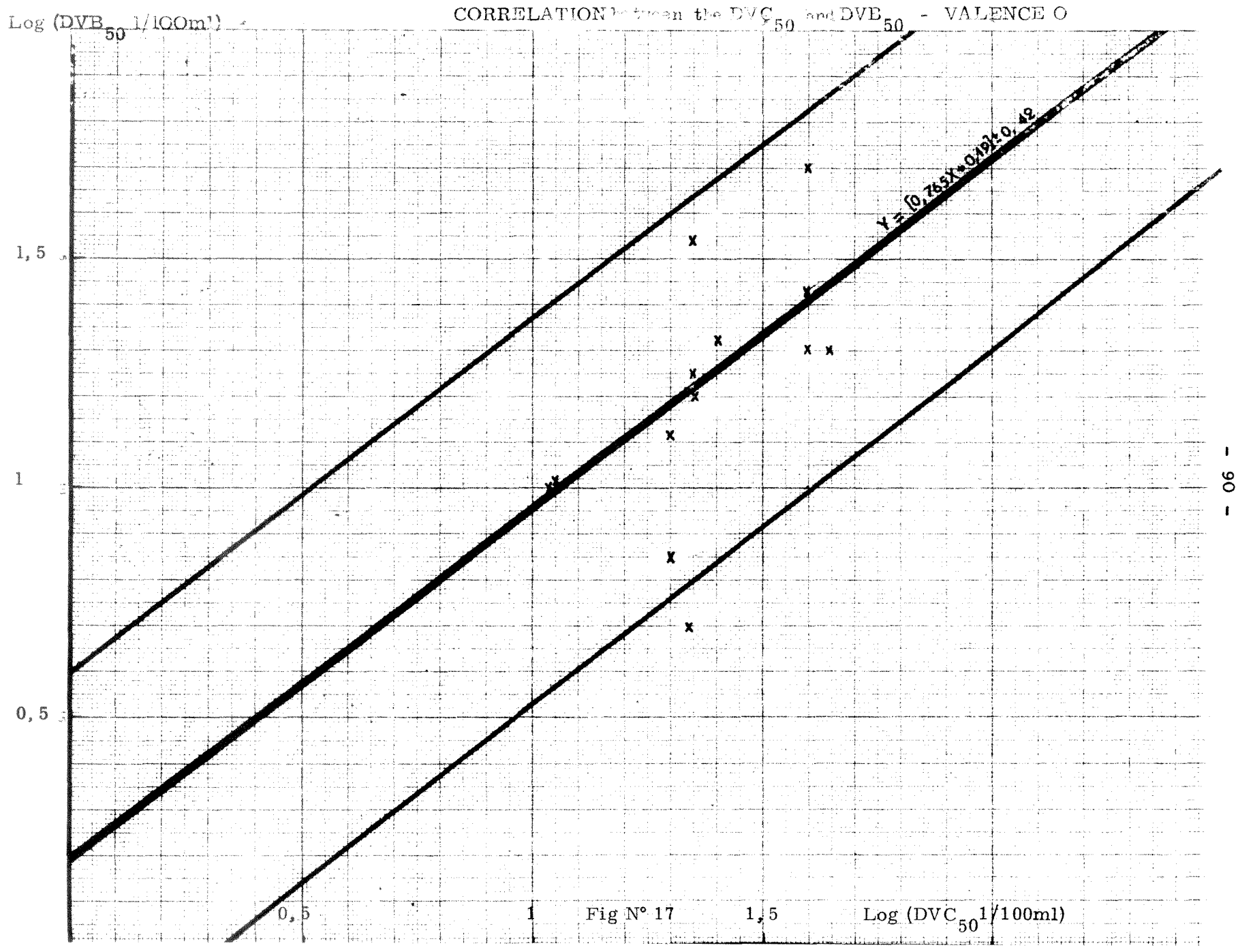
CORRELATION between the DVC50 and DVB50 - VALENCE O
Log (DVB50 1/100ml)
1,5
1
0,5
0,5
1
1,5
Log (DVC50 1/100ml)
Y = (0,765X+0,19)±0,42

Fig N° 17

Correlation between the DVC_{50} and the DVB_{50} : VALENCE A

Vaccine No.	DVC_{50}		DVB_{50}	
	in ml	$X = \log.(\frac{1}{100}$ ml)	in ml	$Y = \log.(\frac{1}{100}$ ml)
5220	0,20	1,30	0,62	1,80
5224	0,09	0,95	0,066	0,82
5226	0,15	1,18	0,09	0,95
5227	0,14	1,15	0,25	1,40
5228	0,12	1,08	0,12	1,08
5233 (x)	0,06	0,80	0,75	1,87
1208	0,06	0,80	0,07	0,85
1209	0,10	1,00	0,07	0,85
1210	0,06	0,80	0,20	1,80
1211	0,05	0,70	0,07	0,85
1212	0,04	0,60	0,20	1,30
1213	0,08	0,90	0,30	1,50
1216	0,07	0,85	0,07	0,85
1217	0,14	1,15	0,82	1,62
1218 (1)	0,07	0,80	0,20	1,30
1218 (2)	0,09	0,95	0,20	1,30
5221	0,11	1,04	0,20	1,30
5229	0,10	1,00	0,07	0,85
5230 (x)	0,08	0,90	0,50	1,70

(x) Aberrant case, not used in our calculations: (further work on it in progress)

$r = 0,665$ - significant at $p = 0,99$ - ddl = 15

Formula of law of correlation: $Y = (1,12\ X + 0,11) \pm 0,52$ à $p = 0,95$

Variance : $s^2y = 0,064$ $\sigma y = 0,26$

Figure N° 18

CORRELATION between the DVC_{50} and DVB_{50} - VALENCE A

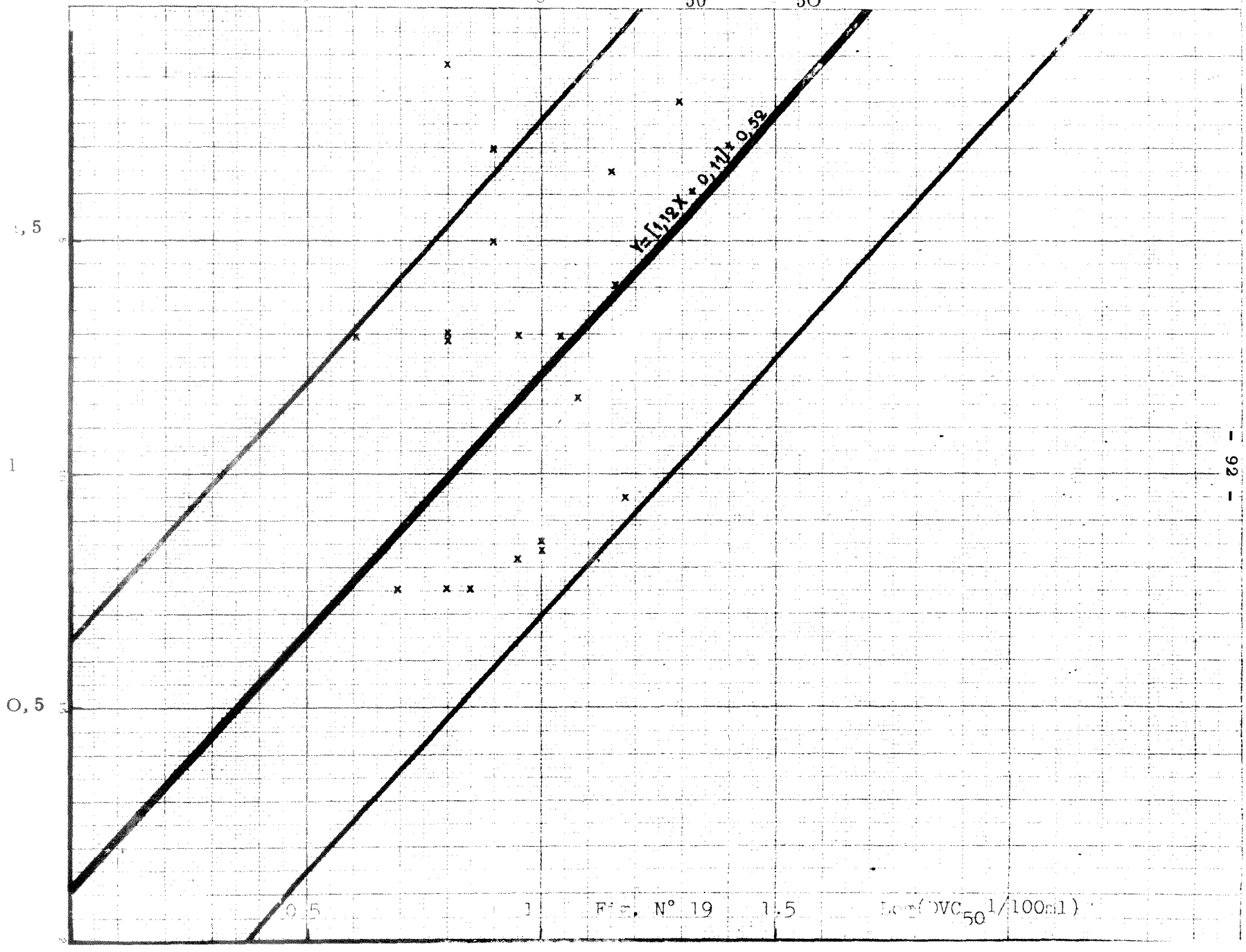

Fig. N° 19

CORRELATION between DVC 50 and DVB 50 - VALENCE C

Vaccine No.	DVC 50		DVB 50	
	ml	$X = \log(\frac{1}{100}\ ml)$	ml	$Y = \log(\frac{1}{100}\ ml)$
354	0,10	1,00	0,07	0,85
355	0,10	1,00	0,07	0,85
1307	0,25	1,40	0,50	1,70
1308	0,25	1,40	0,20	1,30
1309	0,10	1,00	0,20	1,30
1311	0,10	1,00	0,07	0,85
1312	0,20	1,30	0,07	0,85
1313	0,35	1,55	0,20	1,30
1315	0,09	0,95	0,07	0,85
1316	0,06	0,80	0,07	0,85
1317	0,12	1,08	0,16	1,19
5319	0,13	1,11	0,20	1,20
5320	0,20	1,30	0,12	1,08
5322	0,07	0,85	0,18	1,25
5324	0,27	1,43	0,43	1,63

$r = 0,75$ significant at $p = 99,9\ \%$ ddl = 13

Formula of correlation law - $Y = (0,94\ X + 0,08) \pm 0,39$ à $p = 0,95$

Variance $s^2y = 0,038$ $\sigma y = 0,195$

Figure N° 20

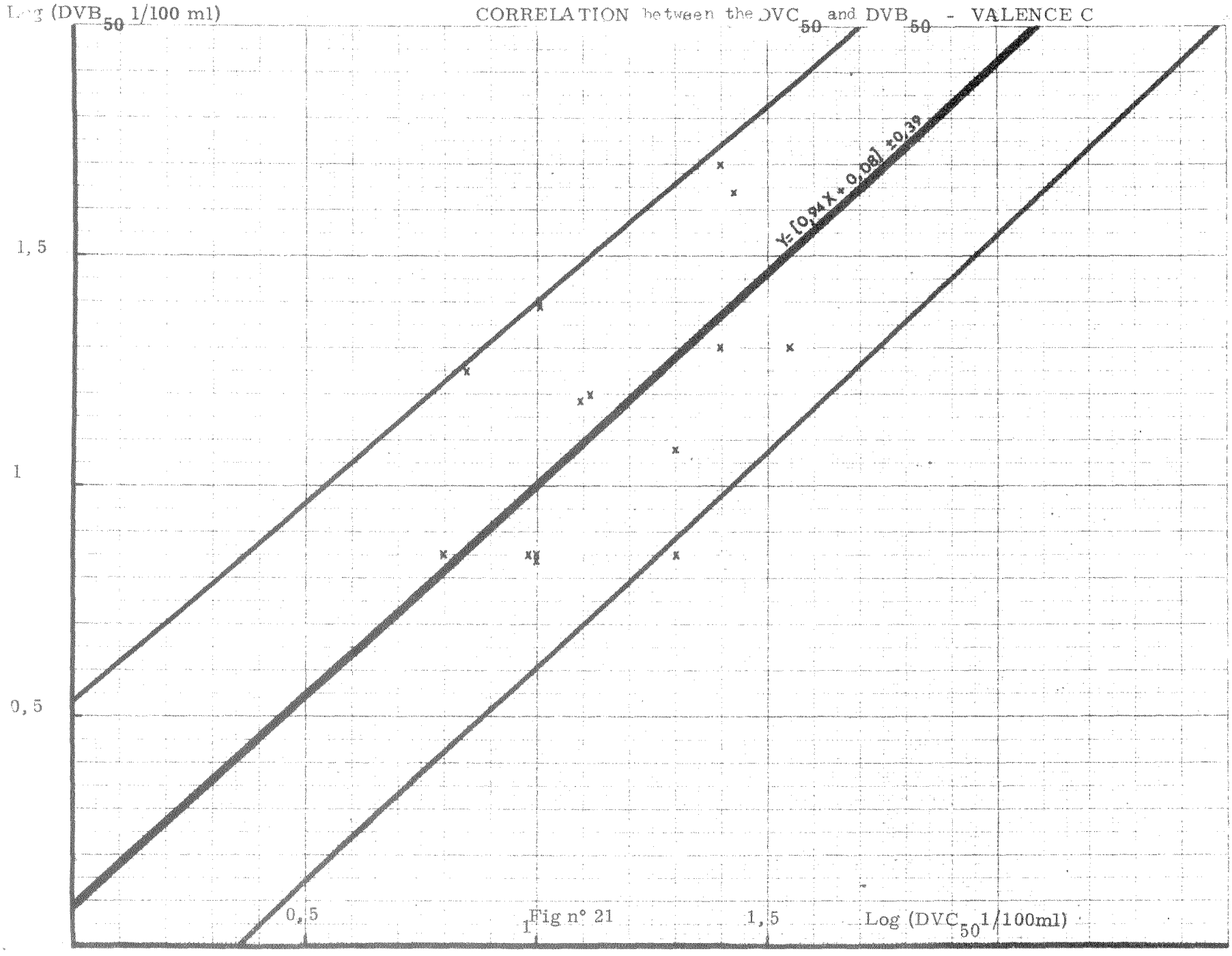
CORRELATION between the DVC50 and DVB50 - VALENCE C
Log (DVB50 1/100 ml)
1,5
1
0,5
0,5
1
1,5
Log (DVC50 1/100ml)
Y= [0,94 X + 0,08] ±0,39

Fig n° 21

We are justified in estimating the DVB_{50}, using the DVC_{50}, taking into account the related variance, i.e. as we mention above, the precision of the DVB_{50} estimated by this technique should take into account two errors in our measurements (cattle and guineapigs): we therefore lose precision of the DVB_{50} (Figure No. 22).

In actual fact, knowing the DVC_{50} of a vaccine of a given valence, the most probable DVB_{50} can be estimated ($p = 0.95$) by the aid of the formula of one of the laws (Figure No. 22) or by the aid of Figures Nos. 17, 19, 21.

To illustrate our conclusions, we will interpret the three examples in Figures Nos. 16, 18, 20.

Examples:

"O" Vaccine No. 105

$DVC_{50} = 0.40$ ml with $X = 1.60$ (log. 40)

Replace in the formula $Y = (0.77X + 0.19) \pm 0.42$,X by its value, whence $Y = 1.41 \pm 0.42$

$1 < Y < 1.83$

whence the theoretical $DVB_{50} = 0.26$ included between 0.10 and 0.70 ml. The experimental value found in cattle:

- at the first titration: 0.27 ml included between 0.14 and 0.51 ml
- at the second titration: 0.21 ml included between 0.09 and 0.51 ml.

"A" Vaccine No. 220

$DVC_{50} = 0.20$ ml with $X = 1.30$ ml.

Replace in the formula $Y = (1.12X + 0.11) \pm 0.52$,X by its value

whence $Y = 1.56 \pm 0.52$

$1.04 < Y < 2.08$

whence the theoretical $DVB_{50} = 0.36$ ml included between 0.11 ml and 1.20 ml. Experimental value found in cattle: 0.62 ml included between 0.31 and 1.24 ml.

"C" Vaccine No. 322

$DVC_{50} = 0.07$ with $X = 0.85$

Replace in the formula $Y = (0.94X + 0.08) \pm 0.39$,X by its value

whence $Y = 0.88 \pm 0.39$

$0.49 < Y < 1.27$

whence the theoretical $DVB_{50} = 0.075$ included between 0.035 ml and 0.19 ml. Experimental value found in cattle : 0.18 ml included between 0.09 and 0.35 ml.

Figure No. 22

Correlation law giving the DVB_{50} and its precision in terms of the DVC_{50}

Valence	Number of Vaccines	Formula of the law	Precision p = 0.95 2σ	Threshold of significance r
O	12	Y = 0.77 X + 0.19	± 0.42	0.95
A	17	Y = 1.12 X + 0.11	± 0.52	0.99
C	15	Y = 0.94 X + 0.08	± 0.39	0.999

Y = DVB_{50} in log (1/100 ml)

X = DVC_{50} in log (1/100 ml)

(b) Comparison of the laws of correlation between O, A and C valences.

The three laws are mathematically different and it is difficult to compare them statistically (Figure No. 22).

The formulae of the laws are Y = a X + b.

We compared the "a" slopes between the three valences and calculated: $F^2 26 = 0.3$: thus there is no significant difference between these slopes at the threshold of p = 0.95.

Factor "b" is more difficult to compare because we do not know its variance.

We think it preferable to consider the formulae of the laws of the three valences as being different, because we lose precision if we calculate only one law for the three values. In fact, in this case, the variance of the DVB_{50} becomes equal to : $s^2 = 0.06$ ($2\sigma = \pm 0.50$).

(c) Abnormal (aberrant) cases.

In our tests, we had three aberrant cases in 47 pairs of points (DVC_{50} and DVB_{50}) : we did not include them in our present calculations, because they are now being more fully studied.

3. Correspondence between the DVC_{50} and the average percentages of protection in cattle.

(a) DVC_{50} and the average percentage of cattle protection.

Taking into account the correlation between the DVC_{50} and the DVB_{50} (Figure No. 22), as well as the relationship between the DVB_{50} and the average percentage of cattle protection (Figure No. 12), it is possible to establish the correspondence between the DVC_{50} and the average percentage of protection

of cattle for each valence (Figure No. 23). (Note: Figure No. 4 made from different data, is comparable with Figure No. 23).

(b) Comparison of the average percentages of cattle protection estimated in cattle and in guineapigs.

In Figure No. 24, which concerns a series of 15 vaccines, there are shown the average percentages of cattle protection estimated in both cattle and guineapigs: in general, these two estimations are comparable.

Correspondence between the 50% guineapig vaccinating dose (DVC_{50}) and the average percentage of cattle protection

Average DVC 50 (in ml)			Average percentage P of cattle protection
Valence O	Valence A	Valence C	
0,43	0,16	0,30	99,4
0,80	0,24	0,47	97
1,00	0,28	0,59	95
1,50	0,35	0,80	90
1,80	0,42	0,95	85
2,10	0,47	1,10	80
2,80	0,56	1,40	70

Figure N° 23

Comparison of the Estimation of Average Percentages of Cattle Protection, using the DVB_{50} and the DVC_{50}

Vaccine N°	Average Cattle Protection	
	Estimation in Cattle	Estimation in Guineapigs
Monovalent O		
32	≥ 99,4	≥ 99,4
19	≥ 99,4	≥ 99,4
18	≥ 99,4	≥ 99,4
05	≥ 99,4	≥ 99,4
31	98,2	≥ 99,4
Monovalent A		
24	≥ 99,4	≥ 99,4
26	≥ 99,4	≥ 99,4
28	≥ 99,4	≥ 99,4
27	≥ 99,4	≥ 99,4
20	90	≥ 99,4
Monovalent C		
20	≥ 99,4	≥ 99,4
17	≥ 99,4	≥ 99,4
22	≥ 99,4	≥ 99,4
19	≥ 99,4	≥ 99,4
24	97	≥ 99,4

Figure N° 24

DISCUSSION

The main objects of the experimental work in this report are: -

1. Attempts to titrate the activity of a monovalent foot-and-mouth disease vaccine by establishing the DV_{50} in cattle (DVB_{50}).

2. The use of guineapigs for similar titration (DVC_{50}).

3. Establishment of the parallelism between the guineapig and cattle vaccinating doses.

4. To make conclusions and practical applications.

If we analyse the results obtained, in how far have these objectives been met?

1. With regard to the establishment of the DV50 in cattle, we were able to confirm the value of the method first put forward by Henderson and Galloway. Titrating 15 monovalent vaccines (five each of types O, A and C) in 300 cattle, we were able not only to obtain the DVB_{50} for each vaccine, but also to establish, by our system, the slope of the dose-effect for cattle (Figure No. 12), i.e. the immunological response of cattle to different doses of a vaccine. We have been able to conclude that, according to our system, there is a general law for all vaccines and that from this slope it is possible, knowing the number of DVB_{50}s of a vaccine, to evaluate the percentage of protection it will confer on cattle, as shown in Figure No. 25.

These values reflect the immunity obtained 21 days after vaccination and have a static character, because after having reached a maximum at about the 30th day, the immunity decreases and the DVB_{50} of the vaccine will be greater if the challenge test is made say 60 days after the vaccination, as we already showed in 1959 (11). If we can trace the kinetics of the DVB_{50} from the results of the experiments now in progress, it would then be possible to give a dynamic character to the titration of the vaccine and, knowing the results of the control, to deduce thereby the duration of the immunity conferred by it.

From the establishment of the dose-effect slope, a vaccine can be titrated, using not four dilutions as previously in say 20 cattle, but only two dilutions in 10 cattle and obtain sufficient accuracy ($\pm$ 0.37) at 95 percent probability, for which the method may be used in present practice.

Again, we can compare two vaccines and state which is the better, taking into account the separating power of our system, with an average of 2.7, always with 95 percent probability. We can therefore state that a vaccine is 2.7 times better than another. Above this limit, we cannot have any sure indices. It is evident that if we accept a greater risk, the separating power will become better (e.g. for a one to three chance, a separating power of 1.6), for there is a compromise between the property of definition of the method and the accepted risk.

Values of DVB_{50} in terms of minimum percentage of cattle protection

Minimum percentage of protection (S = 3, 14)	Number of DVB_{50}s in th full dose of vaccine
50	1
70	1, 85
72	2
75	2, 3
78	2, 5
80	2, 7
83	3
85	3, 4
86	3, 5
88	4
90	4, 5
92	5
95	6, 7
96	8
97, 5	10
99	15
$>$ 99, 4	20

Figure N° 25

It must be pointed out, finally, that all our calculations have been made, taking into account only generalization foot lesions. It is very probable that by considering other criteria, e.g. the number of feet affected, it would be possible to have more precision. In addition, the titrations of the antibodies in cattle used in establishing the DVB_{50} could also provide complementary elements.

As this work has not yet been completed, we cannot take this into account at this time.

2. The use of the DV_{50} % in guineapigs (DVC_{50}) has given us encouraging results. Guineapigs become well vaccinated with divided doses of vaccine and their immunological response is consistent, regular and reproducible. For this, however, certain conditions have to be fulfilled: -

- The animals must come from a known source, must be of similar weight and in a state of good health.

- The results are capable of comparison on the condition that the same virus is used in the preparation of the vaccine and in the challenge tests and the technique of preparation of the vaccines is exactly similar.

- Guineapigs do not react similarly to different viruses. We have had many examples when adapting a natural virus. It is therefore probable that something similar concerns the immunological response to the injection of an antigen. A change in the challenge virus, or in the virus used in the preparation of the vaccine or even in the technique, introduces a new element which has to be examined each time by cattle tests.

- In order to study the correlation between DVC_{50} and DVB_{50}, the laws relating to each type of virus have been established separately. It is very probable that within the same type, the same separation has to be made for each strain.

- In our study, guineapigs were subjected to challenge tests ten days after vaccination : comparison of tests at ten and 21 days is now being carried out.

3. In establishing the correlation between the DVC_{50} and the DVB_{50}, it must be emphasized that we are looking for correlation guineapig $\longrightarrow$ cattle, i.e. in estimating the activity value of a vaccine, we wish to deduce the percentage of protection in cattle, as expressed by the DVC_{50}.

Figure No. 4 shows, for each type of virus, the mass percentages of protection obtained in cattle, in terms of the DVC_{50}. We reproduce this in another form below. Vaccines with a DVC_{50} below or equal to 0.4 ml are not included. For each type of vaccine we give the number of cattle vaccinated with these vaccines and which resisted challenge. The percentages of protection have been deduced.

Vaccine Type	Number of batches of vaccines tested	Number of cattle examined	Number of cattle protected	Percentage of protection
O	24	130	127	97.6
A	33	175	169	97.6
C	31	160	159	≃ 100

In consideration of these results from the control of the produced vaccines, it is permissible to conclude that a vaccine which has a DVC_{50} below or equal to 0.4 ml gives about 100 percent protection in cattle and that this applies to the three types of the virus: however, Figure No. 23 and the results obtained with current vaccines during the past year cause us to introduce a restriction insofar as A and C valences are concerned. In fact, it seems that it may be advisable to have as the limit of the value of the DVC_{50}, 0.15 ml for A valence and 0.3 ml for C valence, in order to be certain of obtaining protection in cattle approaching 100 percent. This precaution is even more recommendable when Figure No. 18 is considered: here, among 17 monovalent A vaccines which were titrated in both cattle and guineapigs, there were two "pairs" of aberrant results which we have not included in our calculations but which we are now studying in greater detail.

If, in our opinion, it is now possible to deduce from the DVC_{50} (0.4 - 0.3 or 0.15 ml according to the types) that a vaccine is good and that the protection it gives to cattle approaches 100 percent, it would be unwise, at least at the present time, to draw any other conclusions from titrations in guineapigs. There has to be taken into account the fact that two different systems "cattle and guineapig" are used, each having its own statistical lack of precision. In establishing the correlation, there are two errors attached to each other with the result that the insecurity zone is greater than in the case of each separate system (Figure No. 22). It is, in fact, this quite large insecurity zone which imposes the limits of the method. This does nothing to limit the value of the guineapig insofar as concerns complementary qualitative control of vaccines, but we must guard against too extensive conclusions and narrower correlations which would be attempted to be made by considering Figures Nos. 16, 18 and 20. It is possible that experimentation and accumulation of results may lead to supplementary precisions but it is wise at present to continue with the norms already indicated.

PRACTICAL APPLICATIONS

This experimentation has enabled us to pass from qualitative controls in cattle which did not give us precision, to quantitative control in guineapigs by the establishment of a correlation between the DVB_{50} and the DVC_{50} of a vaccine: cattle being always the animals for reference. At the present time control in guineapigs is used in the Institute for two objectives: -

1. For the monovalent vaccines in routine production.

(Monovalent cattle dose: 1.66 ml).

If the DVC_{50} of monovalent "O" vaccine is less than 0.4 ml, of monovalent "C" vaccines less than 0.3 ml and of monovalent "A" vaccines less than 0.15 ml, we consider them satisfactory and use them to prepare trivalent mixtures which are systematically controlled in cattle.

When the value of the DVC_{50} is near the limits which we have fixed, we carry out quantitative titration in cattle, using two dilutions.

Thus, while economising in the use of cattle, we obtain a quantitative answer for each monovalent vaccine and can make our production very homogeneous.

2. For experimental vaccines.

It is perhaps in connection with these vaccines that this technique renders us the greatest service: as a matter of fact, for economic reasons, it has not been possible up to the present time to carry out all the indispensable controls for our different tests. The establishment of the DVC_{50} in guineapigs enables us to have a quantitative answer for each vaccine and to compare vaccines: they are grouped according to their activity value and then we have only to carry out quantitative titrations in cattle using a representative of each group in order to ascertain the correlation between the DVC_{50} and the DVB_{50} and to have an exact answer. Thus, there is great progress in that a practical method of quantitative control has been placed in the hands of research workers.

Certainly, there are still many points to be clarified: we are sure, however, that the technique used within the limits as indicated, is of much value in the control of vaccines.

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Deterioration of the Value of the Foot-and-Mouth Disease Antigen already evaluated in In-Bred Mice.

by Georges Gayot, Léone Dhennin and Louis Dhennin.

We have already shown that when mice C57 $B_1/6$, 12 weeks old are vaccinated against foot-and-mouth disease and challenged 15 days later by the inoculation of mouse-adapted virus, the doses of antigen and virus being properly chosen, it is possible by studying the results by the probit method, to obtain a dose-effect law with the m average at probit 5 corresponding to the dose which protects 50 percent of the animals against a determined dose of virus.

This method of determining the antigenic potential of inactivated foot-and-mouth disease vaccines may be applied many times and following its use during the last year in other experiments on the evaluation on three different occasions of the same vaccine kept in the refrigerator at an average temperature of +4°C., it seemed that its value decreased in the course of time and that it was possible to calculate this loss in quantity in terms of the time.

It is this experimental work which we propose to report. Work done at the Laboratoire Central de Controle et de Recherches du Service Vétérinaire (Directeur : Dr.A. Lucas).

Table I shows the results of titrating the challenge virus in baby mice born from white Swiss mothers four - six days old and randomised. The 50 percent lethal dose (LD_{50}) was calculated by the Spearman-Kärber method : this we may mention, is justified since there are the same numbers of mice per dilution and the 0-100 percent mortality doses are included in the results and, corresponding to the average LD_{50} there is a replica of the deviation of the average, intended to determine the 95 percent reliance and it is evident that the titrations do not give different results. Mice C57 B1/6 vaccinated with 1 ml. of a 10^{-5} suspension were challenged in the different experiments with 2,100, 2,200 and 1,200 LD_{50}, which were not significantly different doses and may, in fact, be considered identical.

TABLE I

Dilutions:	-4	-5	-6	-7	-8	-9	m	2 Sm
Exp.I	6/6	6/6	6/6	5/6	0/6	0/6	7,33	0,33
Exp. II	7/7	7/7	7/7	4/7	2/7	0/7	7,36	0,55
Exp. III	7/7	7/7	7/7	4/7	0/7	0/7	7,07	0,40

Titrations in mice with challenge virus (O 169 S). For each inoculation the volume was 0.1 ml.

Table II shows the results of the three experiments. It may be said that if the first two averages are quite justified, the determination of the third may to some extent lend itself to criticism, because at the dose of 0.16 ml. with 31 percent survival with probit 4.51 the threshold of the average, probit 5 is not reached. However, the slight amount of extrapolation which we have given, is largely justified because this determination was part of a series of experiments in which different forumlae of the same vaccine were being compared and the usual formula being supposed and shown to be the least active, it seemed right to have somewhat lower protection titres for it.

TABLE II.

Dose of Vaccine in 0.01 ml. / Survivals	4	6	8	12	16	24	m b
Exp.1 : 15.4.64	2/11		4/15		8/15		1,1871
Exp.2 : 16.6.64		0/13		5/13		9/16	1,3059
Exp.3 : 14.8.64	1/16		2/16		5/16		1,4038

Experimental results of the dose-effect laws with the same vaccine at intervals of two months on three occasions.

From these results it is possible to make out a regression of the 50 percent vaccinating dose with the time as has been done in Table III where "y" is the log of the 50 percent vaccinating dose and "x" the time in months, the first experiment having "x" = o.

TABLE III

	x	x²	y	y2	xy
15/4	0	0	1,1871	1,4092	0
18/6	2	4	1,3059	1,7053	2,6118
12/8	4	16	1,4038	1,9706	5,6152
Σ	6	20	3,8968	5,0851	8,2270

$$r = \frac{0,4334}{\sqrt{8 \times 0,0235}} = 1^{**}$$

$$y - 1,2989 = 0,0541 \ (x - 2)$$

$$y = 0,0541 \ x + 1,1907$$

Regression of the 50 percent vaccinating dose with time.** The position arrived at is r = I significant to 1 percent for 1 degree of freedom (n-2) and from this, the linear relation may be expressed where the value of the LD_{50} ("y") evolves in terms of the time in months ("x") in accordance with y = 0.0541 x + 1.1907 and which is represented graphically in Figure 1.

The average slope of the three dose-effect laws, balanced by the inverse of their variances is established at 1.9798 (say 2) so well that a law constructed with this slope in a graph (Figure 2) with the abeissa of logs of DV_{50} and the ordinates of the percentages of protection expressed as probit, considering that the degradation of 0.05 per month allows the percentage of protected animals to be forecast at any time during the period of study, by the vaccines the specimens of which under study are representative.

Thus, a vaccine whose DV_{50} is 1 and which at zero day, with a dose log of 1.4 gives rise to 79 percent protection (probit 5.80) produces four months later and after preservation at an average temperature of +4°C., at the same dose log of 1.4 no more than 65 percent protection (probit 5.4), i.e. an average loss of 15 percent of activity.

These results, it should be noted, hold good only for the mouse and for a period of only four months which should take into consideration the date of the preparation of the vaccine : they indicate, however, a certain feeble loss of activity which, nevertheless, is not negligible.

(Central Control and Research Laboratory of the Veterinary Service - Director: Dr.A.Lucas)

SUMMARY

In a commercial trivalent vaccine whose 50 percent vaccinating dose has been determined in C57 B1/6 in-bred mice on several occasions, it was shown that the antigenic potential became significantly reduced (**r=1**) during preservation of the vaccine over a period of four months at +4°C., this reduction of activity being capable of being calculated quite simply.

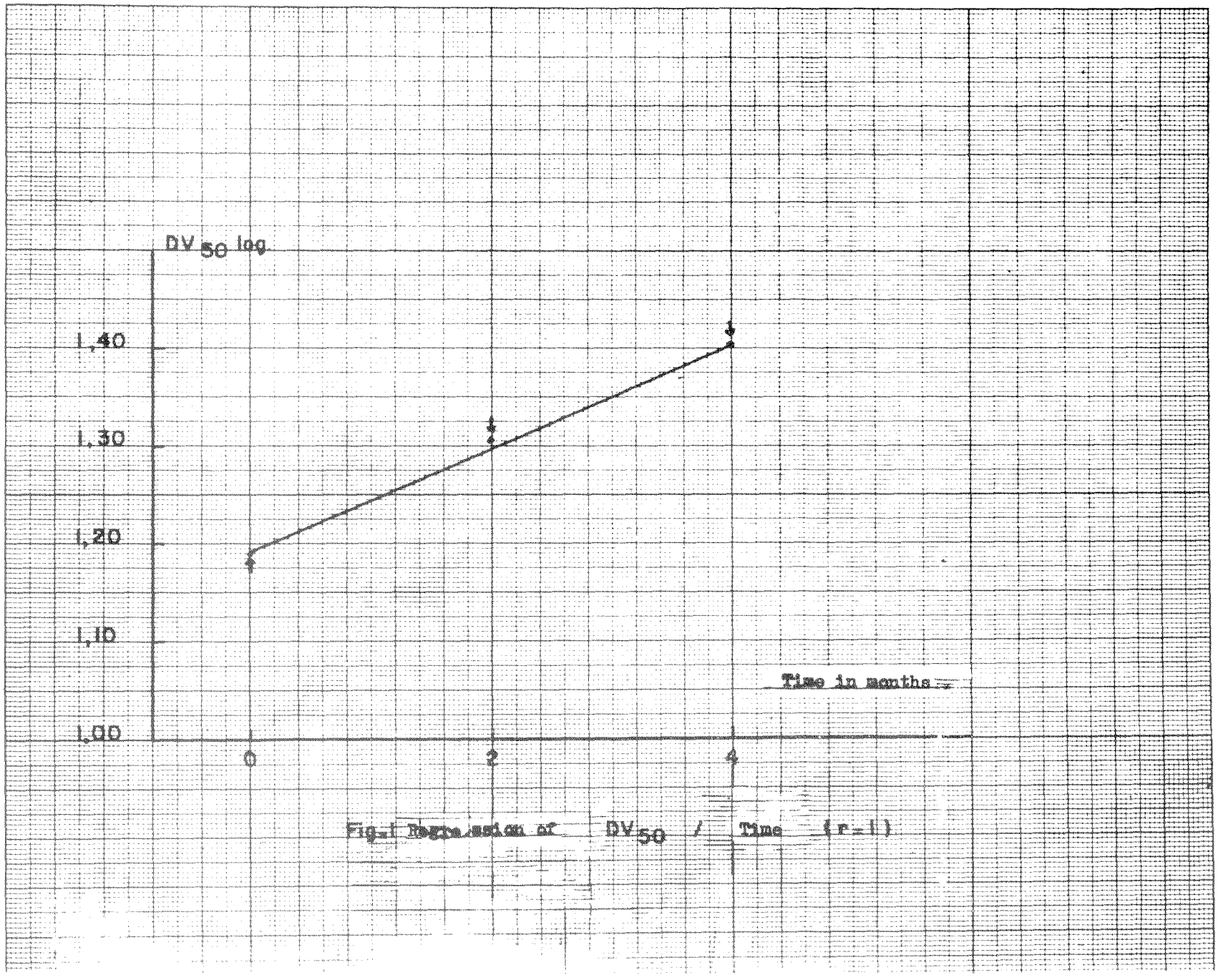

Fig. 1 Regression of DV50 / Time (r = 1)

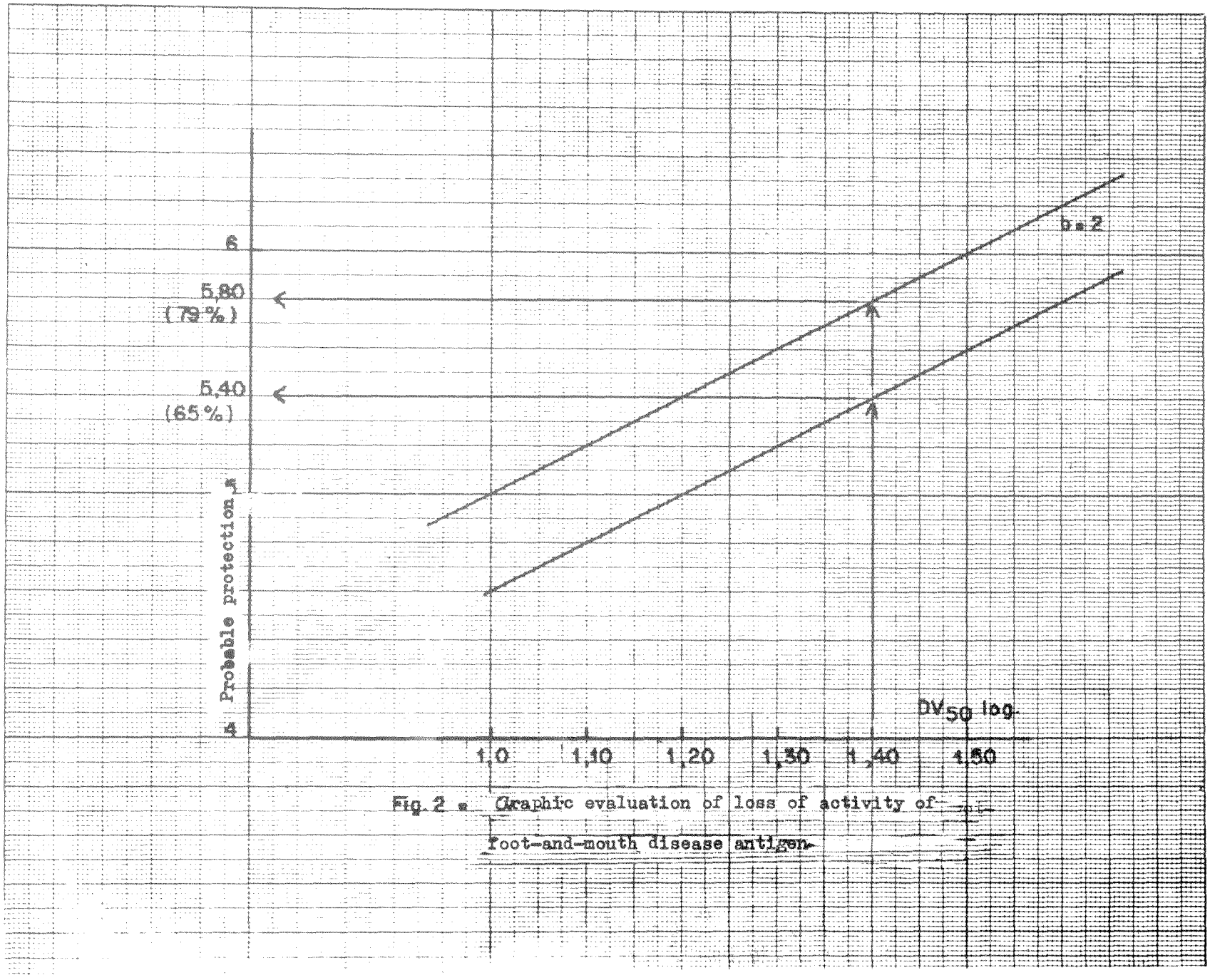

Fig. 2 = Graphic evaluation of loss of activity of foot-and-mouth disease antigen

PRELIMINARY NOTE ON THE QUANTITATIVE CONTROL OF FOOT-AND-MOUTH TRIVALENT VACCINE IN CATTLE BY THE SIMULTANEOUS INOCULATION OF THE THREE TYPES OF VIRUS

by

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In a former note (6) we reported the results of study of the law on dose of monovalent vaccine-protection of cattle. .This present paper deals with work carried out on establishment of this same relationship for trivalent vaccine.

For some time (4) we have been working on the possibilities of the control of trivalent vaccine by simultaneous challenge with the three viruses in the same cattle. The encouraging results led us to undertake a systematic study which is now in progress. We felt it would be useful to give an account of this work, always remembering that it is to be regarded as preliminary only.

It is of course quite clear that before embarking on this problem it would first be necessary to study the behaviour of our three strains of virus inoculated simultaneously in susceptible, non-vaccinated cattle.

In 1958, CUNHA et al. (3) showed that infection could be established simultaneously at different points of the tongue where the three types of the virus O, A and C are injected. The disease then developed in the classical way. These authors were unable to recover the three viruses in the secondary foot lesions of the same animal in any of the six cattle used in this work.

In a similar experiment, HENDERSON, quoted by CUNHA, succeeded in demonstrating two of the three viruses in a mixed sample of blood of six injected animals.

In 1959, ARMBRUSTER et al. were unable to show simultaneous multiplication of their three O, A and C strains of the virus. In fact, the strain O_2 which they used dominated their C and A strains. These authors came to the conclusion to inject the viruses in the order of A, C and O.

In this present article, after dealing with the disease following the simultaneous inoculation of the three types of the virus (O, A and C), we will report, in the second part, the preliminary results of the quantitative control of trivalent vaccine in cattle.

I. Diseases after Inoculation of the three viruses in cattle

A. Materials.

Details concerning:

1. The cattle and their susceptibility to foot-and-mouth disease

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* Work done by the Institut de la Fièvre Aphteuse, Lyons
Director: Dr. Vre C. Mackowiak.

viruses O, A and C, and

2. The challenge virus

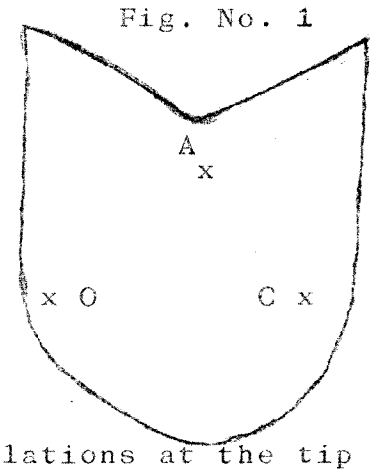
are described in a former article (6) and will not be dealt with here.

B. Methods.

1. Challenge.

The animals are brought to isolation stables at the Institute. The three viruses are injected simultaneously at three separate points of the tongue. The volume of each suspension of virus injected intradermolingually is 0.1 ml. containing about 5,000 DIB_{50} (DIB_{50} = 50% cattle infecting doses, titrated by the method according to HENDERSON). Figure No. 1 shows the points of the injections.

Fig. No. 1



We avoid making inoculations at the tip of the tongue so that reactions will not be squeezed when reading results. The viruses are injected in three separate spots and so the development of the primary vesicle from each strain can be followed.

2. Demonstration of the virus.

(a) Point of injection: primary vesicles.

Specimens.

Experiments in 17 cattle have shown that, under our conditions, readings should be made at an average of 24 hours after the inoculation. This seems a little bit early with our "O" strain (virus Flanders O) but it seems to be the maximum time in the case of the "C" strain and especially the "A" strain, if the running together of the primary vesicles is to be prevented.

Examination

Each specimen taken from the points of inoculation (which are clearly marked) is subjected to the complement fixation test.

(b) Feet: secondary foot lesions.

Specimens

The animals are slaughtered on the 4th or 5th day after the inoculation, depending on the individual case, and specimens of the vesicles from each foot are taken. Because of precautionary measures in force, we are not permitted to take specimens on several occasions from the same animal.

Examination

These specimens are subjected to the complement fixation test: 100% haemolysis (2) or 50% haemolysis (5).

(c) Blood: viraemia.

Specimens

On the occasion of each challenge, 15 ml. of blood are drawn into flasks containing 15 ml. Alsever solution. Centrifugation at 4000 r.p.m. for 10 minutes is carried out and then the plasma is collected.

In the preliminary study, 6 cattle were used and samples of blood from them were collected at 24, 48 and 72 hours after the inoculation. The plasma was frozen at -30°C before being tested.

Then 9 cattle were studied in exactly the same way, but blood was collected at the 18th, 24th, 40th, 48th, 64th and 72nd hours. Titration of the plasma was carried out at once without its being frozen.

Examination

Monolayers of pig kidney cells in Earle medium containing 0.5% lactalbumen hydrolysate and 5.0% calf serum, 200,000 cells per ml., are used.

For the plasma, the medium is Hank's medium, containing 0.5% lactalbumen hydrolysate but serum is not added.

Two techniques have been tried successively:

1. Serumneutralization of two viruses to encourage multiplication of the third virus.

Plasma, diluted to 1/10 and 1/100 are mixed with three groups of two anti-foot-and-mouth disease sera prepared in cattle (O + A; A + C; O + C). These pairs of sera are used in dilutions of 1/8 and 1/32. Following this serumneutralization, the virus present in the positive tubes (*) cannot be directly demonstrated by complement fixation reaction because the presence of the bovine antisera gives rise to anticomplementary results. Passage of the virus without antiserum in the same cell

(*) By "virus in the positive tubes is meant, culture fluid of the tubes where a typical cytopathogenic effect is seen.

system is necessary which, theoretically, should enable isolation of the non-neutralised virus to be made. The problem, however, is more complex, because the titres of each of the viruses change with each specimen and it is impossible to know the titres which each one will have. It is clear that, under such conditions, the neutralization reaction cannot be strictly balanced for the three viruses brought into contact with a pair of antisera. The presence of a virus with a high titre requires the use of only little diluted antisera and, under these conditions, non-specific neutralisation will occur for a virus with a slight titre, of a different type. On the other hand, if the antiserum is diluted too much, it may be that, after eliminating these non-specific phenomena, the virus of the same type as the serum will be incompletely neutralized. It is not surprising to observe, therefore, that following passage in the positive tubes, the virus as shown by the results of complement fixation may not automatically be that which should be found.

Because of the results which have been obtained, we have given up this method and have tried a simpler way of demonstrating the three viruses in plasma.

For practical reasons we have not been able, up to the present time, to take up this technique with fresh plasma which has not previously been frozen.

2. Direct titration of a fresh plasma.

The plasma is diluted 4-fold. Six tubes of culture are each inoculated with 1 ml. of each dilution. By working quickly, coagulation phenomena are avoided. The virus in the positive tubes can be identified directly by the complement fixation reaction.

In rare instances of anticomplementary obility, i.e. sometimes with low dilutions of plasma, it is necessary to carry out a second passage in the same cell system, which entails the trouble of selecting a virus with the highest titre. This virus infects the majority of the cells and because of this, multiplication of the other two viruses is hindered and their presence is not shown by the complement fixation reaction. In order to have a positive result in this reaction, a minimal concentration of about 10^6 $DECP_{50}$/ml* is required. Below this concentration, it is difficult to demonstrate the virus by the complement fixation reaction.

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* $DECP_{50}$/ml: the dose having a cytopathogenic effect in 50% of the tubes.

C. Results

1. Multiplication of the virus at the point of inoculation

Figure No. 2.

Multiplication of the three viruses at the point of intradermolingual inoculation

Types of virus	Clinical vesicles (No. of animals with lesion / total number of observations)	Specificity confirmed by complement fixation (100% haemolysic)	Notes
O	13/17	12/13	One specimen was accidentally destroyed, but "O" virus was found in foot lesions on the 5th day.
A	17/17	16/17	One specimen was very anticomplementary. There was a suspicion of "A" virus in foot lesions on the 5th day.
C	16/17	15/16	One specimen was accidentally destroyed, but "C" virus was found in tongue and foot lesions on the 5th day.

The cattle in which there were no primary vesicles from viruses O and C will be referred to later.

2. Spread of the viruses in the animal body.

Preliminary work was carried out with 6 cattle. Examinations were made for the presence of virus:

(a) at the points of inoculation after 24 hours;

(b) in the plasma after 24, 48 and 72 hours;

(c) in the secondary foot lesions on the 5th day.

Figure No. 3

Spread of the three viruses in the animal body after their simultaneous inoculation by the intradermolingual route

Number (cattle)	Primary vesicles after 24 hours			Viraemia after 24, 48 or 72 hours (mass results)			Secondary Foot vesicles after 5 days		
670		A	C		A	C		A	C
674		A		O	A			A	C
514	O	A	C	O		C			C
831	O	A	C	O	A	C		A	C
832	O	A	C	O	A	C		A	C
833	O	A	C	O	A	C	O	A	C

In two cattle, only two of the three viruses were demonstrated as having spread (viraemia and secondary foot lesions). Serumneutralization was used in studying the viraemia in them (Nos. 670 and 514). As we have already said, however, this technique does not now seem to us to be highly sensitive. Again, in the second animal, "A" virus multiplied at the point of inoculation and there was suspicion of its presence in the foot lesions. These encouraging results led us to perfect the technique for studying virus throughout the animal body, by increasing the number of blood specimens, by preventive coagulation of the plasma and by using 50% haemolysis in the technique for virus in the foot lesions.

Later work was carried out with 9 cattle.

Figure No. 4

Invasion of the animal body following simultaneous inoculation of the three viruses by the intradermolingual route

Number (cattle)	Tongue lesions (4th day)	Viraemia (hours) 18	24	40	48	64	72	90	Secondary Foot Lesions (4th day)
1240	AC	OAC	O	O C	O	O C	O	O	O
1260	OAC	O C	OAC	O C	O C	O C	OAC	AC	AC
1267	OAC	O C	AC	O C	O C	O C	AC	C	OAC
1637	OAC	OAC	AC	C	AC	AC	AC	A	AC
1639	AC	C	C	O C	C	C	C	AC	C
1643	OAC	C	O C	O C	O	O C	OA	AC	OAC
1666	AC	OAC	AC	O C	OAC	OAC	OAC	C	C
1684	Anticompl.	OAC	C	OAC	OAC	O C	C	AC	OAC
1687	OAC	OAC	OAC	OAC	OAC	OAC	OAC	OAC	OAC

D. Discussion

1. Multiplication of the virus at the point of inoculation.

Virus A

Multiplication was constant for our strain in 17 cattle tested.

Virus C

There was one wrong result (No. 674 in Figure 3). However, although no macroscopic primary vesicle was recorded at the 24th hour, virus C was recovered from the secondary foot lesions.

Virus O

In 4 of the 17 cattle, we did not find evidence of multiplication of this virus. In the first animal (No.1684, Figure 4), the virus was demonstrated as a viraemia and in the foot lesions; in the second animal (No. 674, Figure 3), only as a viraemia; in the third (No. 670, Figure 3), the technique used was not very sensitive (three blood samples, freezing of plasma, serumneutralization) and perhaps was incapable of detecting the virus. The viraemic stage of the 4th animal (Figure 2) was not examined, only serum-neutralization tests with foot vesicles being carried out.

2. Spread within the animal body.

By this, we mean the presence of virus in the blood and the foot lesions. In the preliminary work with the 6 cattle (Figure No.3), the results show that the three viruses were found only four times in the same animal. On the other hand, using the technique described in the second part of the viraemia work, the three viruses were found in all the 9 cattle (Figure No.4).

From the results shown in Figure No.4, it is seen that the type of the virus in successive specimens is not always the same for the individual animal. The titres of the three types of the virus in the viraemic phase varied according to the time when they were collected. The sensitivity of the technique provided a certain threshold and because of this, it may happen that a virus present in the blood when collected could, because of its low concentration, be masked by the others. This is more likely to occur when there are few blood samples and when the plasma is frozen before inoculation. Serological consideration of the secondary foot vesicles shows it is less sensitive than that of the viraemia but still it is a very convenient and sure method.

Conclusion

This experiment with 25 cattle enabled us to:

1. Point out the techniques for identifying the viruses in specimens taken from cattle inoculated simultaneously with our three strains of types O, A and C.

2. Show that simultaneous multiplication of the three viruses at the points of inoculation and in the animal body occurs in the same animal. It was not possible to show any evidence of a systematic interference phenomenon: multiplication of a strain of the virus being able to be prevented purely and simply in an animal.

In the present stage of our research, the fact that the failure to demonstrate a virus seems to us to lie rather in a lack of sensitivity of our methods than in an interference phenomenon.

II. Quantative Control of Trivalent Vaccine

A. Material

The cattle and the challenge viruses have already been described.

The Trivalent vaccine No. 2067 was prepared by mixing equal parts of monovalent vaccines O (No.119), A (No.220) and C (No.319) whose DVB_{50} (50% cattle vaccinating dose) (6) we know. The average dose for cattle of this vaccine is 5 ml.

B. Methods

1. Vaccination

The cattle were vaccinated subcutaneously with different doses of the vaccine, but in similar volumes of 5 ml. made up with the same buffer as for the DVB_{50} (6). The vaccine, diluant and later, the diluted vaccine were kept at refrigerator temperature.

The dilutions were 4-fold. Four groups, each of 5 cattle were used, each animal receiving in a volume of 5 ml. the following amounts of the vaccine:

Group I	-	4.8 ml.
Group II	-	1.20 ml.
Group III	-	0.3 ml.
Group IV	-	0.075 ml.

The cattle were kept under observation for 22 days on our farms.

2. Virulent Challenge

On the 22nd day after vaccination, all the cattle were placed in isolated stables at the Institute where blood samples were taken and then the virulent challenge made by intradermolingual inoculations, either at two points of 0.1 ml. of a suspension of bovine virus representing 2 x 5,000 DIB_{50} for animals challenged by one virus, i.e.

by "monovalent challenge"; or at three points of 0.1 ml. for animals challenged by our three types of virus as described in the first part of this article i.e. "by trivalent challenge".

For each challenge 2 control cattle were used, the high sensitivity of our animals to our strains of virus being taken into account (6).

3. Reading of results

The animals were slaughtered 5 days after the virulent challenge. The tongue, head and foot lesions were noted in the way adopted at the Institute with monovalent challenges.

Concerning the notation of the trivalent challenges, in the first three squares are recorded the primary tongue lesions. The secondary tongue and mouth lesions are recorded in the 4th and 5th squares respectively. The four last squares show the readings concerning each of the four feet.

Each lesion was systematically controlled by complement fixation.

An animal was considered to be "non-vaccinated" if it showed one or several foot lesions. This is the only criterion used in this work.

4. Methods of calculation

We used the Litchfield method, whereby we could define the following four parameters:

DVB_{50} - 50% cattle vaccinating dose

fD - Precision of DVB_{50} ($p = 0.95$)

S - Slope

fS - Precision of the Slope S ($p = 0.95$).

C. Results

In Figure No. 4 there are collected the results of the readings of the titration of monovalent O vaccine No. 119 which have already been published elsewhere (6) and those of the O valence of trivalent vaccine No. 2067. Figures Nos. 6 and 7 give the same information on A and C valences respectively. Finally, in Figure No. 8 there are the results of the control of trivalent vaccine No. 2067 by "trivalent challenge". In this challenge, the control animals showed generalisation by the presence of lesions in the four feet. In Figure No. 9 the values of the different parameters for these 7 titrations are collected together.

D. Statistical Analysis

Statistical analysis of the results obtained up to the present stage of our research show:

1. The collected 7 titrations (Figure No. 9) form a coherent system which is not significantly different (at $p = 0.95$) from that laid down

for the monovalent vaccines controlled in cattle (6).

2. There are no significant differences (at $p = 0.95$) between the values of the DVB_{50} obtained for a monovalent vaccine and the value of the DVB_{50} (expressed as the volume of monovalent vaccine) of the same valence in the trivalent mixture No. 2067 (Figure No. 9).

3. The value of the DVB_{50} obtained by the trivalent challenge is not significantly different from the greatest value of the DVB_{50} found in this case for A valence (Figure No. 9).

In Figure No. 8, there may be seen in the groups of cattle vaccinated with the full and the 1/4 dose one and four animals respectively with generalized foot lesions: "A" virus is prominent in these lesions. If these results are compared with those of the monovalent challenges (Figures 5, 6 and 7), it will be seen that they are of the same order of magnitude as those found in the A valence control (Figure No. 8).

The mean value of the DVB_{50} found with the cattle which received the trivalent challenge is 0.80 ml. The mean value of the DVB_{50} for the A valence is about 0.60 ml: it is less although not statistically different. In our work, therefore, the trivalent challenge is as severe as the most severe monovalent challenge.

Figure No. 9

Values of the different parameters of the monovalent vaccines, the valences of the trivalent vaccine controlled separately and of the three valences controlled simultaneously.

Vaccines	Challenge virus	DVB_{50} (ml)*	fD	DVB_{50} limits* (ml)	S	fS
Monovalent O (No. 119)	O	0.10	1.9	0.05-0.19	1.7	1.3
O Valence(No.119) in trivalent mixture (2067)	O	$\frac{0.45}{3}$ = 0.15	2.0	0.07-0.30	2.25	1.5
Monovalent A (No. 220)	A	0.62	2.0	0.31-1.24	2.3	1.5
A Valence (220) in trivalent mixture (2067)	A	$\frac{1.77}{3}$ = 0.59	2.0	0.29-1.18	2.7	1.5
Monovalent C (No. 319)	C	0.20	2.2	0.09-0.44	2.3	1.5
C Valence (319) in trivalent mixture (2067)	C	$\frac{0.96}{3}$ = 0.32	1.9	0.17-0.61	2.1	1.5
O, A & C Valences in trivalent mixture (2067)	D+A+C	$\frac{2.4}{3}$ = 0.80**	2.0	0.4 -1.6	2.3	1.5

* DVB_{50} values are collected in monovalent doses for convenience in studying the results.

** This DVB_{50} concerns "A" virus, the lowest valence in the mixture.

General Conclusions

In the first part of this report, we dealt with demonstrating that multiplication of our three strains of the virus was possible in the same susceptible bovine animal.

Development of the three viruses at the points of inoculation was generally found. Invasion of the animal body by the three virus strains, often fairly simultaneously, was shown by study of the viraemia and by serological work with the secondary foot vesicles, caused by the three different types.

In the second part, we give an account of the preliminary work carried out on titrating the activity of trivalent vaccine No. 2067, using in all, 126 cattle, excluding the control animals. This vaccine, composed of three monovalent vaccines of known DVB_{50} was titrated:

(a) for each valence, separately by monovalent challenge of three series of vaccinated cattle;

(b) for the three valences, by the simultaneous inoculation of the three types of virus in one series of vaccinated cattle.

The following conclusions can be drawn from this work:

1. The activity of each valence in a trivalent mixture seems to be of the same order as that of the corresponding monovalent vaccines.

2. The trivalent challenge gave results in keeping with those found for the lowest valence.

3. Serological work with foot vesicles of cattle in which the disease has generalized, enables the lowest valence of the trivalent vaccine to be identified.

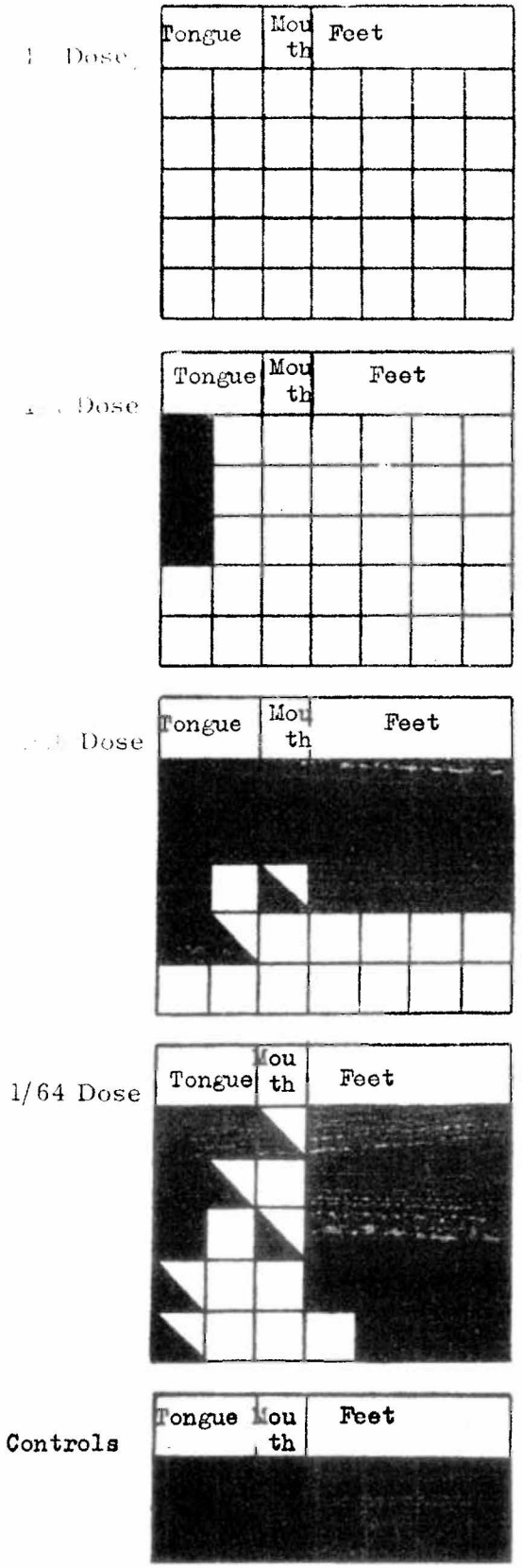
4. From this first bit of work, we were unable to show either synergy or competition (at $p = 0.95$) between our strains of virus in trivalent challenge.

If further work confirms these first results, it would be possible to control quantitatively the activity of a trivalent vaccine, using a limited number of cattle.

Indeed, in the control of a trivalent vaccine, if one of the valences does not correspond with the minimum demanded, the vaccine should be rejected, even if the other two valences are satisfactory. The trivalent challenge enables the lowest valence in a mixture to be identified and its DVB_{50} to be established and, therefore its minimal protection to be estimated. Thus, if this valence corresponds to the established norms, it can be accepted as quite certain that the other two are greater.

MONOVALENT O TEST

Monovalent vaccine O n° 119



Monovalent vaccine n° 2067

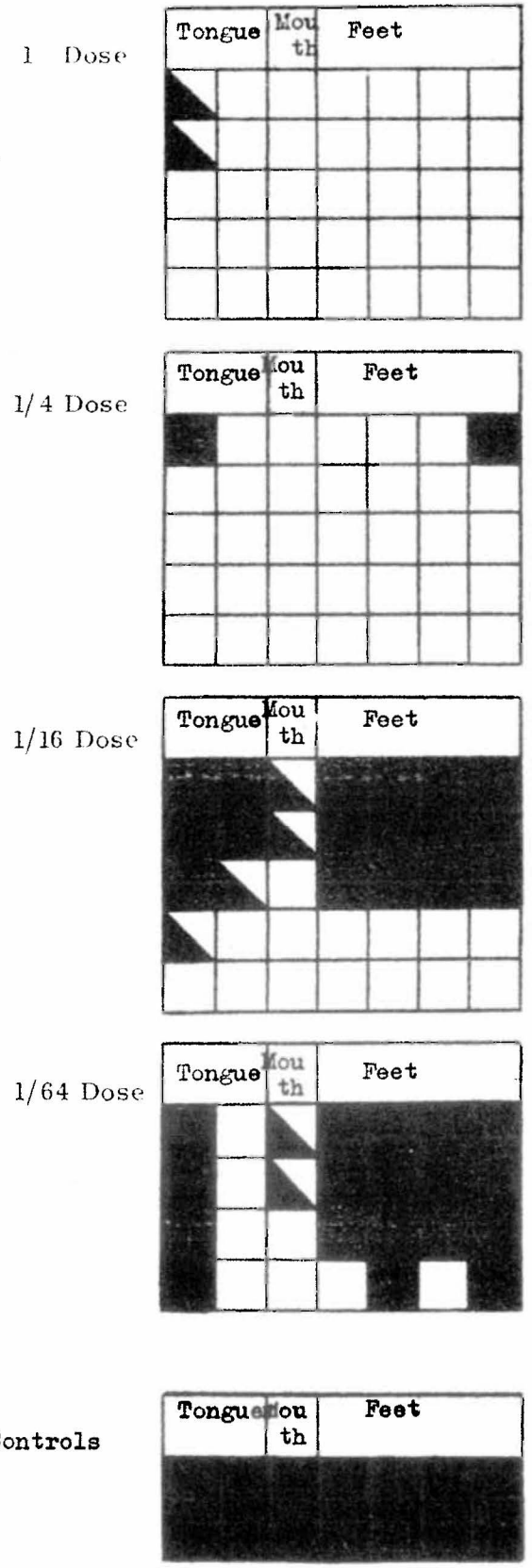
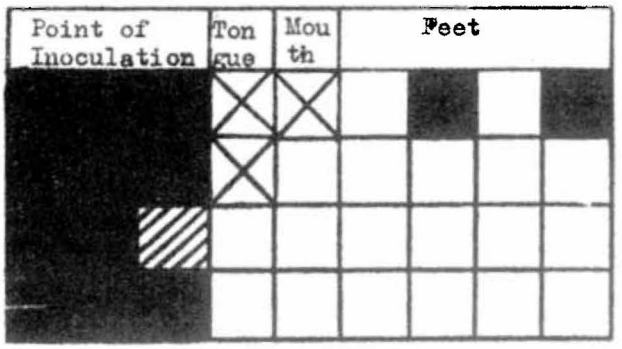


Fig. N° 5

Trivalent vaccineN° 2067

1 Dose



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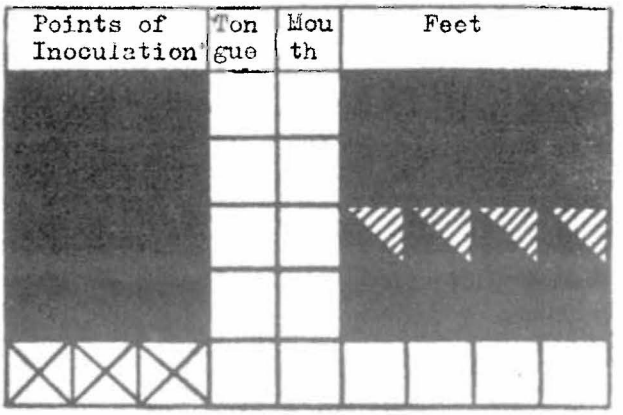

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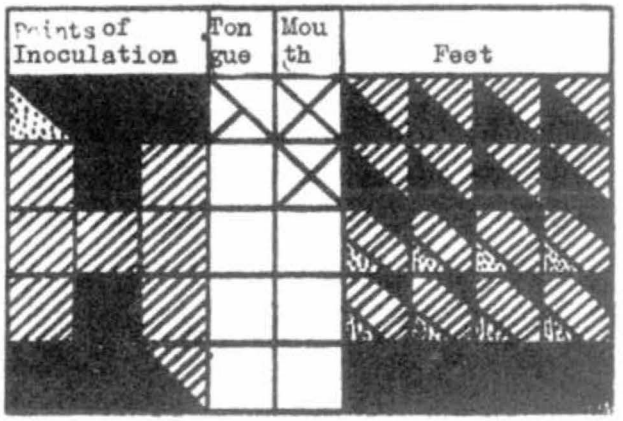

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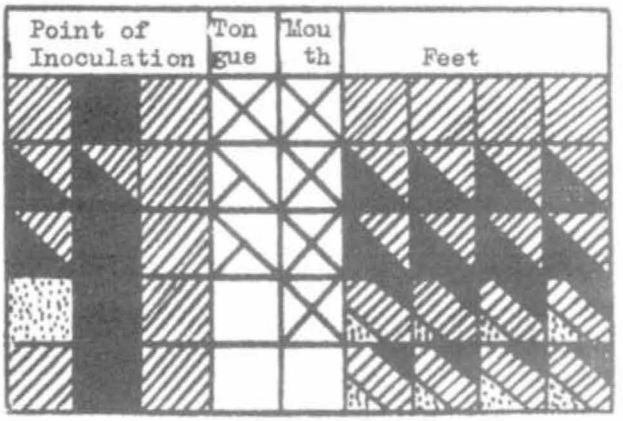

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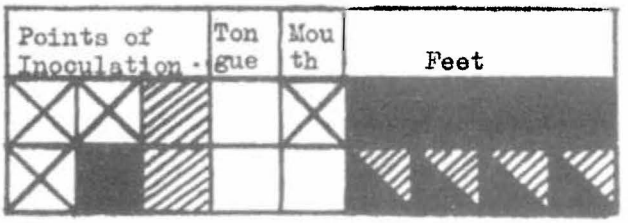


Legend :

Virus O

Virus A

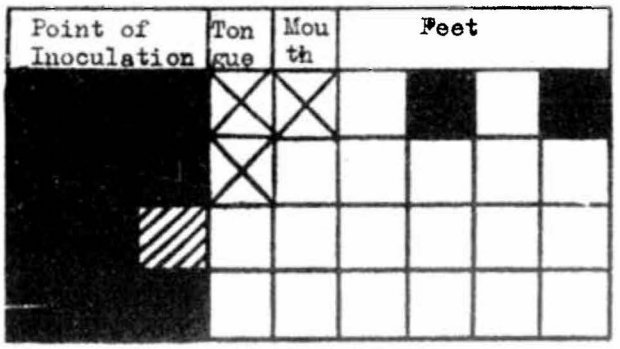
Virus C

Presence of a lesion but of non-identified type

Fig 8

Trivalent vaccine N° 2067

1 Dose



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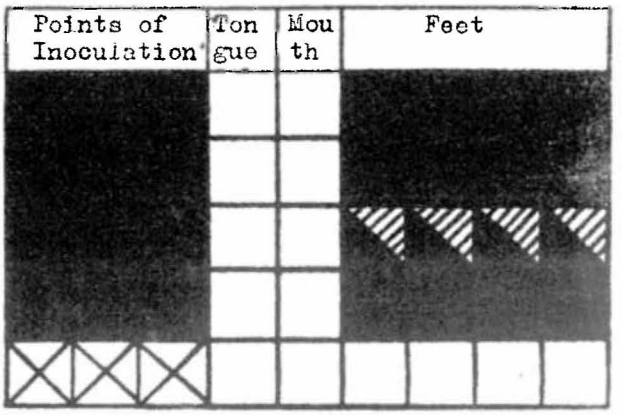

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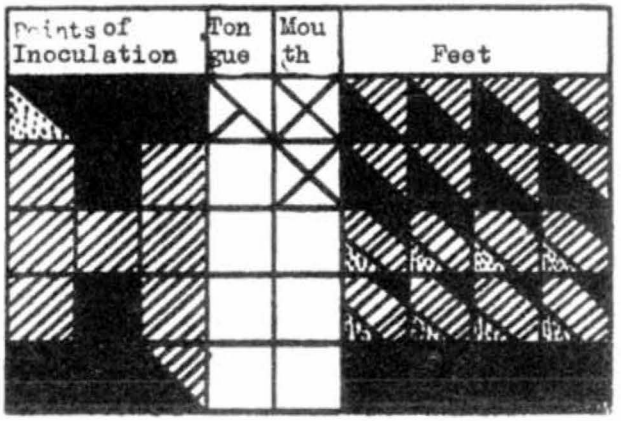

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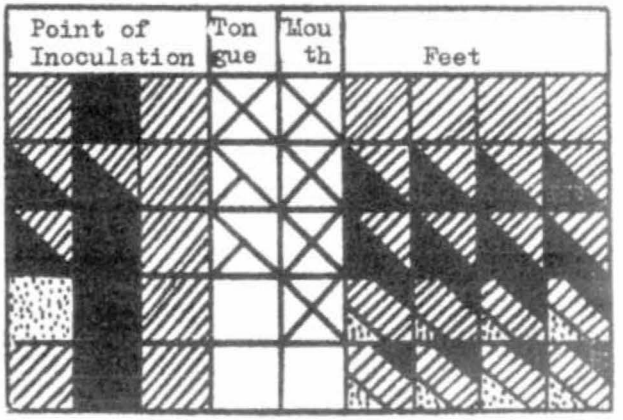

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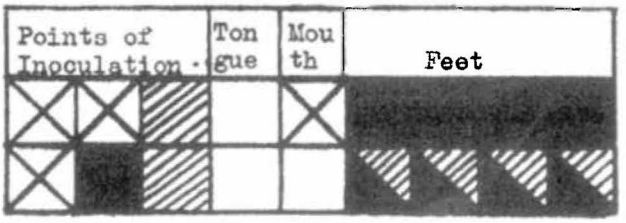

Legend :

Virus O

Virus A

Virus C

Presence of a lesion but of non-identified type

Fig 8

Figure No. 9

VALUES of the DIFFERENT PARAMETERS of MONOVALENT VACCINES, of the VALENCE of TRIVALENT VACCINES CONTROLLED SEPARATELY and of the 3 VALENCES CONTROLLED SIMULTANEOUSLY

VACCINES	TEST VIRUS	DVB_{50} in ml (x)	fD	Limit of DVB_{50} n ml (x)	S	fS
Monovalent O (119)	O	0,10	1,9	0,05 - 0,19	1,7	1,3
Valence O (N° 119) in the trivalent mixture N° 2 067	O	$\frac{0,45}{3}$ = 0,15	2,0	0,07 - 0,30	2,25	1,5
Monovalent A (N° 220)	A	0,62	2,0	0,31 - 1,24	2,3	1,5
Valence A (N° 220) in the trivalent mixture N° 2 067	A	$\frac{1,77}{3}$ = 0,59	2,0	0,29 - 1,18	2,7	1,6
Monovalent C (N° 319)	C	0,20	2,2	0,09 - 0,44	2,3	1,5
Valence C (N 319) in the trivalent mixture N ° 2 067	C	$\frac{0,96}{3}$ = 0,32	1,9	0,17 - 0,61	2,1	1,5
Valences O, A and C in the trivalent mixture N° 2 067	O + A + C	$\frac{2,4}{3}$ = 0,80 (xx)	2,0	0,4 - 1,6	2,3	1,5

(x) The DVB_{50} values are reduced to monovalent doses in order to facilitate the study of the results.

(xx) These DVB_{50}s concern the A virus which is the weakest in the mixture.

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VACCINATION OF SHEEP AGAINST FOOT-AND-MOUTH DISEASE

RESEARCH ON A METHOD FOR DEMONSTRATING IMMUNITY

by

J. Fontaine, C. Dubouclard and P. Bornarel (*)

There are not many references in the literature to experimental studies on foot-and-mouth disease or on vaccination against it in sheep.

VIVIANO (7) studied more particularly the localization of lesions during the course of the naturally occurring disease and the appearance of virus in the blood.

DELLERS and HYDE (1) studied the development of the experimentally produced disease, working on the viraemia and on complement-fixing and neutralizing antibodies in the serum of recovered or sick animals.

WYNOHRADNYK and POPOVICI (8) showed the presence of complement-fixing antibodies in sheep which were convalescent or which had been immunized with a saponin inactivated vaccine.

More recently RIVENSON, SEGURA and ZAKIN (4) worked on neutralizing and complement-fixing antibodies in the serum of convalescent and vaccinated animals.

For our part, we wished to study vaccination of sheep against foot-and-mouth disease and the first problem to settle was a method of demonstrating immunity, if possible by some direct method of test. The main difficulty we met at first was the irregularity of the clinical lesions observable in generalized foot-and-mouth disease in inoculated susceptible sheep: for this reason, we worked on a surer method of demonstrating immunity, viz. virus in the blood of infected animals.

Our report, mainly concerning virus type C, will follow the under-mentioned plan:-

(*) Thanks are given to Mr. Dupasquier for his technical collaboration.

I. MATERIAL AND METHODS

1. Sheep

2. Vaccines

3. Virus

A. Research on the route of inoculation

- Virus type C
- Virus type O
- Virus type A

B. Adapting virus to sheep

- Virus type C
- Virus type O
- Virus type A

C. Methods of demonstrating viraemia

D. Preparation of virus for use in tests to demonstrate immunity

E. Methods of demonstrating immunity

(a) Vaccination

(b) Virulent challenge

(c) Reading the results

II. RESULTS

1. Foot-and-mouth disease lesions in inoculated sheep

2. Viraemia

A. Non-vaccinated control animals

(a) Regularity of viraemia

(b) Duration of viraemia

(c) Degree of viraemia

3. Immunity resulting from vaccinating sheep

A. Trivalent, single dose, vaccination

B. Monovalent vaccination, using different doses: determination of the 50% vaccinating dose.

III. DISCUSSION

IV. CONCLUSION

I. MATERIAL AND METHODS

1. Sheep. Adult animals which had not been vaccinated against foot-and-mouth disease, bought in the market in lots as homogeneous as possible.

2. Vaccines. These were inactivated vaccines prepared for use in cattle. The dose of the trivalent vaccine for cattle of 500 kg. weight is 5 ml and for sheep 2 ml. The average dose of monovalent vaccine for cattle is 1.6 ml.

The trivalent and monovalent vaccines are tested out in cattle by challenge with virulent materials: in addition, monovalent vaccines are tested by ascertaining the 50 percent vaccinating dose in guineapigs (5).

3. Virus.

A. Research on route of inoculation

Our first work was to find a route of inoculation whereby foot-and-mouth disease lesions would be produced in 100 percent of the treated animals.

(a) Virus type C.

Virus was inoculated by 6 different routes:

- 3 sheep - into the coronary band,
- 3 sheep - between the two digits,
- 3 sheep - intradermally on the inside of the thigh,
- 3 sheep - intradermally on the muzzle,
- 3 sheep - intradermally on the udder,
- 3 sheep - into the tongue mucosa.

The C type virus had been passaged 3 times in cattle and its titre was $10^{8.2}$/g. Each sheep received 10,000 DIB 50 in a volume of 1 ml (see table No. 1). (DIB 50 = 50 percent cattle infecting dose- Henderson's method). The interdigital or the tongue inoculations produced the most clean-cut results. Experiments were then carried out with virus types O and A.

(b) Virus type O

Ten sheep were inoculated between the two digits of the fore feet with 10,000 DIB 50 of virus which had been passaged 6 times in cattle: only two of the sheep, when slaughtered 5 days after the inoculation, showed lesions of foot-and-mouth disease.

(c) Virus type A

Ten sheep were inoculated with virus passaged 3 times in cattle, the route being as that used for virus type O: 5 of the sheep developed lesions of foot-and-mouth disease.

From these observations we concluded that to set up the disease regularly, these viruses require to be adapted to sheep.

B. Adaptation of the viruses to sheep

(a) Virus type C

Using virus passaged 3 times in cattle, successive passages were made in sheep as follows:

Vesicular material is ground up and suspended in phosphate buffer at pH 7.5 containing antibiotics, in a proportion of 1 gr. to 9 ml buffer. The suspension is centrifuged for 10 minutes at 6,000 r.p.m. and 2 - 3 ml of the supernatant fluid is injected at several points of the tongue mucosa of 2 - 3 sheep. In 20 - 24 hours, the well developed vesicles with the containing lymph are collected and are used for the next passage inoculation. After 8 such passages, generalisation of the disease does not occur regularly in all the inoculated sheep: we then verify that generalization has occurred by looking for virus in the circulating blood.

After the 10th passage, we regularly find a viraemia in the inoculated non-vaccinated sheep. We consider that at the 13th passage, the virus is sufficiently adapted to sheep for use in immunity challenge tests.

(b) Virus type O

Virus strain "O Espagne" isolated in 1964 and passaged twice in cattle (50 percent cattle titre = $10^{8.2}$/gr.) was inoculated as above into two sheep. As early as the first passage a viraemia was regularly found. Adaptation of this virus to sheep was stopped after the 4th passage.

Table 1

Inoculation of foot-and-mouth Disease virus by different routes: lesions observed

Inoculation route	No. of sheep	Lesions after different times following inoculation: 48 hours	3 days	5 days	Max. Temperature	Lesions found at autopsy 6 days after the inoculation
Coronary bands of the fore feet	32	nil	tongue	tongue	41.6°C	Tongue and rumen
	33	nil	tongue	tongue	40.6°C	Tongue, 1 foot and rumen
	34	nil	nil	tongue	41.4°C	Gums and rumen
Interdigital route	35	Two fore feet	2 fore feet	1 fore foot	40.8°C	Tongue, gums, 4 feet and rumen
	46	Right fore foot	1 fore ft., tong	1 fore foot	41.2°C	Tongue, 4 feet, rumen
	47	Left fore foot	1 fore ft., tong	1 f.ft., tongue	41.2°C	Tongue, gums, rumen
Intradermal route (inside the thigh)	48	nil	nil	nil	42.2°C	Tongue and rumen
	49	nil	nil	nil	39.5°C	No lesions
	50	nil	nil	one hind foot	41° C	Tongue, gums and rumen
Intradermal route (upper lip)	51	Tongue) All very small vesicles	nil	nil	41.2°C	Tongue and rumen
	52	Tongue)	tongue	nil	40.9°C	Tongue and rumen
	53	Tongue and muzzle)	muzzle + tongue)	nil	41.2°C	Tongue, gums and rumen
Intradermal route (udder)	54	nil	nil	nil	40.9°C	Tongue, one foot, rumen
	55	nil	nil	nil	41.2°C	no lesions
	56	nil	tongue	nil	41.6°C	Tongue, gums, 4 feet, rumen
Tongue mucosa	57	Tongue	tongue	tongue	41.2°C	Tonguem gums, rumen
	58	Tongue	tongue	tongue	40.8°C	Tongue, gums, rumen
	59	Tongue	tongue	tongue	41.2°C	Tongue, gums, rumen

(c) Virus type A

The virus used had been passaged 3 times in cattle (50 percent cattle titre = $10^{6.7}$/gr) and its adaptation to sheep was carried out as above. Viraemia was present in all the animals after the 6th passage and adaptation to sheep was stopped after the 9th passage.

C. Methods of examination for viraemia

A sample of blood from the jugular vein in Alsever anti-coagulant is centrifuged at once for 10 minutes at 4,000 r.p.m. Several drops of the plasma are then placed in each of 4-6 tubes of monolayer pig kidney cell culture and the cytopathogenic results are examined at 24, 48 and 72 hours later. The type, etc. of the virus is confirmed by complement fixation.

D. Preparation of the virus for immunity challenge tests

Up to the present, our work has been mainly with virus type C. The tongue vesicular material of 3 sheep inoculated with the 12th passage virus is ground up in Hank's medium to which lactalbumin hydrolysate is added: the ratio of vesicular material to medium is 1 gramme to 9 ml. The supernatant is introduced to a culture of sheep kidney cells and left in contact for 10 minutes; then this liquid is removed and replaced by Hank's medium + lactalbumen. The cytopathogenic effect is completed in 22 hours and the liquid of the culture is now dispensed into small flasks and frozen. A flask is used for each test.

The 50 percent titres of this virus (per ml) are:-

- $10^{7.2}$ on sheep kidney cells
- $10^{7.4}$ on pig kidney cells
- $10^{5.2}$ on cattle tongue (Henderson's method).

E. Method for estimating immunity

(a) Vaccination

The vaccine is injected subcutaneously in the breast. If a fixed dose is being used, it is 1.6 ml for trivalent vaccine, i.e. approximately one third of the cattle dose.

In order to establish a 50 percent vaccinating dose, the vaccine is always injected in a volume of 1.6 ml, the dilution being made with a simple buffer of the following composition:

Distilled water	10 litres
Na_2CO_3	0.530 gr
$NaHCO_3$	16.800 gr
NaCl	85 gr

As we have already said (5) we prefer not to use diluent containing adjuvant substances, so that the true value of the vaccine may be accurately ascertained. Blood samples for examination for some neutralizing antibodies, are taken before vaccination and again before the challenge test.

(b) Challenge

This is carried out 3-4 weeks after vaccination. In our early work we injected 10,000 DIB 50 cattle virus between the claws of the fore feet. Now, we use virus produced on sheep kidney cultures, inoculated into the tongue mucosa. A dose of 10,000 DECP 50 (i.e. 50 percent cytopathogenic effect, titrated on pig kidney cells), in a volume of 1 ml is injected at about 20 places. Daily temperatures of the injected sheep are recorded.

(c) Reading Results

In the earlier tests, in which the sheep were inoculated with cattle virus, only primary or secondary foot-and-mouth disease lesions were noted, post-mortem examination being carried out 5 days after challenge. In the method now used, samples of blood are taken 24, 48 and 96 hous after the challenge for examination for the presence or absence of virus. In sheep in which the samples taken at 24 hours show viraemia, no further blood samples are required.

II. RESULTS

1. Foot-and-Mouth Disease Lesions in Inoculated Sheep

We have given up consideration of visible macroscopic lesions as criteria, because of the irregularity of the occurrence of secondary lesions at 4-6 days after challenge. We found that 22 non-vaccinated sheep injected with sheep-adapted type C virus into the tongue mucosa developed inoculation lesions, but only in 12 of them did secondary lesions occur. On the other hand, 7 sheep injected with the C type virus between the claws, developed both primary and secondary lesions. As we have already stated, the inoculation of A and O viruses gave unsatisfactory results. We have, therefore, adopted the study of viraemia for indicating infection.

Indeed, nearly all of the non-vaccinated sheep injected with foot-and-mouth disease virus develop high temperatures and lose their appetite while a large proportion of them show no visible clinical lesions at post-mortem examination.

2. Viraemia (Virus type C)

A. Non vaccinated control animals

(a) Regularity of viraemia

Among 54 sheep injected into the tongue mucosa with 10,000 $DECP_{50}$ sheep-adapted type C virus, viraemia was found in 53, viz:-

in 43, there was viraemia 24 hours after inoculation; in 10 of the remaining 11 sheep, viraemia was found 48 hours after the inoculation. It is to be noted, however, that in some of the sheep positive at 24 hours, the viraemia had disappeared when the further examination at 48 hours was carried out.

(b) Duration of viraemia

To obtain information about the duration of the viraemic phase we inoculated 5 sheep and took blood samples every 24 hours afterwards, with the following results:-

24 hours after inoculation - viraemia in 5 sheep,

48 " " " - " " "

72 " " " - " in 4 sheep

96 " " " - no viremia in any of the sheep.

These results are in agreement with those recorded by other workers (1).

(c) Degree (intensity) of viraemia

Several titration (on pig kidney cells) of the plasma of inoculated sheep at 24 hours after injection showed that the average titre varied from $10^{3.5}$ to $10^{4.5}$. The results of titrations of the mixed plasma of 6 sheep injected into the tongue mucosa were:-

Samples 23 hours after infection - $10^{4.8}$DECP 50/ml

" 50 " " " - $10^{6.2}$ DECP 50/ml

" 110 " " " - nil.

B. Vaccinated animals

Animals which received normal doses of vaccine (1/3 - 1/4 cattle doses) showed no evidence of viraemia.

In immunity experiments on 138 sheep with fractions of these doses (see the next section), 34 showed viraemia in 24 hours and 55 in 2 days after challenge, only 2 later samples showing viraemia.

We, therefore, examined for viraemia at 24 and 48 hours after challenge and, to be on the safe side, at 4 days.

3. IMMUNITY OBTAINED BY VACCINATION OF SHEEP

A. Trivalent vaccine in a single dose

(a) In a preliminary experiment, we injected 10 sheep with 1/3 cattle dose (1.6 ml) subcutaneously of a currently produced trivalent vaccine. These animals together with 4 controls were challenged by injection of 10,000 DIB_{50} type C virus between the claws 14 days after vaccination.

Blood samples were taken before vaccination and after challenge and the serum was examined for neutralizing antibodies. The results, 5 days after challenge, are given in table No. 2.

(b) In a second experiment, 19 sheep were vaccinated as those in (a) and were challenged with 10,000 $DECP_{50}$ virus C prepared on sheep kidney cells. Immunity was examined by observations concerning viraemia after 24, 48 and 96 hours. Lesions were noted at post-mortem examinations carried out 4 days after challenge (Table No. 3).

It may be concluded that there was no viraemia in the vaccinated sheep; and one trivalent vaccination accords very effective protection from the 14th day after the vaccine is injected.

B. Monovalent vaccination, using different doses: determination of the 50% vaccinated dose

After having established a good method of determining immunity by examination of viraemia, we began a quantitative study of the vaccination of sheep. We used monovalent C vaccine, No. 19; the results of its quantitative estimations in cattle and guineapigs are given in Table No. 4 (2, 3, 5).

In the first instance, we investigated the size of the 50% vaccinating dose of this vaccine for sheep, then the development of its value. In this work, 250 sheep were vaccinated with different doses of monovalent type C No. 19 vaccine and the 50% vaccinating dose for sheep was established month by month for 3 months by virulent challenge and examination for viraemia (Table No. 5). From the results, we were able to establish the duration of immunity in terms of the number of 50% vaccinating doses. The 50% vaccinating dose for sheep is about 10 times less than that for cattle.

III. DISCUSSION

Study of foot-and-mouth disease vaccination of sheep was upset, in our early work, because of the great difficulty in setting up easily and regularly an observable disease in non-immunised animals. After studying different methods aimed at producing foot-and-mouth disease lesions in all control animals (by successive passages in sheep, different routes of inoculation, etc.), direct observation of lesions following virulent challenge appeared to be inadequate. Macroscopic foot-and-mouth disease lesions have, therefore only a relative value in interpreting results. Titration of serological antibodies, while of much interest, is only a semi-quantitative method.

Investigation of the presence or absence of viraemia in inoculated sheep seems to be a sure and relatively easy method of determining the susceptability of sheep to foot-and-mouth disease. By determining the 50% vaccinating dose, we have been able to make a quantitative evaluation of foot-and-mouth disease immunity of sheep.

The position is that every animal whose serological antibody titre is measurable, fails to show viraemia after the inoculation of a pathogenic virus. On the other hand, animals with virus in their circulating blood after such inoculation, always show some symptoms for several days: high temperature, dejection, loss of appetite, etc.

By considering the 50% vaccinating dose for sheep of a vaccine whose activity is well known for cattle and giuneapigs, we have something whereby the immunogenic power can be compared in these three species. On the other hand, by determining the evolution of the value of this 50% vaccinating dose over a period, we believe that precise information can be obtained on the duration of immunity in terms of the immunogenic power of the vaccine.

IV. CONCLUSION

Clinical generalized foot-and-mouth disease lesions are not always present in sheep and, therefore, cannot be used as evidence of the disease in all susceptible animals.

By study of the viraemia in animals inoculated with a sheep-adapted virus, distinction can easily be made between protected and non-protected animals.

By the establishment of the 50% vaccinating dose for sheep of a vaccine of known quality, the quantitative value of the immunity of sheep from foot-and-mouth disease vaccination can be ascertained.

From the evolution over a period of this 50% vaccinating dose, the duration of immunity against foot-and-mouth disease can be ascertained in terms of the immunogenic value of the vaccine used.

From the Institut Français de la Fièvre Aphteuse
(Director - C. Mackowiak)

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Table No. 2

Challenge of 10 sheep 14 days after trivalent vaccination by inoculation of 10,000 DIB_{50} between the claws

Sheep	Lesions				Antibodies*		
	Feet 1. 2. 3. 4.	Rumen	Tongue	Gums	Anti-O	Anti-A	Anti-C
No. 91 vaccinated	Nil	Nil	Nil	Nil	≤ 0.4	1.6	0.7
92 "	Nil	Nil	Nil	Nil	1.3	1.5	1.2
93 "	Nil	Nil	Nil	Nil	1.3	1.9	0.8
94 "	Nil	Nil	Nil	Nil	0.8	1.5	≤ 0.4
95 "	Nil	Nil	Nil	Nil	1.7	2.3	1.4
96 "	Nil	Nil	Nil	Nil	1.5	2.2	1.0
97 "	Nil	Nil	Nil	Nil	1.7	1.9	≤ 0.5
98 "	Nil	Nil	Nil	Nil	2.0	2.1	0.8
99 "	Nil	Nil	Nil	Nil		Serum lost	
100 "	+ + - -	Nil	Nil	Nil	≥ 2.7	2.2	0.7
Control I	± - - -	±	+	-			
Control II	Nil	-	+	-			
Control III	Nil	+	+	-			
Control IV	Nil	+	+	-			

± = only one small vesicle
\+ = several vesicles
* Titres expressed as the log of the dilution of the serum neutralising 100 $DECP_{50}$
Results read 5 days after the challenge

Table No. 3

Time between vaccination and challenge	Sheep	Viraemia: Time after challenge				Lesions		
			48 hours	72 hours		Tongue	Rumen	Feet
	895 vaccinated		-	-		-	-	-
	896 "		-	-		+	-	-
	897 "		-	-		+	-	-
	998 "		-	-		-	-	-
14 days								
	271 control		-	-		+	-	-
	272 "		-	+		+	-	-
	273 "		+	+		+	-	+
	274 "		+	+		+	-	-
	275 "		+	+		-	+	-
		24 hours	48 hours	72 hours	96 hours			
	882 vaccinated					+		
	885 "					-		
	887 "					+		
	888 "	Nil	Nil	Nil	Nil	+	Nil	Nil
	891 "					+		
	892 "					+		
	893 "					+		
28 days								
	276 control	+	+	+	-	+	-	
	277 "	+	+	+	-	+	-	
	278 "	+	+	+	-	+	+	Nil
	279 "	+	+	-	-	-	-	
	280 "	+	+	+	-	+	-	
		24 hours	48 hours	5 days	6 days			
	881 vaccinated					-		
	883 "					-		
	886 "					-		
	889 "	Nil	Nil	Nil	Nil	-	Nil	Nil
	894 "					+		
	899 "					-		
	900 "					-		
35 days								
	288 control	+	+			+	-	
	229 "	+	+			-	+	
	230 "	-	+			-	-	Nil
	231 "	+	+			+	-	
	232 "	+	-			+	-	

+ = vesicles

Table No. 4

Tests in Cattle					Tests in Guineapigs			
DV_{50}* value	Doses** of vaccine injected	No of cattle with lesions of general-isation	Total number of cattle	Neutralising*** antibodies	DV_{50} value	Doses of vaccine injected	No of guineapigs with lesions of general-isation	Total number of guineapigs
0.2 ml	1.6 ml	-	5	2.28	0.13 ml	0.4 ml	2	8
	0.4 ml	1	5	1.54		0.2 ml	3	8
	0.1 ml	4	5	1.02		0.1 ml	3	8
	0.025 ml	5	5	0.68		0.05 ml	7	8
						0.025 ml	8	8

* DV_{50} = 50% vaccinating dose: DV_{50} for cattle - $0.09 < 0.2 < 0.44$ ml
DV_{50} for sheep - $0.09 < 0.13 < 0.2$ ml

** The valume injected was always 1.6 ml, the virus being diluted in buffer solution

*** Mean of the logs of the serum dilutions neutralising 100 $DECP_{50}$ in 50% of the cell culture tubes (2).

Table No. 5

1st Test: 1 month after vaccination			2nd Test: 1 month after vaccination			Test 2 months after vaccination			Test 3 months after vaccination		
Dose of vaccine (ml)	Number of sheep*	Mean Anti-body Titre	Dose of vaccine (ml)	Number of sheep*	Mean Anti-body Titre	Dose of vaccine (ml)	Number of sheep*	Mean Anti-body Titre	Dose of vaccine (ml)	Number of sheep*	Mean Antibody Titre
0.006	7/8	< 0.4	0.006	6/6	< 0.4	0.006	7/7	≤ 0.45	0.012	8/8	≤ 0.16
0.025	1/8	< 0.4	0.012	6/7	< 0.4	0.012	5/7	≤ 0.45	0.025	3/7	≤ 0.28
0.1	0/8	0.8	0.025	3/7	≤ 0.7	0.025	4/7	≤ 0.5	0.050	3/8	≤ 0.31
0.4	0/8	1.15	0.05	0/7	≤ 0.8	0.050	2/7	0.9	0.1	2/8	≤ 0.4
1.6	0/8	1.5				0.1	0/7	1.05	0.2	1/9	0.71
Controls	10/10	< 0.4	Controls	7/7	< 0.4	Controls	7/7	≤ 0.26	Controls	7/7	< 0.15
**0.006 < 0.012 ml < 0.027			**0.0136 < 0.021 ml < 0.034			**0.017 < 0.025 ml < 0.033			**0.031 < 0.047 ml < 0.07		

* $\frac{\text{Number of sheep with viraemia after test}}{\text{Total number of sheep}}$

** Value of DV_{50}

TEST OF INFECTIVITY AND DOSAGE OF FOOT-AND-MOUTH DISEASE IN SHEEP

by

Dr. J. Cardassis, Dr. C. Pappous, Dr. D. Brovas, Dr. P. Stouraitis and Dr. A. Seimenis

The dosage of foot-and-mouth disease vaccine for sheep constitutes a serious problem which, up to now, as we know, has not been dealt with systematically as can be seen from the lack of research carried out on the subject and from the divergency of the vaccinating dose recommended by the institute producing the vaccine (dose of vaccine being 1/3, 1/2 or one cattle dose).

For Greece, with more than 10,000,000 sheep and 5,000,000 goats (as opposed to 1,200,000 cattle), the problem of vaccinating these animals which very often are responsible for the spread of the disease in particular during movement to summer pastures, assumes aspects of much importance, since it often necessitates the availability of enormous quantities of vaccine. In association with F.A.O., we have carried out several experiments on this subject and the preliminary results are given in this report.

Technical and material difficulties as well as the invasion of Greece by the type A virus (Middle East strain) has had a bearing on the realization of this research which constitutes only a small contribution to the study of the problem. It represents mainly orientation for future research.

ASPECTS OF THE PROBLEM

Before approaching the problem of dosage of foot-and-mouth disease vaccine for sheep, there had to be resolved, in the first place, the question of experimental susceptibility of this animal which at one time was considered only slightly susceptible to the foot-and-mouth disease virus; an animal in which foot-and-mouth disease develops in a mild form which may pass unobserved, there being either isolated lesions in the feet or in the mouth, with only rarely the complete clinical picture.

In the very few studies which we found reported in the literature on the experimental infection of sheep (Stockmann and Minett[1], Viviano[2], Dellers and Hyde[3], Rivensen et al.[5]) use was made of only a very small number of animals, or the authors dealt with other aspects of the problem (viraemia, route of inoculation, antibodies, etc.) On the other hand, with regard to the vaccination of sheep, the studies of Wynohradnyk and Popovici[4], Rivensen et al.[5] are limited to serological research and do not include tests for comparative virulence.

From these biological data, it can be seen that little work has been carried out in sheep, that this animal is difficult to infect, at least in so far as the clinical disease is concerned, and that in no case, has the efficiency of foot-and-mouth disease vaccine been controlled experimentally by comparative virulent tests. The problem

with which we were confronted was mainly the following:-

(a) to chose a route of inoculation of the foot-and-mouth disease virus into sheep which gave the best chance of producing the clinical disease;

(b) to establish a criterium for the infection of sheep for the sub-clinical and the clinical disease; and finally

(c) to establish a 50% (DP 50)protective dose of the foot-and-mouth disease vaccine for sheep.

MATERIALS AND METHODS

1. Sheep aged 2 to 6 years; they came from the Greek islands which have been free from foot-and-mouth disease for many years. They did not show the presence of any serum-neutralizing antibodies or even an insignificant titre.

2. Virus. For the inoculation of the animals, vaccinal strains of virus types O and A were used, which were maintained in our Institute, passaged in cattle. The tongue cattle epithelium was minced, centrifuged and diluted to 1/10th in phosphate buffer solution and had a titre of 10^{-8}/gr (type O) and $10^{-7.50}$/gr (type A) in baby mice (DL 50). Each animal received 1 ml of the diluted virus at one, two or several places, according to the route of inoculation used, with the exception of animals 811 and 812 which, for the purpose of orientation, received lower quantities of virus (N°. 812 received 3,000 DL).

3. Routes of inoculation. For the experimental reproduction of the disease, the following routes were tried out:- aphtization, intralingual injection alone or combined with intramuscular injection, injection into the interdigital space or the coronary band, injection under the mucous membrane of the upper lip with and without simultaneous intramuscular injection, intratracheal injection. When two routes were used simultaneously, the virus was divided equally in these routes (0.5 + 0.5 ml). For the intratracheal route, the virus was diluted in 4 ml phosphate buffer solution.

For the challenge of vaccinated animals and the controls, the labial sub-mucous route alone or simultaneously with the intramuscular route was preferred.

4. Vaccines. We used monovalent O and A vaccines of current production in our Institute containing 57% cell culture virus of titre $10^{-7.12}$ (type O) and $10^{-7.62}$ (type A) in baby mice and 40% of aluminium hydroxide, inactivated by 1/1000 commercial formalin (at 40 vol.) and kept at a temperature of 26° C for 46-48 hours. Our vaccinating dose is fixed at 15 ml for cattle and 5 ml for sheep.

For the dosage of the vaccine in sheep, two series of animals

were vaccinated (one for each type of vaccine). Each series comprised 3 groups of 5 animals and each animal in the respective groups received 0.50, 1 and 4.5 ml of vaccine O or 1, 3 and 9 ml of vaccine A. A group of 5 animals for each series served as control animals for the challenge carried out 21 days after vaccination.

5. Viraemia. Viraemia was determined in 14 animals inoculated with O virus after 19, 48 and 72 hours following the inoculation. The blood in sodium citrate (10 mg/ml) was injected, undiluted and diluted (1/10 - 1/100) by the subcutaneous route into 5 groups of mice. The 50% infective titre was calculated according to the Reed and Muench technique.

6. Serum-neutralization. Serum-neutralization was carried out with the sera of the animals before experimental infection or vaccination and on the 21st day after vaccination using stationary tubes of primary calf kidney cell cultures, according to the technique of fixed virus (100 DICT 50/ ml) and progressive two-fold dilutions of serum. The virus-serum mixture, maintained for 60 minutes at 26°C, was introduced in a dose of 0.2 ml into each tube of cell culture containing 1 ml of culture medium (Hanks medium without serum).

The titres were calculated according to the method of Reed and Muench and expressed as the decimal of the logarithm of the inverse of the dilution of the serum giving a 50% protection.

RESULTS

The results of our experiments are shown in tables I and II. They have to be examined separately.

A. Experimental infection

(a) Value of clinical symptoms. The analysis of clinical symptoms observed after the inoculation of the foot-and-mouth disease virus in non-immune sheep shows that it is difficult to obtain a complete clinical picture of the disease in all the inoculated animals, despite the use of large amounts of foot-and-mouth disease virus up to 10^7 DL 50. On this point, it may be said that during the experimental disease, we observed, perhaps to a lower degree, the same findings as in natural disease, i.e. we found some animals which did not show any lesions, animals which showed lesions at the point of inoculation (mouth or feet) and animals which, in addition to the primary lesions, showed secondary localizations of generalization. We consider such secondary lesions as those which occur away from the points of inoculation (for instance lesions in the mouth or in the feet other than those inoculated when the inoculation was made into the interdigital space, foot lesions following inoculation in the mouth, etc.).

These differences of generalization of foot-and-mouth disease

from the inoculation of the virus into sheep do not seem to depend on the route of inoculation, but rather on the particular behaviour of certain individual animals.

In the third experiment, using the labial route of inoculation, we had 4 out of 5 sheep which showed generalization on the feet. In the fourth experiment, under the same conditions, 3 out of 5 animals showed similar generalisations. It is impossible, therefore, to take as a basis the presence or the absence of generalized lesions in judging whether a sheep has really become infected following challenge. This holds good also for the primary lesions, which are more often found. In these cases of asymptomatic development or without generalization of the infection, other tests have to be used, as for instance the determination of viraemia and mainly the titre of neutralizing antibodies of the inoculated animals in order to evaluate their degree of infection.

With regard to body temperatures of inoculated animals, we did not include them in table I, because they were so irregular in individual animals and varied greatly from animal to animal. While the temperature in one or the other of our animals reached 41.7°C - 41.8°C, in others it remained below 40° or rose above this figure to a small degree even though often generalization of the disease was evident in the inoculated animal.

(b) Value of viraemia. We examined 14 animals for viraemia and found it in 11 at significant titres within 19 and 48 hours after inoculation of the animals. In one animal (No. 998) despite generalization of the disease, the virus could not be found in the blood. Since we have also seen fluctuations of viraemia in the hours following the inoculation of the animals and because of difficulties of technique, we consider this test as an auxilliary in examinations for the state of infection of the animal.

(c) Value of the titres of neutralizing antibodies. Titration of neutralizing antibodies was carried out on the 21st day in order to observe the same conditions as in the vaccinated animals before challenge. Dellers and Hyde (3), however, report a maximum titre on the 10th day after inoculation of the animals, and indicate a slight decrease between the 10th and the 35th day and a further rise on the 91st day. The titration of antibodies can, therefore, be carried out sooner without a great variation of titre.

As can be seen from table I, in the animals inoculated with foot-and-mouth disease virus, the antibody titres are always very high. The bottom limit obtained was about 1.94 (despite generalization) and the top limit 2.80. The most frequent titres were higher than 2.50. It has to be pointed out, however, that animals in which there was no generalization showed the same quite high antibody titre (2.70 - 2.50 etc.). It is, therefore, a good test of infection in the animal which does not show clinical symptoms among animals inoculated with foot-and-mouth disease virus and could render good service in

epizootological research.

Dosage of vaccine

The results of dosage of foot-and-mouth disease vaccine in sheep, according to the Henderson and Galloway method (6) (determination of DP 50) were somewhat inconclusive in view of the small number of animals used in these experiments, as well as the lack of generalization of the disease in all the control animals, despite the use of high doses of foot-and-mouth disease virus (10^7 DL 50) inoculated by the sub-labial route (as in the vaccinated animals).

As can be seen in the example in table II, in the animals which received 0.50 ml of vaccine, we had two out of five protected (40% protection), with a dose of 1.50 ml, three out of five protected (60% protection) and with a dose of 4.5 ml, which is about the usual dose of our vaccine for sheep (5.0 ml), there were four out of five protected (80% protection). On the other hand, in the control group, only three of the five animals developed generalized lesions (foot), that is, to the same extent as the animals which received 0.5 ml of vaccine. Thus, the principle of the Henderson and Galloway method which, in addition to the considerably greater number of animals, requires generalization in all the control animals, cannot be considered in this experiment. Because we have not found the clinical disease in all the inoculated sheep, the Henderson and Galloway method seems at once to be ruled out as being applicable to sheep. Furthermore, the fact that certain sheep have not the tendancy to develop lesions of generalized foot-and-mouth disease is a serious obstacle in interpreting the results of challenge of vaccinated animals with virulent virus. It cannot be explained whether the failure of generalization in vaccinated animals is because of their state of immunity or whether it is simply a coincidence concerning the individual. Under these conditions, the dosage of foot-and-mouth disease vaccine in sheep by the method of the DP 50 determination and on the basis of clinical symptoms in challenged and control animals seems risky.

We believe, however, that this trouble may be surmounted by considering the titres of the neutralizing antibodies of the control and the vaccinated animals. In our experimental work, the experimentally injected animals all showed antibody titres of high order, mostly round about 2.50. It would appear that titres above 1.94 constitute a good index of infection whether generalization has or has not occurred.

Interpretation of the significance of antibody titres in vaccinated animals is more difficult. A glance at table II gives the impression that there is a correlation between the antibody titre and the degree of immunity of the animal.

It is seen from table II, in fact, that antibody titres above 1.80 coincide with an immunization which is almost complete, that

titres between 1.80 and 1.40 correspond to a protection against generalization, that those between 1.40 and 1.20 correspond to a doubtful protection and that with titres below 1.20, there is a total absence of protection. Now, when generalization is considered, it is necessary to use a considerable number of animals because our results are evaluated statistically. It is necessary, especially, to increase the number of neutralizing-antibody titrations carried out, as well as the number of the challenges, in order to fix a standard relationship between the neutralizing-antibody titre and the state of immunity of the animals, as in the case of cattle reported by several authors, notably by Mackowiak et al. (7), Lucam and Fedida (8), Gagliardi et al. (9), Frenkel (10) and ourselves (11).

It seems evident that in sheep this work becomes still more commanding because the present experimental data does not permit judgement to be made of the immunity state of the animal on the basis of its antibody titre in the majority of the cases, as we have said, we have been unable to state whether the resistance of an animal inoculated with virus coincides with the titre found or whether it is an individual resistance which is so often found in sheep.

If these problems are solved we believe that we will be able to answer the question of the dosage of foot-and-mouth disease vaccine in sheep with more precision.

SUMMARY AND CONCLUSION

Experimental infection of sheep by different routes (principally sub-labial, intralingual and foot), despite the use of high doses of foot-and-mouth disease virus (up to 10^7 DL 50/ml), was not always followed by generalization of the disease in the inoculated animals, some of the animals having shown asymptomatic infection and others, primary lesions at the site of inoculation.

Titration of serum-neutralizing antibodies, for the purpose of comparison with vaccinated animals on the 21st day after vaccination, showed titres up to 1.94 - 2.80 (mostly above 2.80). Thus, this titration seems to be the best index of the infection of animals, especially in cases where the disease is not apparent.

It seems to us that this titration can also be of value concerning the dosage of foot-and-mouth disease vaccine for sheep as well as for the development of the state of immunity following vaccination.

Seeing that resistance is found in sheep to generalization of lesions after natural or experimental infection, it is difficult to fix the DP 50 of foot-and-mouth disease vaccine for sheep in the same way as the level of antibody titre corresponding to the different degrees of immunity.

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TABLE I

Results of Inoculation of Sheep with foot-and-mouth disease virus Type O.

No. of exp.	No. of anim. used	Ident. No. of anim inoc.	Route of inoc.	Lesions Mouth	Lesions Feet	Viraemia 19h	Viraemia 48h	Viraemia 72h	Antibodies before inoc.	Antibodies 21 days after inoc.	Type of virus inoc.	Titre of virus inoc.
I	2	811	Aphtisation	0 + 0	0000	-	-	-	-	-	0	10^{-8}
		812	I/tongue 3000 DL	0 + 0	0000	-	-	-	-	-		
II	5	813	Interdigit Space	000	000Xi	2,32	-	0	-	2,33	0	10^{-8}
		814	"	000	0XXXi		-	-	-	2,30		
		815	"	000	0XiX0	-	-	-	-	2,60		
		816	"	000	0Xi00	-	-	-	-	2,51		
		817	"	000	XXi0X	-	-	-	-	2,50		
III	5	985	Sub-lab.muc	+00	XX 00	0	2,37	0	0	2,55	0	10^{-8}
		986	" " "	+00	X0 00	0	1,30	0,36	0,36	2,58		
		987	" " "	+00	00 00	2,12	0	1,83	0	2,50		
		988	" " "	+00	0X XX	0	0	0	0,49	2,70		
IV	3	848	Intratrach.	000	XX X0	1,68	3,50	3,20	0	2,80	0	10^{-8}
		849	"	000	0X 00	0	0,86	0	0,30	2,20		
		850	"	000	00 00	0	0	0	0	1,95		
V	4	851	Intraling. I/M	000	00 00	2	-	-	0	2,30	0	10^{-8}
		852	" "	0+0	0X 00	2,23	-	-	0	2,10		
		853	" "	0+0	XX 0X	3	-	-	0	2,60		
		854	" "	0+0	00 00	0	-	-	0	1,94	Culture Virus	DICT 50 $10^{-6,75}$

Legend: Mouth Lesions by order: lips, tongue, gums.

Foot Lesions: Xi = Lesions on inoculated foot

X = Lesions on other feet

- = Not searched for

TABLE II

Example of dosage of foot-and-mouth disease vaccine in sheep.

No. of exp.	Group of animals	No. of animals	Dose of vaccine	Titre of virus of vaccine	Test for virulence		Antibody level	
					Mouth lesions	Foot lesions	before vac.	21 days after vac.
I Type of vaccine O Series 78	A	834	0,50	$10^{-7,12}$	+00	X0X0	0	1,16
		835	"	DL50/ml	000	0000	0,53	1,80
		836	"		+00	0X00	0	0,95
		837	"		+00	0X00	0	1,13
		839	"		+00	0000	0,30	1,80
	B	828	1,50		000	0000	0	1,80
		830	"		+00	0000	0	1,40
		831	"		++0	0XX0	0	1,01
		832	"		+00	0000	0,30	1,35
		833	"		+00	0000	0	1,31
	C	823	4,50		+00	0000	0,37	1,73
		824	"		+00	0000	0	1,75
		825	"		+00	0000	0,49	1,80
		826	"		000	0000	0,37	1,80
		827	"		+00	X000	0	1,38
	D	801	Control	10^{-8}/gr	+00	0000	0	1,95
		804	"	Dose inoc.	+00	XXXX	0	1,94
		807	"	10^{-7}	+00	X000	0,60	2,10
		808	"	Sub-labial route	+00	X000	0	2,55
		810	"		000	0000	0	2,70

THE INFLUENCE OF FOOT-AND-MOUTH DISEASE VACCINATION OF THE MOTHER ON THE LEVEL OF NEUTRALIZING ANTIBODY IN HER YOUNG

by

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At the Central Veterinary Institute, Amsterdam, this question has been investigated for two species, viz. cattle and pigs.

In both of these, the young animal is practically devoid of antibody at the time of birth.

Antibody derived from the mother is usually acquired after feeding with colostrum, absorption from the gut only taking place during the first days of life.

The colostral antibodies appear somewhat different chemically from those circulating in the mother's blood and there is no full agreement as to their origin.

In a group of 10 heifers whose antibody levels varied within a narrow range GRAVES (1) found the correlation between the neutralization indices of the serum and those of the colostrum of the individual cows to be not quite significant. According to this author there was a difference in titres in quarters of the same udder. This suggests a dependency of the colostral antibody content on the activity of the quarter involved.

In one animal the neutralization index of the udder secretion remained high although that of the serum was falling off.

The neutralization index of the colostrum usually was higher than that of the serum.

We studied this question at a time when we had no technique suitable for measuring the neutralizing activity of the colostrum (2).

In a small number of calves the serum antibody in most instances was correlated with that of the mother. The mother's titre usually was somewhat higher than that of their calves, 24 hours after birth.

In pigs a similar relationship existed between the titres of the sows and those of their two week old litters (3).

For instance litters of sows that had redeived two doses of vaccine and had serum titres between 1.4 and 2.2, had mean titres varying from 0.7 to 1.7. In another group that had been vaccinated three times, the titres of the sows varied from 2.1 to 3.1, and those of their litters from beneath 1.7 and 3.4.

We therefore tend to expect a certain relation between the serum titre of the mother and that of her offspring. Exceptions, however, do occur.

According to FEY (4) inadequate absorption of maternal gamma globulins from the intestinal tract is common in calves and may be a major factor in the pathogenesis of colibacillosis. Our own experience shows that some new born calves, although fed with colostrum from immunized cows, have little or no serum titre.

WELLMANN et al. (5), working on swine erysipelas, report [illegible] that some sows, although themselves having high antibody levels, are poor transmitters of immune proteins.

We had little opportunity to study different litters of the same sows, but can confirm that not all mother pigs do transfer antibody equally well. We further repeatedly observed a difference between pigs of the same litter. Some piglets acquired much lower serum titres after feeding with colostrum than did their litter mates.

The effect of the vaccination status on the level of immunity transmitted to the offspring was particularly well illustrated in one experiment in which two litters farrowed by the same sow, were available for swine fever experiments.

The first litter was born three weeks after a vaccination of the sow. The injection of a second or third dose of crystal violet vaccine causes a marked serological response and in this case, this was reflected in the values found for the sera of the baby-pigs.

Whether the colostral antibodies are produced in the udder or elsewhere, they are of maternal origin and it therefore seems logical to expect a relationship between the vaccination history of the dam and the amount of antibody available for its young. Our experience so far does not contradict this hypothesis.

Consequently any factor affecting the immune status of the mother may be expected to influence the level of antibody that may be acquired by its offspring.

This will for instance apply to the type and dosage of vaccine, the number of injections and their spacing, the interval between administration of the vaccine and parturition and the extent to which the individual animal is able to respond to vaccination. What happens in the following phase of the absorption of the antibody from the intestinal lumen of the young animal, is largely unknown. It is, however, apparent that under certain conditions this process does not take its normal course.

Such a blocking will in its turn adversely affect the quantity of immune proteins that passes into the circulation of the young individual.

Both in cattle and pigs the absorption ends after two or three days. The antibody molecules are slowly broken down and consequently the serum titre drops and, unless the animal is actively stimulated with specific antigen, eventually reaches zero. The rates of decline are different for various species.

The half life of maternal F.M.D. antibodies in calves is stated by GRAVES as varying from 15 to 19 days (1). Our own estimate from different experiments is from 12 to 24 days (2).

Using a neutralization index measure in surviving tongue epithelium cultures traces of antibody could usually be detected up to an age of 60 to 160 days in calves born from cows that had been vaccinated repeatedly under field conditions. Some animals never showed any antibody.

The half life of maternal antibody in baby pigs may be estimated at 13 to 14 days (3). There are, however, individual variations and within litters the variability was seen to increase with age.

With the colour test, antibodies were found up to about 16 weeks post partem in pigs from convalescent sows. With some exceptions litters from sows that had received two injections of monovalent type C vaccine became negative after six to ten weeks, but after three or more vaccinations antibody might persist for as long as twenty weeks and occasionally longer.

From the above it will be apparent that only very general rules can be given for the effect that vaccination of the dam may have on the serum titre of her young.

Factors such as vaccination history, interval between injection of vaccine and parturition, etc., introduce such a wide variation that only rather vague upper limits can be set. Under our conditions, these could be defined as follows:

In young pigs and calves from mothers that have been vaccinated under field conditions, i.e. repeatedly, traces of antibody may persist for as long as 150 to 160 days.

In young from dams that have been vaccinated only once we would expect these periods to be shorter by 30 days or so.

However, it should be pointed out that this is only an estimate for an upper limit and that any values between this maximum and zero may occur.

REFERENCES

1) Graves, J.H. (1963), J. Immunol., 91 251.
2) van Bekkum, J.G., Thesis, Utrecht, 1959.
3) Nathans, I., Thesis, Utrecht, 1965.
4) Fey, H., Bull. O.I.E. (1964), 62, 627.
5) Wellmann, G., et al., Zentralbl. f. Bakt. Orig. (1961), I, 183, 217.

Table I

INACTIVATION OF FMDV STRAIN SA13/61 10th TONGUE EPITHELIUM TISSUE CULTURE AT 25°C. pH 7.6

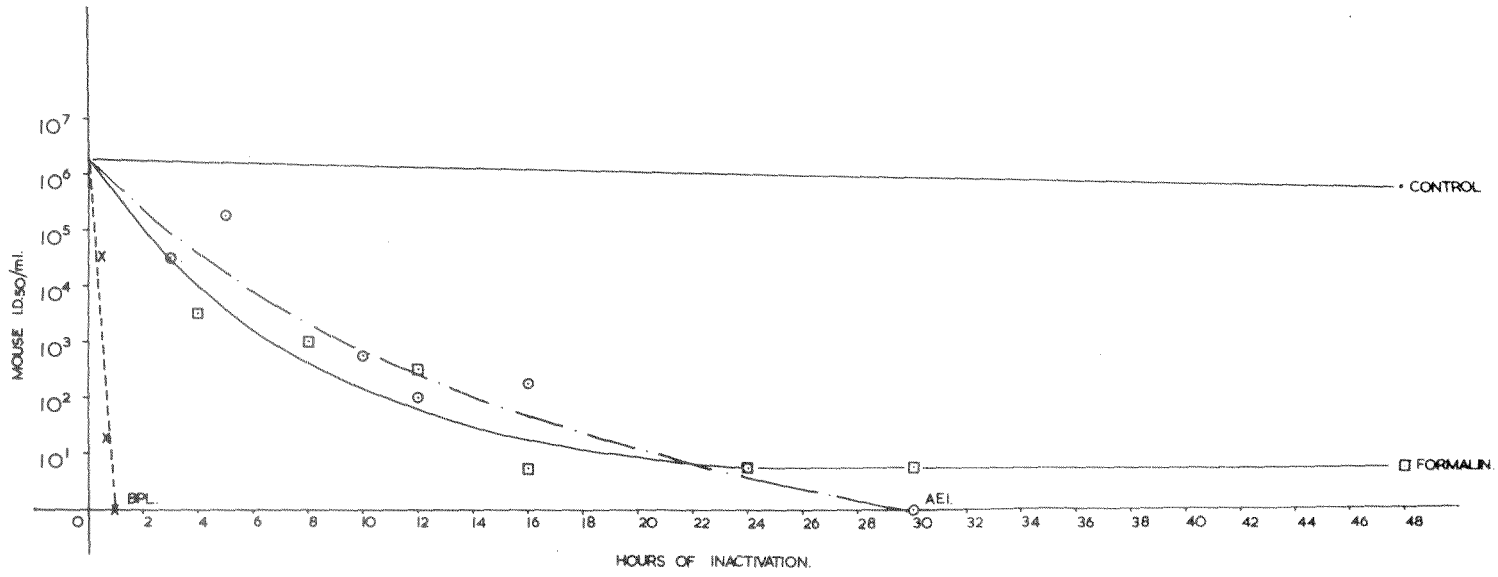


Table II

INACTIVATION OF FMDV. STRAIN SA13/61 10th. TONGUE EPITHELIUM TISSUE CULTURE AT 25°C. pH 9.0.

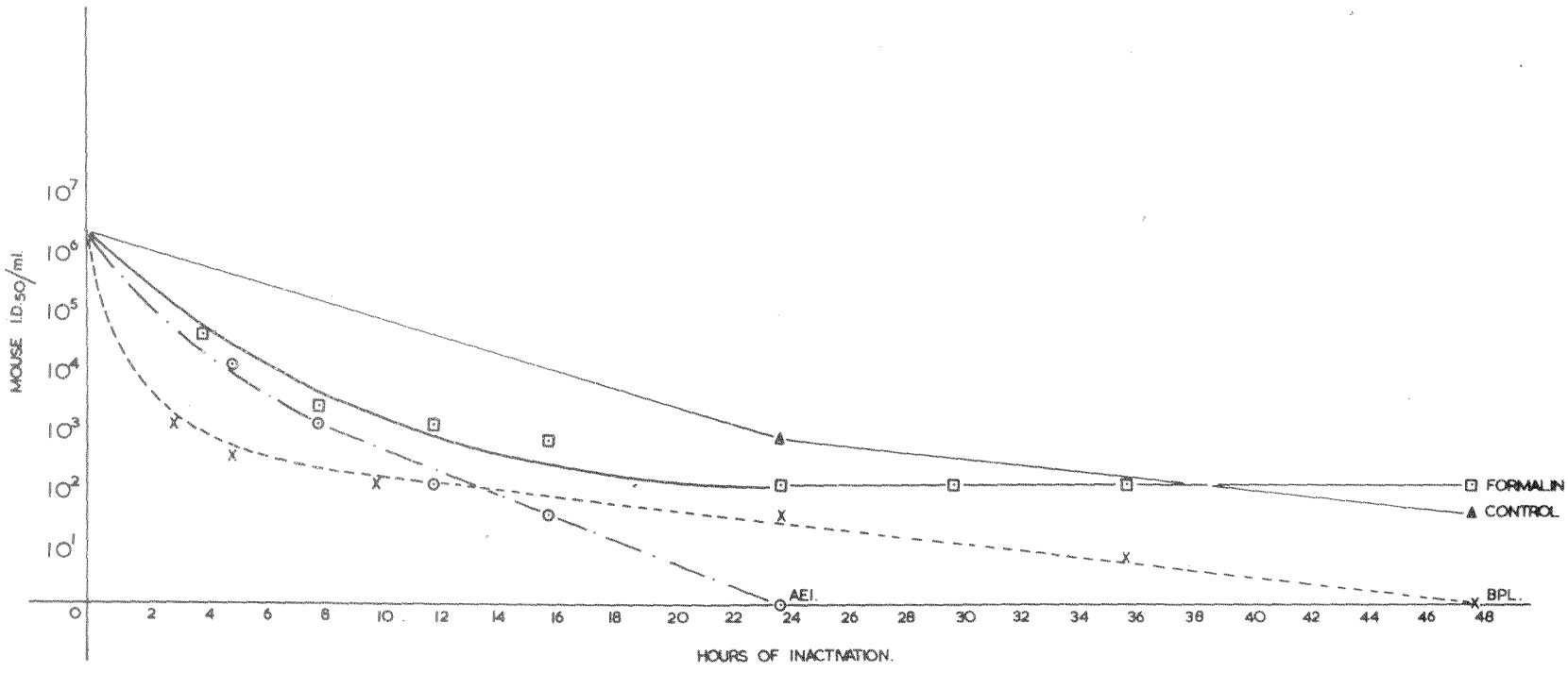


Table III

INACTIVATION OF FMDV. STRAIN SA13/61 10th. TONGUE EPITHELIUM TISSUE CULTURE AT 37°C. pH 7.6

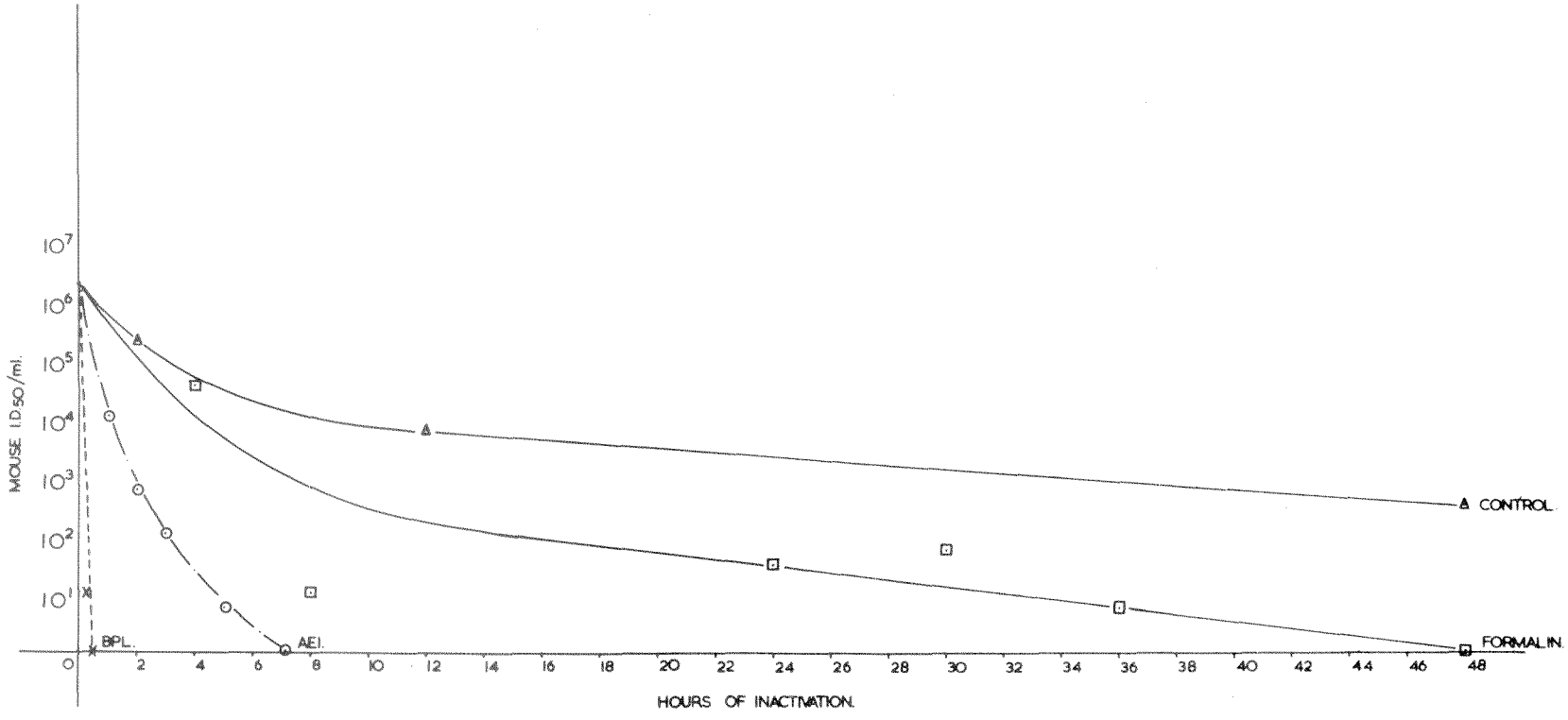


Table IV

INACTIVATION OF FMDV STRAIN SA13/61 10th. TONGUE EPITHELIUM TISSUE CULTURE AT 37°C pH 9.0

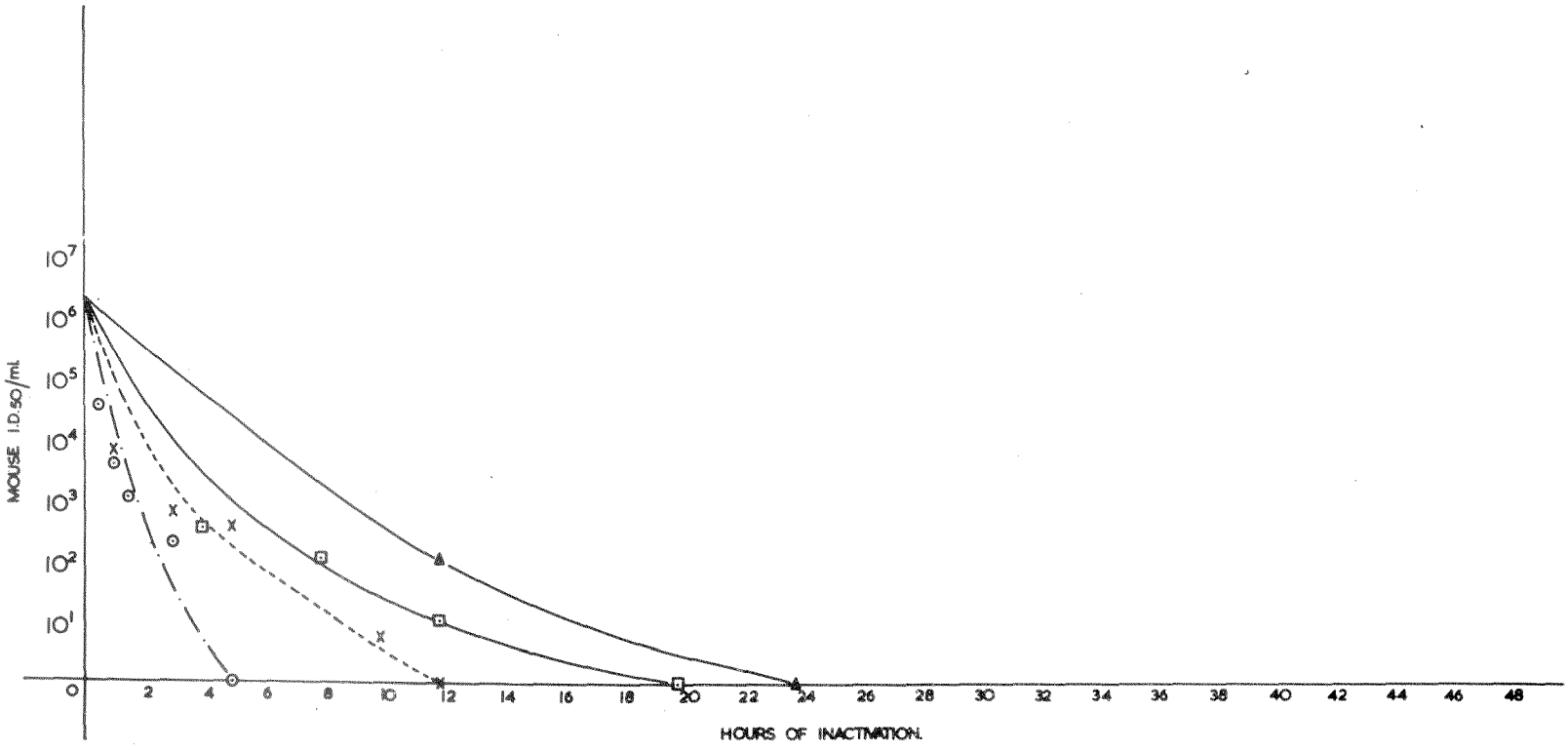


Table V

INACTIVATION OF FMDV. STRAIN SA13/61 10th. TONGUE EPITHELIUM TISSUE CULTURE AT 25°C. pH 9.0 + 5% BOVINE SERUM.

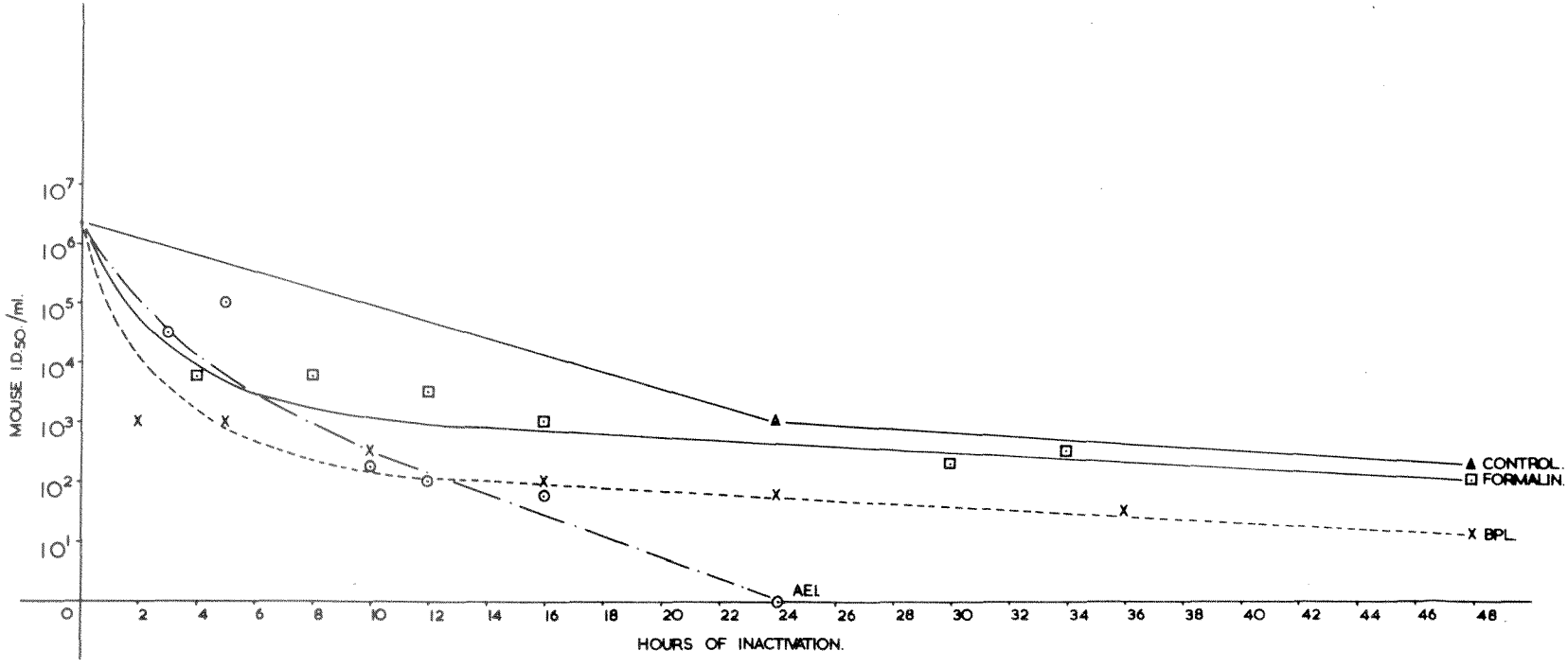


Table VI

pH OF VIRUS SUSPENSIONS INACTIVATED BY BPL AT 25°C. WITH M/25 PHOSPHATE OR 2 M. GLYCINE BUFFER.

ALUMINIUM HYDROXIDE ADDED BEFORE INACTIVATION.

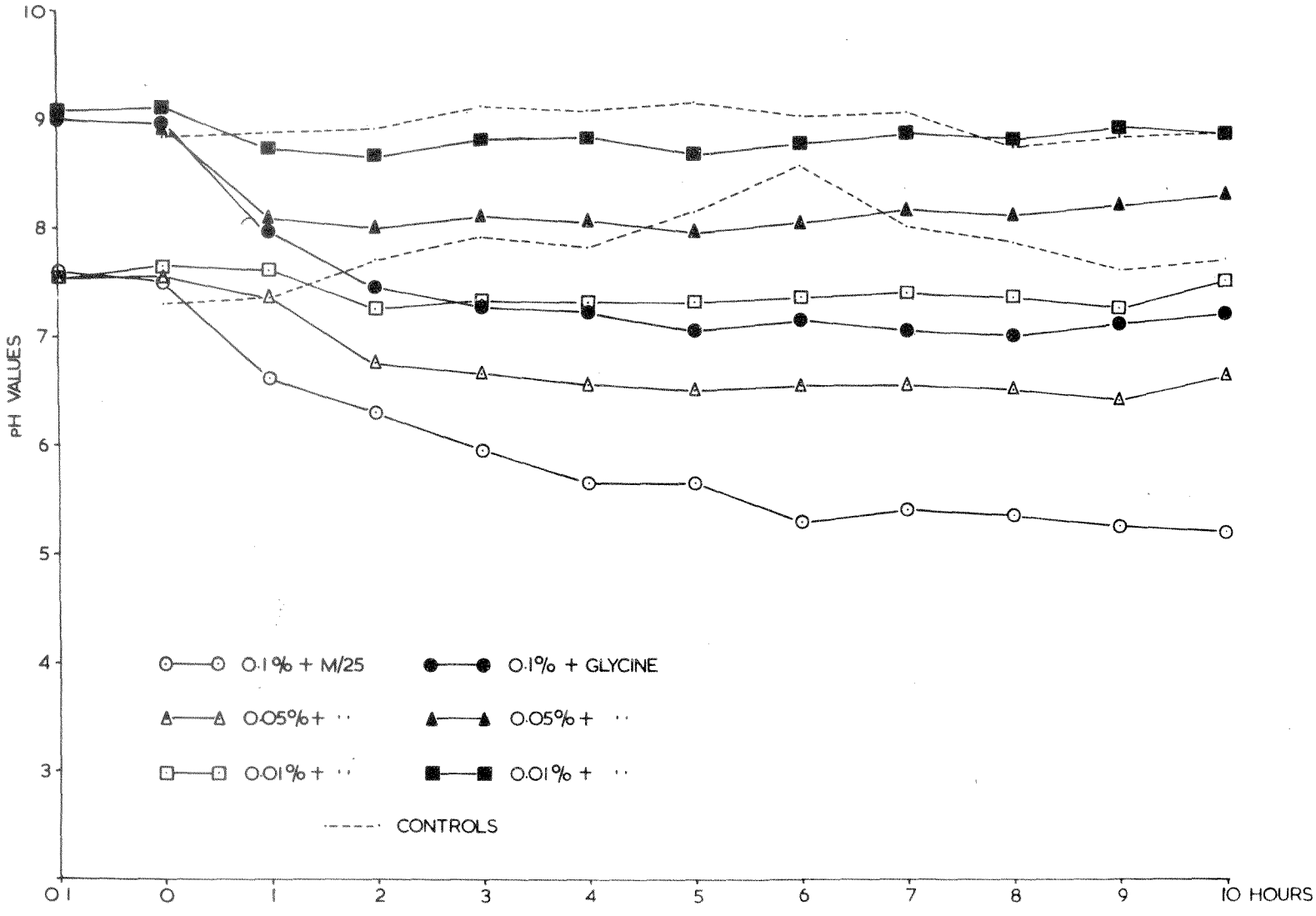


Table VII

PH OF VIRUS SUSPENSIONS INACTIVATED BY BPL AT 25°C. WITH M/25 PHOSPHATE OR 2M. GLYCINE BUFFER.

ALUMINIUM HYDROXIDE ADDED AFTER INACTIVATION

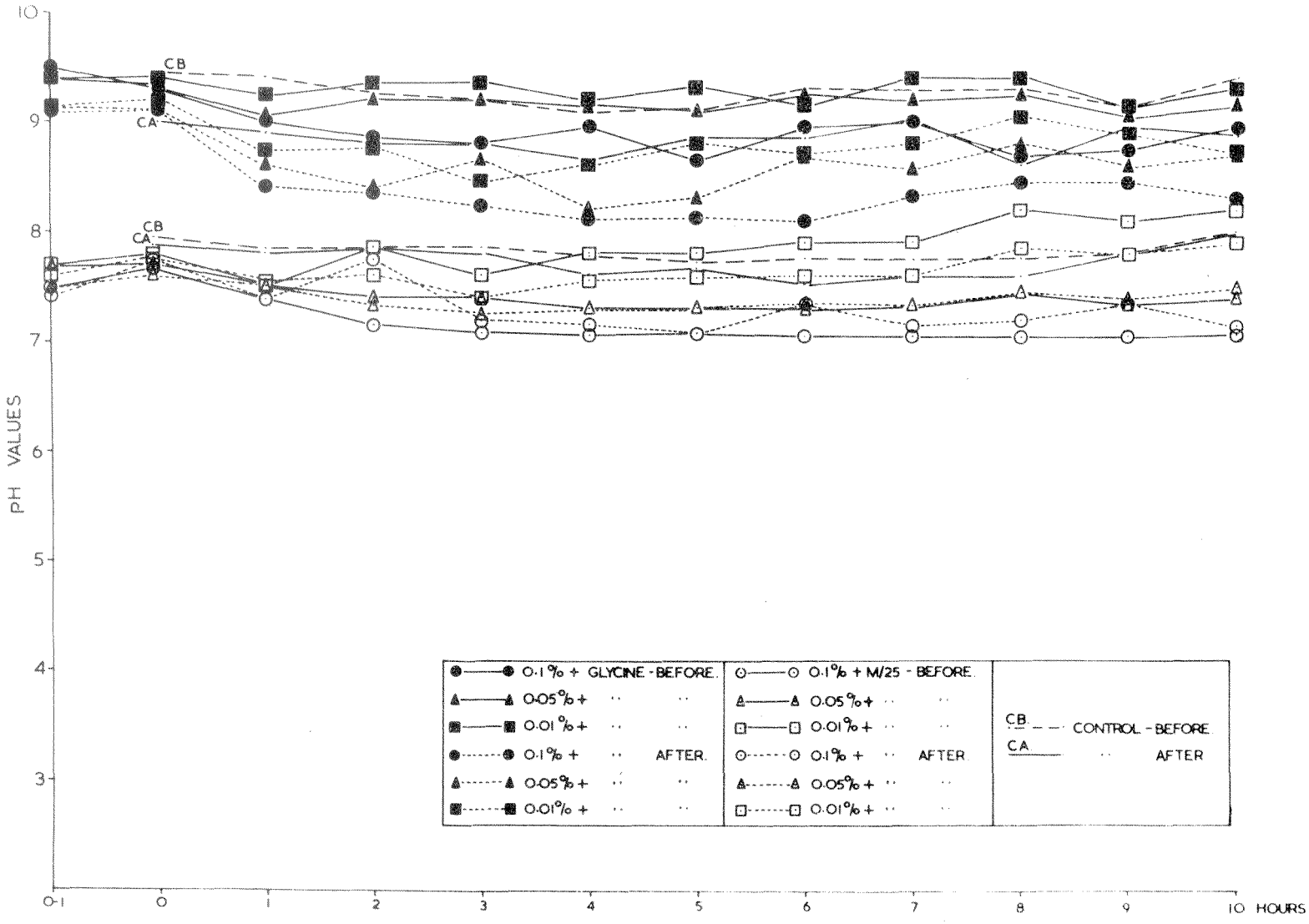
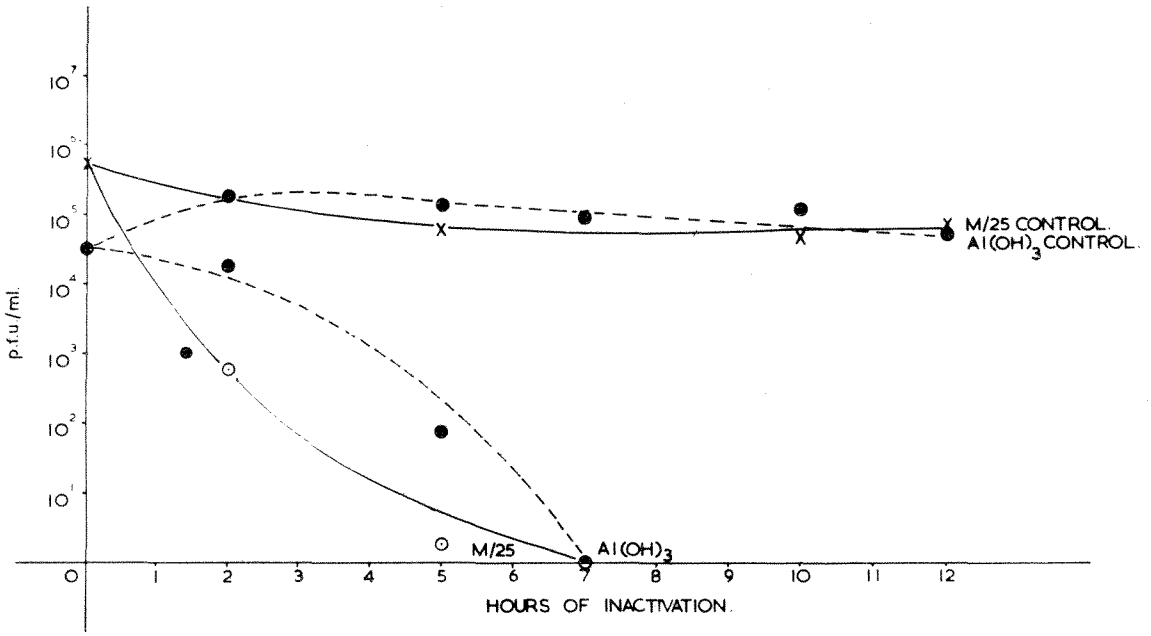
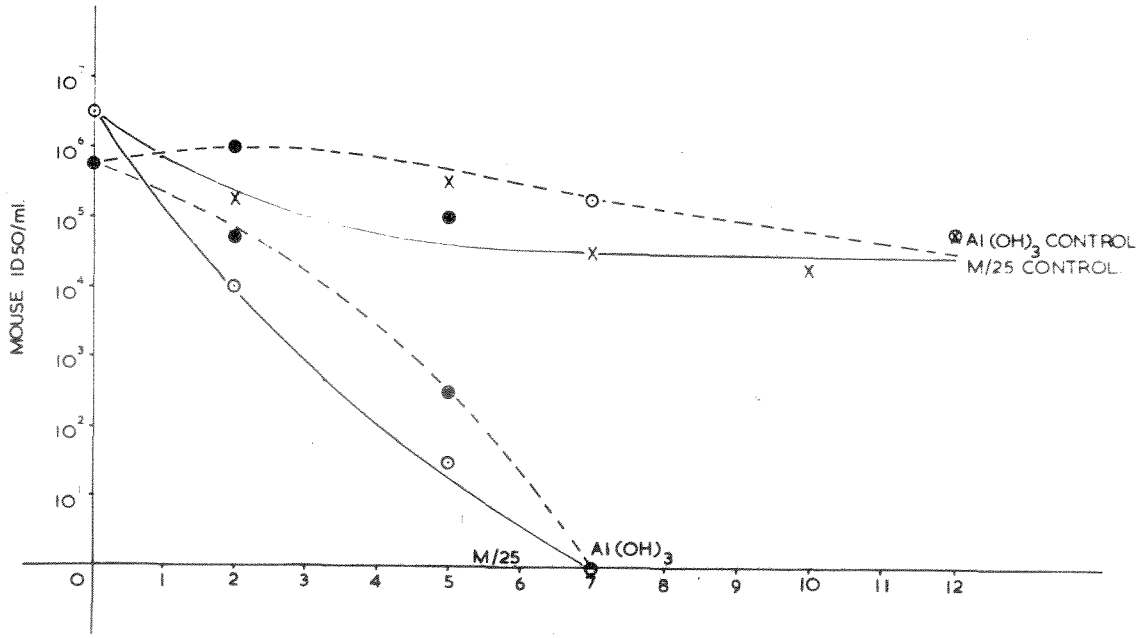


Table VIII

EFFECT OF ALUMINIUM HYDROXIDE ON AEI INACTIVATION AT 37°C pH 7.6

A. PLAQUE ASSAY



B. MOUSE ASSAY.