A COMPARISON OF DIFFERENT ROUTES OF INOCULATION OF CATTLE FOR DETECTION OF THE VIRUS OF FOOT-AND-MOUTH DISEASE

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Very small quantities of foot-and-mouth disease virus can only be detected by inoculation of a susceptible host. In general, the most sensitive host is one to which the particular virus strain under investigation has been adapted either by passage under natural or field conditions or by serial passage under experimental or laboratory conditions. For example, the maximum infective dilution of a bovine strain of virus may be from ten to one hundred times greater for cattle than for guinea-pigs, and, conversely, a guinea-pig-adapted strain may be more infective for guinea-pigs than for cattle (Henderson, 1949). Brooksby (1950) has recorded observations, based on experiments carried out under controlled laboratory conditions, on two strains of virus recovered from field outbreaks in swine which showed a marked degree of species adaptation to swine. An exception would appear to be the use of 1-week-old unweaned mice, for Skinner (1951) has shown by titration experiments that intraperitoneal inoculation of such mice with certain virus strains of cattle or guinea-pig adaptation gives as high an end-point as inoculation of the tongues of cattle or of the plantar pads of guinea-pigs respectively. This does not happen if older mice are used. Conversely, strains of virus passaged serially in unweaned mice show higher titres when titrated in such mice than when titrated in cattle or guinea-pigs.

Most workers would agree that when cattle are employed intradermal inoculation of the tongue is the most certain method of producing infection, but there is little information available on how much more virus is required to produce an infection if given by another route. Bedson, Burbury & Maitland (1925) inoculated guinea-pigs subcutaneously, intramuscularly, intraperitoneally and by scarification and intradermal inoculation of the plantar pads. They found the subcutaneous route very uncertain and, of the others, preferred intradermal injection of the pads but noted that scarification of the pads was almost as satisfactory. Working in this laboratory, Aramburu (1949) has shown by quantitative studies that multiple intradermal tracking is the most sensitive method of plantar-pad inoculation in the guinea-pig. Brachmann (1925) compared intradermal inoculation of the plantar pads with intraperitoneal and subcutaneous inoculation. He found that less virus was required to infect by the intradermal route.

In seven experiments with cattle, six strains of virus have been used for the quantitative comparison of intradermal inoculation of the tongue with subcutaneous inoculation; in one experiment intracutaneous inoculation was also

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compared and, in another experiment, intravenous inoculation. Two experiments are also described in which intranasal instillation of the virus was used as a means of infection. The different end-points obtained in titrations of virus suspensions in which these different routes were used were compared to give some measure of the relative facility with which infection could be induced.

METHODS

The supply and housing of the cattle used in these experiments

The cattle used were Devon steers from 1½ to 2 years old. When required for experiment they were brought to the Research Institute from the premises of a cattle dealer who had obtained them in various parts of southern England. Because of the favourable conditions prevailing in Great Britain with regard to foot-and-mouth disease it is always possible to obtain cattle with a 'clean' history. The low incidence due to its occasional introduction from abroad and the early elimination of infection by application of the rigid control measures involved in the 'stamping-out' policy, which includes the slaughter of all susceptible animals infected or exposed to the risk of infection, makes it a certainty and not merely an assumption that the cattle have not passed through infection with the virus of foot-and-mouth disease nor have they been vaccinated. The same assurance of the initial susceptibility of the cattle used for experiment cannot always be claimed in countries where the disease is endemic, where the slaughter policy is not used or where there is a programme of vaccination.

The cattle used for intradermal inoculation of the tongue were usually housed in pairs, but those used for inoculation by any other route were always housed singly. The cattle loose boxes are so designed that the animals can be watered and fed without the attendant having to enter the box. Those cattle inoculated subcutaneously, intracutaneously or intravenously when the incubation period might be about a week instead of the few hours following tongue inoculation were, in all but the first experiment, left in the boxes without anyone entering for some days. The possible dangers of accidental infection were fully appreciated, and the maximum practicable precautions were taken in the way of personal disinfection during the course of the experiment.

Preparation of inocula

A portion of freshly collected vesicular epithelium from the tongue of an infected steer was minced with scissors and ground with sand in a mortar and a 1 in 10 or a 1 in 25 suspension prepared using equal volumes of M/25-phosphate buffer solution and Hartley's digest broth, pH 7·6. The suspension was then clarified by centrifugation and the supernatant fluid passed through either a sand and paper-pulp filter followed by a Gradocol membrane (Elford) of between 0·5 and 0·59μ A.P.D. or a Seitz EK filter pad. Dilutions of the filtrates were prepared using M/25-phosphate buffer solution, pH 7·6.

Intradermal inoculation of the tongue

The filtrates were titrated in our routine manner (Henderson, 1949), using four dilutions and five sites per dilution on each tongue. Not more than 0·1 ml. was injected into each inoculation site. The cattle were narcotized before inoculation by the intravenous injection of Thiopentone Sodium, B.P.

Subcutaneous, intracutaneous and intravenous inoculation

Usually three cattle were inoculated with each dilution of virus filtrate, and 5 ml. of the appropriate dilution was inoculated into each animal. Subcutaneous inoculations were made in the dewlap in the region of the brisket, intracutaneous inoculations were made on the side of the neck and intravenous inoculations were made into the jugular vein.

Intranasal instillation

1 ml. of the appropriate dilution of virus filtrate was sprayed into each nostril by means of a nose-and-throat spray of a variety made for human use (Cavendish 'A' Brand All-glass Spray no. 2). In earlier experiments the cattle were narcotized with thiopentone, but when it was found that the spraying could be done equally well without this restraint, no narcotic was used.

Calculation of end-points

The method of Reed & Muench (1938) was used for calculating the 50 % positive end-points.

RESULTS

(i) *Strain A Cor, Vallée A type, an Argentine strain of foot-and-mouth disease virus received in* 1944. *A comparison between intradermal inoculation of the tongue and subcutaneous inoculation*

A filtrate was prepared from tongue epithelium of the 6th passage in cattle of strain A Cor. This filtrate was titrated by the simultaneous inoculation of the 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilutions at five sites each on the tongues of four cattle and by the subcutaneous inoculation of the 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions using 4, 4, 8, 8 and 8 cattle respectively for each dilution.

The intradermal end-point was $10^{-4\cdot1}$ (see Table 1). Details of the reactions in the groups inoculated subcutaneously are summarized in Table 2. The boxes housing these cattle were entered daily, as it was necessary, as part of another experiment, to make use of the reactors as virus donors. Although every precaution was taken in the way of personal disinfection, the possibility cannot be excluded that accidental infection might have accounted for the development of lesions in some animals somewhat later than the majority, i.e. on the 8th, 9th, 10th, 12th and 14th days. If these animals are counted as being negative, the 50 % positive end-point would be $10^{-4\cdot4}$. If, however, they are regarded as being positive the end-point is $10^{-5\cdot2}$. It is probably justifiable to regard them as positive in view of the precautions taken to prevent spread of the disease. Furthermore, the

incubation period is not unduly long for minimal infective doses of virus. In subsequent experiments the added precaution was taken of preventing anyone from entering the boxes housing cattle inoculated subcutaneously, intracutaneously or intravenously until about a week after inoculation. In this experiment no opportunity occurred at an appropriate time to test the susceptibility of the non-reactors.

Table 1. *Summary of intradermal tongue titrations of virus filtrates. Experiments* i–vii

	Dilutions												
	10^{-2}		10^{-3}		10^{-4}		10^{-5}		10^{-6}		10^{-7}		
Exp. no.	+	−	+	−	+	−	+	−	+	−	+	−	End-point
i	.	.	.	.	11	9	0	20	0	20	0	20	$10^{-4.1}$
ii	.	.	20	0	15	5	8	12	0	20	.	.	$10^{-4.6}$
iii	.	.	20	0	17	3	9	11	0	20	.	.	$10^{-4.8}$
iv	.	.	20	0	17	3	8	12	0	20	.	.	$10^{-4.7}$
v	.	.	20	0	20	0	8	12	1	19	.	.	$10^{-4.9}$
vi	30	0	29	1	24	6	9	21	.	.	.	.	$10^{-4.6}$
vii	.	.	.	.	.	.	25	0	21	29	4	21	$10^{-5.9}$

Note. The figures in the table refer to the observed results, not to the accumulated results from which the end-points are calculated.

Table 2. *Subcutaneous titration of a filtrate of strain A Cor. No. of days from inoculation to reaction and extent of lesions*

10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
3. T, L, 3F	4. T, 4F	3. T, 4F	4. T, 4F	6. T, 4F
3. T, L, 4F	5. T, 4F	3. T, L, 4F	4. T, L, 4F	6. T, 4F
6. T, 4F	6. T, 4F	3. T, L, 4F	5. T, L, 1F	6. T, 4F
6. T, L, 4F	Negative	3. T, Ls, 4F	5. T, 4F	9. T, L, 4F
		4. T, 4F	5. T, L, 4F	12. T, 1F
		5. T, 4F	8. T, 3F	14. T, 4F
		5. T, 4F	10. T	Negative
		5. T, L, 4F	Negative	Negative

In this and subsequent tables the extent of the reaction is denoted by T for tongue, L for lip and F for foot.

In the intradermal inoculation of the tongue each site received not more than 0·1 ml., and in the subcutaneous titration each animal received 50 times more, i.e. 5 ml. The subcutaneous end-point must be reduced, therefore, by a factor of 50 times (log 50 = 1·7) before it can be compared with the intradermal end-point. Thus $10^{-4.1}$ intradermally has to be compared with $10^{-3.5}$ (i.e. $1/10^{5.2-1.7}$) subcutaneously. This is a difference of 0·6 on the logarithm scale to the base 10, that is, four times more virus is required to infect by the subcutaneous route than by the intradermal tongue route. If $10^{-4.4}$ instead of $10^{-5.2}$ had been taken as the subcutaneous end-point the difference would be 25 times.

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(ii) *Strain* 39, *Vallée O type, a strain of foot-and-mouth disease virus from an outbreak in cattle in Great Britain in* 1928. *A comparison between intradermal inoculation of the tongue and subcutaneous inoculation*

A filtrate was prepared from tongue epithelium of the 73rd passage in cattle of strain 39. Four cattle were used for intradermal tongue titration of the filtrate using the 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions. The 50% end-point was $10^{-4\cdot6}$ (see Table 1). For the subcutaneous titration of the 10^{-4}, 10^{-5} and 10^{-6} dilutions each dilution was inoculated into three cattle in 5 ml. doses. The boxes housing these cattle were not entered until the 6th day after inoculation. None of these cattle reacted. The period of observation was 15 days. 10^{-4} in the subcutaneous experiment reduced by 50 times, as explained in the description of the last experiment, gives $10^{-2\cdot3}$. As, in fact, no animals reacted to the 10^{-4} dilution when inoculated subcutaneously, more than 200 times more virus would be required, with this particular strain, to infect by the subcutaneous route compared with the intradermal tongue route.

On the 15th day the susceptibility of the subcutaneous non-reactors was tested by inoculating them intradermally on the tongue with strain 39. All reacted with full development of secondary lesions.

(iii) *Strain* 39 *was again used in an attempt to get an end-point for subcutaneous titration, and an intracutaneous titration was done at the same time*

Tongue epithelium of the 75th passage in cattle was used for preparation of the filtrate. Using four cattle for the intradermal tongue titration, an end-point of $10^{-4\cdot8}$ was obtained (see Table 1). The 10^{-2}, 10^{-3} and 10^{-4} dilutions were used for the subcutaneous and the intracutaneous titrations; in each titration each dilution was inoculated into three cattle. The boxes housing the non-reactor cattle were not entered until the 10th day. The reactors were examined when they were seen, through an inspection window, to be obviously affected.

In the subcutaneous group, one animal receiving 5 ml. of the 10^{-2} dilution reacted on the 5th day, the other animals remained free of lesions. Even if the 10^{-1} dilution would have given 100% positive observations the end-point would not have been higher than $10^{-1\cdot8}$.

In the intracutaneous group, the only reactors were two of the animals which had received inoculations of the 10^{-2} dilution and had developed lesions on the 4th day. The end-point was estimated to be $10^{-2\cdot3}$. When these two end-points are adjusted by the 50-times factor we have: intradermal $10^{-4\cdot8}$, subcutaneous $10^{-0\cdot1}$ and intracutaneous $10^{-0\cdot6}$; thus 50,000 times more virus is required to infect by the subcutaneous route and 16,000 times more by the intracutaneous route compared with the intradermal tongue route.

The susceptibility of the non-reactors was tested 17 days later by inoculating them intradermally on the tongue with strain 39. All reacted with full development of secondary lesions.

(iv) *Strain* 119, *Vallée A type, a strain of foot-and-mouth disease virus from an outbreak in cattle in Great Britain in* 1932. *A comparison between intradermal inoculation of the tongue and subcutaneous inoculation*

The filtrate was prepared from tongue epithelium of the 71st passage in cattle of strain 119. The boxes of the group inoculated subcutaneously were not entered until the 9th day, the date of reaction of the reactors being judged from the age of their lesions. The intradermal tongue titration end-point provided by the results from four cattle was $10^{-4.7}$ (see Table 1).

Table 3. *Subcutaneous titration of a filtrate of strain* 119. *No. of days from inoculation to reaction and extent of lesions*

10^{-4}	10^{-5}	10^{-6}
5. T, L, 4F	7. T, L, 4F	8. T, L, 4F
6. T, Ls, 4F	Negative	Negative
Negative	Negative	Negative

The subcutaneous titration result is given in Table 3. This provided an end-point of $10^{-4.8}$, which adjusted by the 50-times factor gives $10^{-3.1}$ compared with $10^{-4.7}$ by the tongue route. Thus, with this strain, 40 times more virus is required to infect by the subcutaneous route compared with the intradermal tongue route.

The non-reactors were found to be fully susceptible when tested at the end of the period of observation by intradermal inoculation of the tongue with strain 119.

(v) *Strain* 149, *Waldmann C type, a strain of foot-and-mouth disease virus from an outbreak in cattle in Great Britain in* 1934. *A comparison between intradermal inoculation of the tongue and subcutaneous inoculation*

The filtrate was prepared from tongue epithelium of the 33rd passage in cattle of strain 149. The boxes housing the group which had been inoculated subcutaneously were not entered until the 8th day.

The intradermal end-point, using four cattle, was $10^{-4.9}$ (see Table 1), and the 10^{-4}, 10^{-5} and 10^{-6} dilutions failed to infect any of the groups of three cattle inoculated subcutaneously. The lowest dilution inoculated subcutaneously, 10^{-4}, adjusted by the 50-times factor gives $10^{-2.3}$, a difference of $10^{2.6}$ from the intradermal result. Thus, without an end-point being reached, at least 400 times more virus is required to infect by the subcutaneous route compared with the intradermal route.

All the cattle inoculated subcutaneously were found to be fully susceptible when tested by intradermal inoculation of the tongue with strain 149.

(vi) *Strain RV* 7, *a* 1937 *Southern Rhodesian strain of foot-and-mouth disease virus belonging to S.A.T.* 3 *type. A comparison between tongue, subcutaneous and intravenous inoculation*

'S.A.T. 3' type is one of three new immunological types identified while examining strains recovered from outbreaks of foot-and-mouth disease in Africa (Galloway, 1950; Galloway, Brooksby & Henderson, unpublished work).

The filtrate was prepared from tongue epithelium of the 10th and 12th passages in cattle of strain RV 7. The intradermal end-point, calculated from the results in six cattle, was $10^{-4.6}$ (see Table 1). The subcutaneous end-point was 10^{-4}, and the intravenous end-point was $10^{-3.5}$ (see Table 4). Adjustment of the subcutaneous and the intravenous end-points by the 50-times factor gives $10^{-2.3}$ and $10^{-1.8}$ respectively compared with the intradermal result of $10^{-4.6}$. With this strain, therefore, 200 times more virus is required to infect by the subcutaneous route and 600 times more by the intravenous route compared with the intradermal tongue route.

Table 4. *Subcutaneous and intravenous titration of a filtrate of strain RV 7. No. of days from inoculation to reaction and extent of lesions*

	10^{-3}	10^{-4}	10^{-5}
Subcutaneous	4. T, L, 4F	5. T, L, 4F	Negative
	5. T, 4F	6. L, 4F	Negative
	Negative	Negative	Negative
Intravenous	6. T, 2F	6. T, 4F	Negative
	7. T, L, 4F	Negative	Negative
	Negative	Negative	Negative

The boxes were not entered until the 7th day, the date of reaction of the reactors being judged from the age of their lesions. No one entered the boxes again until the 12th day, and, finally, on the 14th day, the non-reactors were inoculated intradermally on the tongue with strain RV 7. The result of this susceptibility test was in marked contrast to those of the previous experiments. All the cattle had primary lesions at all the sites of inoculation, but, in the subcutaneous group, three of the five non-reactors had no secondary lesions, one had a lesion on one foot and one had lesions on two feet. In the intravenous group the three non-reactors to the 10^{-5} dilution had secondary lesions on all four feet, the other non-reactors had no secondary lesions.

This apparent lack of susceptibility could be attributed to a number of causes. For example, the cattle may not have been susceptible at the beginning of the experiment, they may have accidentally contracted an inapparent infection during the course of the experiment, they may have developed some immunity as a result of the inoculation of active virus, or strain RV 7 may be exceptional in showing poor development of secondary lesions.

There is little reason to doubt the initial susceptibility of the cattle; the advantage of having a supply of animals with a 'clean' history has been emphasized. Every practicable precaution of personal disinfection was taken during the course of the experiment, and the boxes housing the cattle inoculated subcutaneously or intravenously were entered only twice between the time of the original inoculation and the subsequent test of susceptibility. Because these precautions were taken to prevent the spread of the disease it is unlikely that accidental inapparent infections would account for eight out of eleven cattle developing some degree of immunity. The development of secondary lesions in normal cattle following tongue inoculation with strain RV 7 is quite typical. Of forty-six cattle in other experi-

ments inoculated intradermally, all developed primary lesions and forty-four developed secondary lesions on all four feet, one had lesions on two feet and only one had no feet lesions. The resistance shown among the non-reactors, therefore, was very different from the degree of susceptibility expected in normal cattle. It seems evident that, on this particular occasion, the subcutaneous and intravenous inoculation of these dilutions containing active virus produced in most instances a high degree of immunity.

(vii) *Strain M* 1, *Vallée A type, a strain of foot-and-mouth disease virus from an outbreak in cattle in Mexico in* 1947. *A comparison between intradermal inoculation of the tongue and subcutaneous inoculation*

The filtrate was prepared from tongue epithelium of the 16th passage in cattle of strain M 1. The intradermal end-point calculated from the results in five cattle inoculated with three dilutions was $10^{-5\cdot9}$ (see Table 1). In the group inoculated subcutaneously three of six cattle that received 5 ml. of the 10^{-2} dilution reacted, one on the 4th, one on the 10th and one on the 12th day after inoculation; one of three cattle inoculated with the 10^{-3} dilution reacted on the 5th day and none of three cattle inoculated with the 10^{-4} dilution reacted. The animals that reacted on the 4th and the 5th day were removed on the 6th day when it was obvious that they were affected. The boxes housing the other animals were entered only twice after inoculation, once on the 9th day and again on the 15th day when, after examination, the non-reactors were tested for susceptibility. The date of reaction of the reactors was estimated from the age of their lesions.

The subcutaneous end-point was $10^{-2\cdot2}$ which, adjusted by the 50-times factor, gives $10^{-0\cdot5}$ compared with $10^{-5\cdot9}$ by the tongue route; thus, with this strain 250,000 times more virus is required to infect by the subcutaneous route compared with the intradermal tongue route.

The test of susceptibility showed that seven of the eight non-reactors had a high degree of immunity which, for reasons similar to those discussed in describing the experiment with strain RV 7, was presumably due to the inoculation of active virus. This development of immunity and the fact that so much virus was required to infect by the subcutaneous route is consistent with some of the results obtained in other experiments with this and other Mexican strains of virus. For example, susceptible cattle exposed to infection by contact frequently failed to show any reaction, and when later inoculated on the tongue were found to have a high degree of immunity (see Henderson, Galloway & Brooksby, 1948).

In addition to these experiments comparing the relative sensitivity of different methods of inoculation, experiments have been made with intranasal instillation using two strains of virus. In one experiment with strain 119, a filtrate having an intradermal tongue end-point of $10^{-5\cdot8}$ was used for intranasal instillation in a dose of 2 ml. per animal. The undiluted filtrate infected both of two cattle, reacting on the 2nd and 3rd day respectively; the 10^{-2} dilution infected both of two cattle and both reacted on the 4th day and the 10^{-5} dilution infected two out of six animals, both reacting on the 7th day. On the basis of comparison as used in the inoculation

experiments this would correspond to about 250 times more virus being required to infect intranasally compared with intradermally on the tongue. Using strain 39, 2 ml. of the $10^{-1.4}$ dilution of a filtrate having an intradermal end-point of $10^{-5.4}$ caused reactions in six out of eight cattle after intranasal instillation. This suggests a difference in the infecting dose of about 50,000 times. Although there are few observations on intranasal instillation, they do suggest that a wide variation would occur in the amount of virus required to infect by this route when using different strains of virus.

DISCUSSION

The differences observed in these experiments between the results of inoculating virus by the intradermal tongue route and by the subcutaneous, intracutaneous and intravenous routes are summarized in Table 5. It is necessary to consider the significance that can be attached to these differences. In intradermal titrations the standard deviation of the end-points provided by one of two animals each giving

Table 5. *Summary of comparisons between intradermal tongue inoculation and inoculations by some other routes*

Exp. no.	Strain	Results obtained by comparison of 50% positive end-points	
i	A Cor	Subcutaneous dose	4 times intradermal tongue dose
ii	39	Subcutaneous dose	> 200 times intradermal tongue dose
iii	39	Subcutaneous dose	50,000 times intradermal tongue dose
iii	39	Intracutaneous dose	16,000 times intradermal tongue dose
iv	119	Subcutaneous dose	40 times intradermal tongue dose
v	149	Subcutaneous dose	> 400 times intradermal tongue dose
vi	RV 7	Subcutaneous dose	200 times intradermal tongue dose
vi	RV 7	Intravenous dose	600 times intradermal tongue dose
vii	M 1	Subcutaneous dose	250,000 times intradermal tongue dose
	Bec 1*	Subcutaneous dose	⩽ 50 times intradermal tongue dose

* Result from an experiment described in the subsequent paper (Henderson, 1952).

five observations per dilution is $\pm 0{\cdot}46$ of the logarithm scale to the base 10 of the dilutions (Henderson, 1949). Reducing this deviation by $\sqrt{4}$, as in these experiments not less than four animals were used, gives $\pm 0{\cdot}23$. There are no data on which the error of a subcutaneous, intracutaneous or intravenous titration can be based, but taking the figure of $\pm 0{\cdot}46$ for five observations per dilution in the intradermal test, $\pm 0{\cdot}6$ would be the equivalent figure for the three observations per dilution provided by most of the other titrations. In comparing a result having a standard deviation of $\pm 0{\cdot}23$ with one having a standard deviation of $\pm 0{\cdot}6$, the standard error of the difference is $\sqrt{(0{\cdot}23^2 + 0{\cdot}6^2)} = \pm 0{\cdot}64$. Twice this standard error is 1·28 and $10^{1{\cdot}28}$ is 19. A twentyfold difference, therefore, is the smallest that would be significant. This deduction serves to give some measure of the order of the significant difference between the intradermal and the other end-points. There is no doubt whether the differences observed are significant. The difference in the strain A Cor experiment, (i), is obviously not significant even if the greater difference of 25 times is taken and that in the strain 119 experiment, (iv), is of doubtful significance, but all the rest are highly significant. For example, with strain 39 the difference between the intradermal and the subcutaneous end-points

is more than seven times the above standard error, and with strain M 1 the difference is 8·4 times.

One of the most striking features of these results is the wide variation of this difference between tongue inoculation and inoculation by other routes. When an infective suspension is inoculated intradermally on the tongue the virus is introduced directly into the susceptible tissue where it is able to multiply sufficiently to result in the appearance of a primary lesion, usually within 18–24 hr. When the infective suspension is given subcutaneously, intracutaneously or intravenously, however, some days elapse before lesions are observed at one or more of the sites of predilection, that is, in the mouth or on the feet. Lesions may arise simultaneously at one or more of these sites or a lesion may develop at one site to be followed by secondary lesions at the other sites as a result of dissemination of the virus produced in the primary lesion.

It is of interest to speculate about what is happening to the virus between the time of parenteral inoculation, excluding tongue inoculation, and the appearance of lesions. The lapse of time might be accounted for by slow absorption from the site of inoculation. This would not be so, of course, in intravenous inoculation. It has been shown in cattle in other experiments that, where no local irritation is produced by intracutaneous inoculation, 5 ml. will be largely absorbed within 45 min. (Henderson, 1944). The route of absorption following intracutaneous inoculation appears to be entirely lymphatic and the inoculum can be detected in the regional lymph node within less than 1 min. Although absorption following subcutaneous inoculation is somewhat slower than this, it commences soon after inoculation as is evident with many drugs and, with a 5 ml. dose, an inoculum of 1 % trypan blue in 0·9 % sodium chloride solution has been detected in the regional lymph node in 55 min. (Henderson, 1944). Slow absorption, therefore, does not seem the most likely explanation for the length of the incubation period.

Of more importance is the fact that the absorbed inoculum becomes diluted by the blood and tissue fluids and carried to all parts of the body. In the ox, with a blood volume of 20–30 l., the dilution of 5 ml. will be of the order of five thousandfold. Presumably some time must elapse before enough virus particles have arrived at susceptible cells to set up the disease. With scarcity of virus particles it might well be that the 'primary' lesion would be confined to one cell, so that more time would be required for sufficient cells to be involved and a visible lesion produced. When there were sufficient reactors on which to form a judgement, there was a tendency for the incubation period to become longer with diminishing amounts of virus. In the strain A Cor experiment which provides most observations (Table 2), the mean incubation period following subcutaneous inoculation of the 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions was, respectively, 4·5, 5, 3·9, 5·9 and 8·8 days. During the incubation period it is possible that some strains may be more susceptible to their environment than others. With strain A Cor, for example, where the difference between the intradermal and the subcutaneous end-points was not significant, there can be no appreciable inactivation or loss of the virus inoculated subcutaneously. The 50,000 times and the 250,000 times difference, however, observed with strains 39 and M 1 respectively may in part be due to much

of the subcutaneously inoculated virus becoming inactivated or prevented from gaining susceptible tissue. This is distinct from penetration of and multiplication within the susceptible cell, as the end-points of preparations of these strains titrated by inoculation of the susceptible tissue of the tongue are just as high as those of corresponding preparations of other strains. It is not known, however, if the trauma of intradermal tongue inoculation assists the virus in the process of entering the susceptible cell. If trauma is an aid, then any lesser ability of a particular virus strain to penetrate intact cells would be apparent when the strain is introduced by another route and would be reflected in the greater difference between the dose required to infect by that route compared with intradermal inoculation of the tongue. As a generalization, it would be true to say that subcutaneous, intracutaneous or intravenous inoculations are less certain methods of detecting foot-and-mouth disease virus than intradermal tongue inoculation, and the strain of virus used may greatly influence the readiness with which infection may be produced by inoculation by those routes.

Two of the strains in these experiments, 39 and 119, have been frequently used for other work, particularly in vaccination experiments where the vaccinated cattle and the unvaccinated control cattle have been exposed to infection by contact with reacting animals. In groups of unvaccinated cattle, 13 out of 103 (12·6 %) failed to react to strain 39 infection, whereas with strain 119 only 3 out of 149 cattle (2 %) failed to react. In other experiments using strain M 1, 6 out of 16 cattle (37·5 %) failed to react to contact exposure, and only 1 animal out of 4 developed lesions after receiving three intranasal doses of 4 ml. of an undiluted filtrate having an intradermal end-point of 10^{-4} (Henderson *et al.* 1948). It seems reasonable to conclude that the lower 'invasiveness' of strain 39 and strain M 1 compared with strain 119 is related to the much larger amount of strains 39 and M 1 virus required to infect if inoculated subcutaneously or instilled intranasally.

It is of interest that in the titration experiments with strain 39, tongue epithelium from reactors of the 73rd and 75th passages was used, and with strain 119 the tongue epithelium was from a reactor of the 71st passage. An extended series of passages under experimental conditions cannot necessarily account for the poorer performance of strain 39.

One of the objects of making these comparisons of different routes of inoculation is in connexion with the choice of route for testing 'inactivated' virus vaccines for any survival of traces of active virus—the 'innocuity test' or the test for non-infectivity. From the results observed it would be of little use attempting to detect a trace of active virus of strain 39 or strain M 1 by inoculating the material for test by the subcutaneous route, whereas with strain A Cor there might be an advantage in using the subcutaneous route because of the larger volume that could be inoculated compared with the tongue route. This is discussed in greater detail in the subsequent paper dealing with the significance of tests for non-infectivity of vaccines (Henderson, 1952).

Sufficient mention has already been made of the 'clean' history of the cattle available at this Institute. This is evident in the results of the experiments with

strains 39, 119 and 149, when those cattle that did not react on subcutaneous or intracutaneous inoculation all reacted with development of secondary lesions when later inoculated on the tongue. A number of these cattle, particularly those of experiment (iii) with strain 39, had received appreciable amounts of active virus and yet no detectable immunity was produced as a result of these subcutaneous or intracutaneous inoculations of unmodified antigen. The results of testing the susceptibility of the strain RV 7 and strain M 1 non-reactors were in marked contrast to this and, with strain RV 7, a comparatively small dose of active virus was followed by development of a high degree of immunity. The development of some degree of immunity in the absence of clinical signs of disease following inoculation or exposure to infection with foot-and-mouth disease virus requires further study, and it is beyond the scope of this paper to discuss the significance of the data provided on this problem by the results of testing the susceptibility of the non-reactors to inoculation of active virus.

SUMMARY

Intradermal inoculation of the tongue, subcutaneous, intracutaneous and intravenous inoculation were compared in determining the relative facility with which foot-and-mouth disease infection can be produced in cattle.

In seven experiments using six virus strains, least virus was required to infect by the intradermal tongue route, but wide variation was observed between the amount of virus required to infect by this route and by the subcutaneous, intracutaneous or intravenous routes. The smallest difference between tongue and subcutaneous inoculation was not significant, and the largest difference was that 250,000 times more virus was required to infect by the subcutaneous route. The readiness with which infection is produced by routes other than intradermal inoculation of the tongue may be greatly influenced by the strain of virus used.

Intranasal instillation of the virus was also compared with intradermal tongue inoculation. As with subcutaneous inoculation, more virus was required for infection by the intranasal route and, similarly, the results suggest that considerable variation would be found in the amount of different strains required.

A correlation is suggested between this variation and the variation observed in the 'invasiveness' of strains in cattle exposed to contact infection.

Attention is drawn to the significance of the observations recorded in this paper in relation to tests of foot-and-mouth disease vaccines for non-infectivity.

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