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Studies on the pathogenesis of rinderpest in experimental cattle

III. Proliferation of an attenuated strain in various tissues following subcutaneous inoculation

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The pathogenesis of rinderpest, following experimental infection of cattle with the virulent RGK/1 strain of virus, has been the subject of two recent investigations (Liess & Plowright, 1964; Plowright, 1964). Further experiments have now been undertaken to provide comparative data on infection with a culture-attenuated strain of virus which is widely used as a vaccine for cattle in East and West Africa. It was hoped that the information obtained would be useful in explaining its lack of pathogenicity and high immunogenicity (Plowright & Ferris, 1962*b*). There was also a need to determine how long vaccine virus persisted in the tissues of inoculated cattle, so that meat from potentially infected animals would not be exported to countries free from the disease (see Provost, 1960).

The procedure adopted was to kill experimental animals at daily intervals after administration of vaccine and titrate infective virus in selected tissues.

MATERIALS AND METHODS

Virus

Serial propagation of the virulent Kabete 'O' strain of rinderpest virus in primary calf kidney (BK) cells selected a virus population which, after seventy passages, could be inoculated into cattle without producing pyrexia or other clinical signs (Plowright & Ferris, 1962*b*). This attenuated strain produced a good neutralizing antibody response and is currently used for vaccine production in its 91st to 100th BK passages. For the present experiments virus of the 95th passage was used; it was freeze-dried in glass-sealed ampoules which were stored at $-20°$ C. and constituted a routine production batch of vaccine.

For every cattle inoculation 2 ampoules were each reconstituted in 1 ml. of sterile deionized water and the contents pooled. A twofold dilution was then prepared in tissue culture growth medium (Plowright & Ferris, 1959) and each animal received 2 ml. subcutaneously, on the left-hand side of the neck. The inoculation was made immediately above and in front of the left prescapular (posterior superficial cervical) lymph node, where the point of injection was marked by trimming off a small amount of hair.

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On five occasions during the course of the experiments, portions of the inoculum were titrated in BK cells by the method of Plowright & Ferris (1959, modified 1962*b*). All the results fell within the range $10^{4.0}$ to $10^{4.6}$ TCD 50 per ml. of reconstituted vaccine. It has been shown, for this strain of virus, that one TCD 50 is equivalent to one cattle ID 50 (Plowright & Ferris, 1962*b*); each experimental animal therefore received between 10,000 and 40,000 cattle ID 50 or about 100 times the normal field dose of vaccine.

Experimental cattle

The animals used were grade steers or heifers, the majority being Red Poll or Ayrshire crosses. They were aged between 1 and 3 years, and their sera contained no rinderpest-neutralizing antibody (Plowright & Ferris, 1961). All cattle were segregated after inoculation but they were not housed and had continual access to grass and water. Temperatures were recorded daily before 8.30 a.m., all inoculations being performed before this time. Animals were killed at daily intervals after inoculation; in all instances this was completed before 9 a.m.

Collection of tissues

The technique employed was essentially that described by Plowright (1964). *Blood*, for the recovery of virus and serum, was always collected in the manner detailed in the same paper, as were also portions of the following tissues, viz.: mucosae of the *base of the tongue*, *dorsal turbinate bone*, *abomasum* (*pylorus*), *ileum*, *caecum* and *colon;* one large, or several small *haemolymph nodes* taken from adipose tissue of the left prescapular groove; *spleen*, *bone marrow* and *palatal tonsil; pharyngeal*, *left prescapular*, *right prescapular* and *mesenteric* (jejuno-ileal) *lymph nodes; liver*, *kidney*, *lung*, *myocardium* and *brain*.

Two additional tissues were obtained at autopsy. First, a portion of *muscle* and *subcutaneous tissue* was removed from the neck immediately below the point at which virus was inoculated. Secondly, in seventeen instances, the total lymphoid tissue comprising a *Peyer's patch* was collected; the intestinal contents were first removed from the mucosa by liberal washing with tap water and the tissue of the patch was then dissected clear of the underlying muscle layers.

Subsequent treatment of tissues

The methods used in the preparation of 10-fold dilution series, from the blood, bone marrow and each solid tissue, followed in detail those described by Plowright (1964). In the early stages, dilutions covering the range 10^{-2} to 10^{-5} were prepared from each tissue; concentrations greater than 10^{-2} (w/v) could not be used without inducing cytotoxic changes in the monolayers inoculated. As the experiment progressed it became apparent that a large number of tissues did not support virus multiplication (see Results) and for these the dilution series was first reduced to 10^{-2} and 10^{-3}; later only a 10^{-2} suspension was tested, while finally, in the case of the last three animals employed (nos. 9712, 9716 and 9737), these tissues were excluded.

Where virus proliferation was detected the dilution range extended to a mini-

mum of 10^{-4} and a maximum of 10^{-7}. In a few instances titres were higher than expected and the end-point was not determined.

Viraemia was detected by preparing leucocyte fractions from 20 ml. samples of blood with EDTA and inoculating these into five BK cultures, as already described (Plowright & Ferris, 1962*a*). In addition a 10^{-1} dilution in culture maintenance medium was prepared from blood-EDTA mixtures and inoculated into five additional BK cultures in a dose of 2 ml. All dilutions of solid tissues were inoculated, in the same dosage, into five BK cultures.

Preparation and maintenance of cell cultures

All virus titrations were carried out in 6- to 9-day-old cultures of BK cells in tubes of 160×15 mm. dimensions. For convenience, suspensions of trypsin-dispersed BK cells in growth medium were held at 4° C. for up to 2 days before seeding. The handling of the monolayers before and after inoculation was as described by Plowright (1964). Using the antibiotics described by that author bacterial and fungal contamination were only encountered occasionally and never in serious proportions.

The cultures were examined by low-power microscopy on the 5th, 7th and 9th days after inoculation, final readings being made on the 9th day. Titres were calculated by the method of Thompson (1947) and expressed as $\log_{10}$ TCD 50/g. of solid tissue.

The level of viraemia was never sufficiently high to be expressed as a 50 % end-point; virus was only detectable in the leucocyte concentrates and the results were expressed as the number of positive tubes out of five inoculated (Tables 1–3).

In the case of solid tissues, where only one tube inoculated with a 10^{-2} dilution was positive the result was expressed as a 'trace'. Where two or more were infected it was assumed that all the tubes receiving a hypothetical 10^{-1} dilution would have been positive, and the result was given as a 50 % end-point.

Serum neutralization tests

Quantitative neutralization tests were performed on sera collected from every animal at the time of its destruction. All sera were tested simultaneously against the same stock of virus, yielding $10^{2\cdot6}$ TCD 50 per tube (Plowright & Ferris, 1961).

RESULTS

Clinical signs in cattle

No pyrexial response was recorded and no visible abnormalities were detected in any of the animals while alive. Mild engorgement of carcass lymph nodes was occasionally found at autopsy but otherwise no significant lesions were observed.

Virus proliferation

According to the results, the tissues divided themselves into two clear-cut categories—those in which virus proliferation could be detected, at least occasionally, and those which failed to support virus multiplication at any time. The latter

group included ten tissues, comprising six mucosae and four parenchymatous organs, viz.: the mucosae of the nasal cavity (turbinate bone), base of the tongue, abomasum, ileum, caecum and colon; liver, kidney, myocardium and brain.

Virus multiplication was demonstrated in twelve of the twenty-two tissues

Table 1. *The titre of virus in various tissues of cattle killed on days* 1–4 *after inoculation*

Time after infection ...	1 day		2 days		3 days		4 days	
Animal no. ...	9385	9531	9379	9535	9607	9873	9603	9727
Left prescapular lymph node	0·0	0·0	0·0	0·0	0·0	0·0	Tr.	0·0
Right prescapular lymph node	0·0	0·0	0·0	0·0	0·0	0·0	Tr.	0·0
Pharyngeal lymph node	0·0	0·0	0·0	0·0	0·0	0·0	2·6	Tr.
Mesenteric lymph node	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Peyer's patch	N.T.	0·0	N.T.	N.T.	N.T.	0·0	N.T.	0·0
Tonsil	0·0	0·0	0·0	0·0	0·0	0·0	⩾ 3·2	0·0
Blood*	0/5	0/5	0/5	0/5	0/5	0/5	2/5	0/5
Spleen	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Haemolymph node	0·0	0·0	0·0	0·0	0·0	0·0	⩾ 3·2	1·6
Bone marrow	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Muscle and subcutaneous tissue	0·0	0·0	0·0	0·0	0·0	0·0	⩾ 3·2	0·0
Lung	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0

* Result expressed as number of tubes positive out of five inoculated with each leucocyte fraction.

Table 2. *The titre of virus in various tissues of cattle killed on days* 5–7 *after inoculation*

Time after infection ...	5 days			6 days			7 days		
Animal no. ...	9612	9626	9737	9600	9645	9670	9611	9632	9712
Left prescapular lymph node	2·6	⩾ 4·2	4·0	2·8	2·4	2·4	4·0	0·0	2·4
Right prescapular lymph node	0·0	Tr.	3·2	3·2	3·2	3·2	3·6	⩾ 3·4	⩾ 5·0
Pharyngeal lymph node	2·5	2·8	3·6	2·8	3·4	4·8	⩾ 4·2	3·4	4·4
Mesenteric lymph node	3·0	2·2	2·0	1·6	2·8	0·0	⩾ 4·0	4·6	4·4
Peyer's patch	0·0	N.T.	Tr.	N.T.	3·4	3·2	N.T.	3·8	4·8
Tonsil	2·4	2·6	3·8	3·6	3·8	3·7	3·8	3·0	4·6
Blood*	0/5	1/5	1/5	1/5	2/5	0/5	1/5	2/5	5/5
Spleen	1·7	2·2	1·6	3·0	1·8	3·0	1·8	1·6	3·4
Haemolymph node	Tr.	⩾ 3·2	4·0	3·4	3·8	4·2	5·4	4·6	5·4
Bone marrow	0·0	0·0	0·0	1·6	0·0	0·0	0·0	0·0	1·8
Muscle and subcutaneous tissue	0·0	0·0	0·0	2·2	⩾ 3·2	0·0	0·0	0·0	0·0
Lung	0·0	Tr.	0·0	0·0	0·0	0·0	0·0	0·0	1·8

* Result expressed as number of tubes positive out of five inoculated with each leucocyte fraction.

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tested, viz.: muscle and subcutaneous tissue at the site of inoculation; left and right prescapular, pharyngeal and mesenteric lymph nodes, Peyer's patches, tonsil, spleen and haemolymph node; blood, bone marrow and lung.

Virological events could be roughly divided into three phases, i.e. those of virus eclipse, multiplication and decline.

Table 3. *The titre of virus in various tissues of cattle killed on days* 8–10 *after inoculation*

Time after infection ...	8 days				9 days		10 days	
Animal no. ...	9622	9683	9716	9732	9618	9714	9729	9733
Left prescapular lymph node	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Right prescapular lymph node	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Pharyngeal lymph node	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Mesenteric lymph node	0·0	2·8	4·0	0·0	1·8	0·0	0·0	0·0
Peyer's patch	0·0	Tr.	0·0	0·0	⩾ 5·0	0·0	0·0	0·0
Tonsil	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Blood*	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Spleen	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Haemolymph node	0·0	Tr.	0·0	0·0	3·0	0·0	0·0	0·0
Bone marrow	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Muscle and subcutaenous tissue	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Lung	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0

* Result expressed as number of tubes positive out of five inoculated with each leucocyte fraction.

The eclipse phase

After the inoculations a period of 3 days elapsed during which no virus could be demonstrated in any tissue. This phase ended on day 4, when two animals were killed. In the first (no. 9603), trace amounts of virus were found in both prescapular lymph nodes but more substantial amounts were found in the pharyngeal lymph node ($10^{2\cdot6}$ TCD 50/g.), tonsil ($\geqslant 10^{3\cdot2}$ TCD 50/g.) and prescapular haemolymph node ($\geqslant 10^{3\cdot2}$ TCD 50/g.). A low grade viraemia was present (2/5 tubes infected with a leucocyte fraction) and virus was also detected in the muscle and subcutaneous tissue ($\geqslant 10^{3\cdot2}$ TCD 50/g.).

The second animal killed on day 4 (no. 9727) had a trace of virus in a pharyngeal lymph node and small quantities in a haemolymph node ($10^{1\cdot6}$ TCD 50/g.). No virus could be detected in the blood, prescapular lymph nodes or tissue removed from the site of inoculation.

The phases of virus multiplication and decline in various tissues

Left prescapular lymph node. On day 5 a peak mean titre of $\geqslant 10^{3\cdot6}$ TCD 50/g. was recorded in this structure (Fig. 1). Thereafter the quantity of virus declined, so that on day 7 the mean was $10^{2\cdot1}$ TCD 50/g. (Fig. 1). No virus could be recovered from this node in any of the 8 animals killed on the 8th day or later (Table 3).

Right prescapular lymph node. On day 5 three animals were tested and the mean titre was $10^{1.1}$ TCD 50/g. (Fig. 1). By day 6 the titres had risen considerably (Table 2), whilst the peak mean value of $\geqslant 10^{4.0}$ TCD 50/g. was reached on day 7. This was followed by an abrupt fall in infectivity, as none could be demonstrated in this node on day 8 or on either of the two succeeding days.

Pharyngeal lymph node. The mean titre rose steadily from $10^{1.3}$ TCD 50/g. on day 4 to a peak of $10^{4.0}$ TCD 50/g. on day 7, identical with that obtained in the right prescapular node. As in the prescapular nodes, no virus could be recovered on day 8.

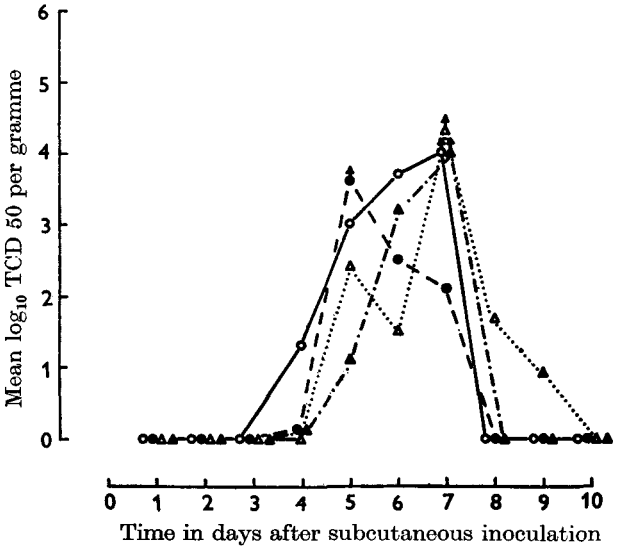

Fig. 1. Proliferation of culture-attenuated rinderpest virus, strain RBOK, in the lymph nodes of cattle. ○——○, Pharyngeal L/N; ● – – ●, left prescapular L/N; △·····△, mesenteric L/N; ▲–·–·–▲, right prescapular L/N.

Mesenteric lymph node. The results for this tissue were more irregular than those for the other lymph nodes studied and virus persisted for a longer time (Fig. 1). Virus multiplication was not detected until day 5 after inoculation, when virus was recovered from all three animals tested (see Table 2 and Fig. 1). On day 6 one of three cattle (no. 9670) had no detectable infectivity, whereas 24 hr. later all animals tested yielded relatively high concentrations of virus; the mean titre on the 7th day was $10^{4.3}$ TCD 50/g., one of the highest recorded during these experiments. On day 8, two cattle, nos. 9732 and 9622, showed no virus in mesenteric lymph nodes, whereas two others had moderate amounts. On day 9, low-titre virus was still present in one of two animals but finally, on the 10th day, neither of the two cattle killed had any demonstrable infectivity in this node.

Palatal tonsil. The tonsil behaved in a very similar manner to the pharyngeal lymph node, virus appearing in one of two animals on the 4th day and disappearing by the 8th (Fig. 2).

Peyer's patches. Virus in this tissue was not present in appreciable quantities until day 6, although a trace was recorded in one of two animals (no. 9737) tested

on day 5. The mean titre rose rapidly to a peak of $10^{4.3}$ TCD 50/g. on day 7. Of four animals tested on day 8 only one (no. 9683) yielded virus and this was in trace amount. However, on the 9th day one animal, no. 9618, showed high-titre virus in a Peyer's patch ($\geqslant 10^{5.0}$ TCD 50/g.) and accounted for the sharp second peak in the mean line (Fig. 2). No virus was recovered from either of the two animals tested on the 10th day post-inoculation.

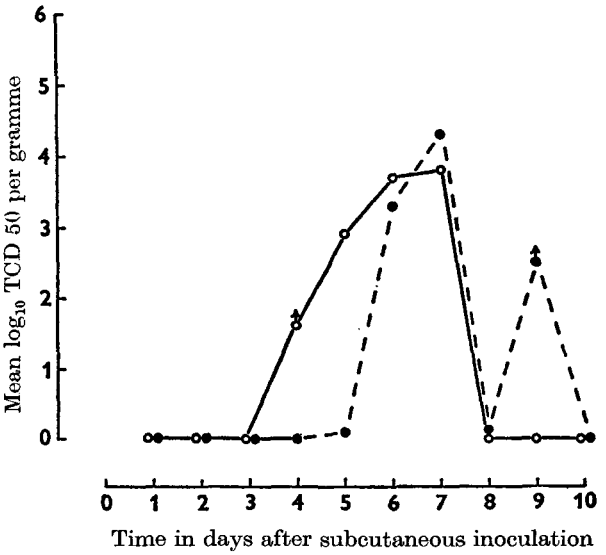

Fig. 2. Proliferation of culture-attenuated rinderpest virus, strain RBOK, in the lympho-epithelial tissues of cattle. ○—○, Tonsil; ● – – ●, Peyer's patch.

Spleen. No virus was detected in this organ until day 5, and the peak mean titre of $10^{2.6}$ TCD 50/g. was recorded on day 6 (Fig. 3). A rapid fall ensued and no virus was recovered from the spleens of four animals killed on the 8th day. Mean titres were the lowest recorded for any of the tissues that regularly supported virus proliferation.

Prescapular haemolymph nodes. Virus was demonstrable in the haemolymph nodes of both animals tested on day 4 (Table 1), the mean titre on this day being $10^{2.4}$ TCD 50/g. On day 5 substantial amounts of virus were found in cattle nos. 9626 and 9737 but only a trace was found in no. 9612 (Table 2). Thereafter the mean titre rose steadily, reaching a peak of $10^{5.1}$ TCD 50/g. on day 7, the highest figure recorded for any tissue (Fig. 3). Only a trace of virus was recorded from one of the four animals tested on the 8th day, but 24 hr. later one of two animals (no. 9618) still yielded $10^{3.0}$ TCD 50/g. (Table 3). No virus was detected in this tissue on day 10.

Blood. As observed in the section on Materials and Methods, the amount of virus in the blood was never sufficiently great to be expressed as a 50 % end-point. Hence, in preparing Figure 3, one positive tube was arbitrarily taken to represent 0·1 $\log_{10}$ units. Virus was first found in the blood on day 4 (no. 9603, Table 1). A low-grade viraemia was also detected in eight of the thirteen animals killed on days

5–8 inclusive. Virus in the blood probably reached a maximum on the 7th day (Tables 2 and 3).

Bone marrow. Virus was recovered from the bone marrow on only two occasions, the titre being low in each instance (no. 9600 with $10^{1.6}$ TCD 50/g. on day 6; no. 9712 with a titre of $10^{1.8}$ TCD 50/g. on day 7).

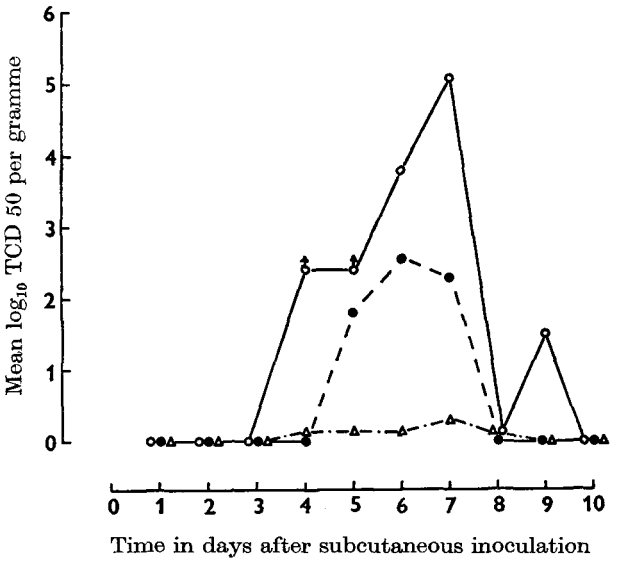

Fig. 3. Proliferation of culture-attenuated rinderpest virus, strain RBOK, in the lymphopoietic tissues and blood of cattle. ○—○, Prescapular HL/N; ● – – ●, spleen; △—·—△, blood.

Lung. Virus was isolated from the lungs of two cattle only, no. 9626 (day 5) and no. 9712 (day 7); in both cases the amount of virus was very small (see Table 2).

Muscle and subcutaneous tissue at site of inoculation. Infectivity was demonstrated in these tissues on three occasions, once on day 4 (no. 9603, $10^{3.2}$ TCD 50/g.) and twice on day 6 (no. 9600, $10^{2.2}$ TCD 50/g.; no. 9645, $\geqslant 10^{3.2}$ TCD 50/g.).

Development of neutralizing antibody

As shown in Table 4, no animal produced neutralizing antibody before day 7. At this time one ox (no. 9712) of three tested, showed a trace of antibody although large quantities of virus were present in many of its tissues. On the 8th day, three of four animals tested had neutralizing antibody in their sera and two of these (nos. 9622 and 9732) were devoid of infectious virus. No. 9683 still had virus in four sites, in spite of the presence of low-titre antibody. One of two animals (no. 9618) killed on the 9th day, had no detectable antibody and virus was still present in three tissues (Table 3); both cattle tested on the 10th day had circulating antibody.

Table 4. *Rinderpest neutralizing antibody in the sera of cattle killed at different times after the inoculation of culture-attenuated rinderpest virus*

Animal no.	No. of days after inoculation	Titre of serum*	Animal no.	No. of days after inoculation	Titre of serum*
9385	1	0·0	9611		0·0
9531		0·0	9632	7	0·0
9379	2	0·0	9712		Trace
9535		0·0	9622		0·8
9607	3	0·0	9683	8	Trace
9873		0·0	9716		0·0
9603	4	0·0	9732		0·4
9727		0·0	9618	9	0·0
9612		0·0	9714		1·0
9626	5	0·0	9729	10	0·8
9737		0·0	9733		0·8
9600		0·0			
9645	6	0·0			
9670		0·0			

* Log_{10} SN 50 at time of death. All sera were tested simultaneously against $10^{2·6}$ TCD 50 of virus.

DISCUSSION

The sequence of events following subcutaneous inoculation of culture-attenuated rinderpest virus was not precisely determined. It is probable that part of the inoculum was first transferred by lymphatic vessels to the local lymph node and proliferated there before reaching the blood through the cervical duct. Some evidence for this was seen in the earlier virus peak in the left than in the right prescapular node (5th *v.* 7th day; see Fig. 1) but no animal was examined in which virus was limited to the node draining the site of inoculation. It may be that some inoculated virus passed through the local node directly into the blood stream; this might account, for example, for higher virus titres in tonsil and pharyngeal node of Ox 9603 (4th day) than in the prescapular nodes of the same animals.

Appreciable quantities of virus (*ca.* $10^{2·2}$ to $10^{3·2}$ TCD 50/g.) were recovered, on three occasions, from muscle and subcutaneous tissues at the site of inoculation. The animals involved were killed on the 4th and 6th days after inoculation and, in view of the lability of cultured rinderpest virus (Plowright & Ferris, 1961), it is almost certain that some local proliferation took place. Difficulties in locating the exact site of deposition of the inoculum could easily account for failure to demonstrate local virus growth in the other animals. There were no indications as to which cells in the muscle and subcutaneous tissue supported virus multiplication.

The viraemia produced by attenuated rinderpest virus differed considerably from that produced by virulent strains. Thus, the Kabete 'O' virus, from which our attenuated variant was derived, reappeared in the blood on the day following its subcutaneous inoculation in large dosage; viraemia reached maximum levels on the 4th day after infection and persisted for 13–14 days (MacOwan, 1956). Recent field strains, of low cattle pathogenicity, produced a viraemia detectable on the

2nd to 4th days after subcutaneous inoculation of about 10^4 to 10^5 TCD 50 of virus; peak blood titres of $10^{1\cdot4}$ to $10^{3\cdot0}$ TCD 50/ml. were usually attained on the 6th or 7th days post-infection, the duration of viraemia varying from 3 to 11, with a mean of over 6 days (Plowright, 1963). The highly pathogenic RGK/1 strain was recovered on the first to 3rd days following parenteral inoculation; pyrexia began on the 3rd to 5th days and blood virus reached a mean of $10^{2\cdot3}$ TCD 50/ml. on the 3rd day of fever (Liess & Plowright, 1964), viraemia lasting until the 12th day after infection (Plowright, 1964).

It is immediately evident that the attenuated virus showed a longer 'eclipse' phase in cattle, being demonstrable at the earliest on the 4th day following inoculation. Infectivity was not regularly demonstrable in the blood, except on the 7th day, when it possibly reached a maximum. The duration of viraemia was only 5 days in the group of cattle studied, although figures for individual animals were not obtained. It is clear that some virus must have circulated before the 96th hour, since spread to and growth in the pharyngeal lymph nodes and tonsil had occurred by that time.

After intranasal inoculation of cattle, the RGK/1 strain produced maximal mean titres of $> 10^7$/g. in the cephalic lymph nodes, tonsil and prescapular haemolymph nodes. Somewhat lower but considerable figures were recorded for the spleen, visceral lymph nodes, mucosae of the alimentary tract (tongue, abomasum, caecum, colon and ileum) and lung, whilst a low level of proliferation was detected in the nasal mucosae, bone marrow, liver and kidney (Plowright, 1964). The proliferation of the virulent Kabete 'O' strain of rinderpest virus in different tissues of cattle has not, apparently, been studied systematically but peak titres of about $10^{6\cdot0}$ ID 50/g. have been recorded in the spleen, body lymph nodes and abomasal mucosa (Scott, 1955; MacOwan, 1956). The attenuated Kabete 'O' strain, used in these experiments, reached maximal mean titres which were about 100-fold to 1000-fold lower in various lymph nodes, tonsil, spleen and haemolymph nodes.

Peyer's patches were not, unfortunately, included in the RGK/1 study (Plowright, 1964), but it was evident that a considerable proliferation of the attenuated virus occurred in them and not in the intervening ileal mucosa or in the mucosae of the tongue, abomasum (pylorus), caecum and colon. As lymphoid follicles, either single or aggregrated, and diffuse infiltrations of lymphoid cells are associated with all the alimentary and respiratory mucosae which failed to support a significant growth of attenuated virus, it is of interest to speculate on reasons for the selective localization observed. It may simply be that the mass of susceptible lymphoid cells in mixed lympho-epithelial structures, such as the palatal tonsil and Peyer's patches, is very much greater than that in the mucosa and submucosa of the base of the tongue, abomasum, etc.; virus would not accordingly reach detectable levels in the latter group of sites. This suggestion is difficult to accept, particularly in the case of the tongue mucosae and lung, where many large lymphoid follicles are present and a trace of virus, at least, would have been expected in some animals. It is possible that lymphoid cells in different situations vary in their capacity to support the growth of rinderpest virus or in their accessibility to infection by virus circulating in the blood. Similar factors may

account for the considerably higher titre of virus in the haemolymph nodes than in the spleen (Fig. 3); the great similarity in histological structure of these tissues (Trautman & Fiebiger, 1952) could have led to the expectation that virus titres attained in them would have been comparable.

The infrequent recovery of small quantities of virus from samples of lung (2) and bone marrow (2), suggested that such results may have been due to the trapping in these tissues of virus-carrier leucocytes, rather than active multiplication in local accumulations of lymphoid cells.

Widespread proliferation of the attenuated strain in lymphopoietic tissues can probably be associated with the solid and lasting immunity which it induces in cattle. Recent work has shown that resistance to severe parenteral challenge lasts for at least 3 years, while the high initial titre and slow rate of decline of neutralizing antibody in the serum, support the belief that it is almost certainly lifelong (Plowright, unpublished). So far as the lack of pathogenicity of vaccine virus is concerned it seems reasonable to suppose that this can be associated with its failure to proliferate in the mucosae of the alimentary tract, or in the nasal mucosa and parenchymatous organs. Attenuated virus is, in fact, strictly lymphotropic, whereas virulent strains multiply in the tissues mentioned and produce lesions there which account for the characteristic clinical signs. The absence of attenuated rinderpest virus from surface mucosae and parenchymatous tissues probably resulted in a failure of virus excretion, which would account for the inability of vaccine virus to spread by contact amongst susceptible cattle (Plowright & Ferris, 1962*b*).

It is interesting in this connection to recall comparable findings with virulent and attenuated strains of other viruses. Thus, a mild strain of Newcastle disease virus differed from a highly virulent one only in the rate of its penetration into and proliferation in the central nervous system of chickens (Karzon & Bang, 1951); a mild strain of fowlpox did not multiply to any extent in the liver and bone marrow of inoculated chicks, whereas a virulent strain did so and subsequently produced a secondary viraemia and generalization (Mayr & Wittman, 1957); finally, Roberts (1963) showed that an avirulent strain of ectromelia virus differed from a virulent one in its reduced ability to infect the liver macrophages and hence liver parenchymal cells.

The abrupt decline of attenuated rinderpest virus between days 7 and 8 was undoubtedly associated with the appearance of antibody at that time; it seems improbable that the supply of susceptible cells was anywhere near to exhaustion, especially as the maximal titres registered were so modest. The 7–10 days before antibody became detectable in different animals was comparable to the 9 days recorded in cattle infected intranasally with the virulent RGK/1 strain (Plowright, 1964) and 6–7 days in others given virulent Kabete 'O' virus subcutaneously (Plowright, 1962). The disappearance of virus from all tissues of cattle by the 10th day after inoculation of a large dose of the attenuated strain indicates that, under field conditions, it can probably be assumed with safety that animals given vaccine 2 weeks previously no longer constitute a risk from the point of view of meat exports (see Provost, 1960).

SUMMARY

Twenty-five grade cattle were infected subcutaneously with $10^{4.0}$ to $10^{4.6}$ TCD 50 of a highly-attenuated strain of rinderpest virus which is used as a vaccine. No clinical reaction was observed but the proliferation of virus was studied in twenty-two tissues harvested at daily intervals from the first to the 10th days after inoculation. Serum samples collected at the same times were examined for rinderpest-neutralizing antibody.

There was an 'eclipse' phase of 3 days during which no infectivity could be demonstrated in any tissue. On the 4th day virus had generalized, as shown by its detection in lymphoid tissues which were not associated with the site of inoculation; occasional animals showed evidence of viral proliferation in the local muscle and subcutaneous tissue. A considerable growth of virus, with peak titres between $10^{4.0}$ and $10^{5.0}$ TDC 50/g., was demonstrated in the prescapular, pharyngeal and mesenteric lymph nodes, also in the palatal tonsils and Peyer's patches of the ileum. Highest titres ($10^{5.4}$ TCD 50/g.) were recorded in the prescapular haemolymph nodes, but less virus (up to $10^{3.4}$ TCD 50/g.) appeared in the spleen.

A low-level viraemia was detected in eight of the thirteen cattle killed on the 5th to 8th days inclusive. Minimal quantities of virus were found on two occasions each in the bone marrow and lung. No virus was recovered from the mucosae of the base of the tongue, abomasum, ileum, caecum and colon; liver, heart, kidney and brain tissue also failed to support its multiplication.

Neutralizing antibody was present in all cattle by the 10th day after inoculation, its appearance being associated with the abrupt decline in virus titres, which was usually demonstrable on the 8th day.

The behaviour of the attenuated virus was compared with that of virulent strains, and it was concluded that its lack of pathogenicity was due primarily to its failure to proliferate in the mucosae of the gastro-intestinal and respiratory tracts. Vaccine virus was, in fact, exclusively 'lymphotropic', a characteristic which may account for the solid, lasting immunity it confers and for the considerable antibody response it provokes in inoculated cattle. Inability to spread by contact amongst susceptible cattle may be a result of the absence of virus in mucosae or parenchymatous organs and hence in excretions.

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