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Studies on the pathogenesis of rinderpest in experimental cattle

II. Proliferation of the virus in different tissues following intranasal infection

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The first paper of this series (Liess & Plowright, 1964) described the results of experiments designed to provide quantitative data on the routes of excretion of virulent rinderpest virus, in cattle infected by a number of different methods. An attempt was made to correlate virus excretion with the appearance and development of clinical signs and viraemia. However, no adequate explanation of the phenomena could be offered without detailed knowledge of the quantitative distribution of virus in various tissues, at increasing intervals after infection. The experiments to be described in this paper were performed to provide the necessary data.

All cattle were infected by the intranasal route, with the same strain of virus as that employed previously (RGK/1). It was considered that these animals would probably behave in a manner closely resembling that following natural infection and hence it should be possible to provide an account of the normal pathogenesis of the disease. The information obtained would be of practical significance in helping to formulate recommendations for the laboratory diagnosis of rinderpest, by the recovery of infectious virus from the tissues of animals in various stages of the disease. It should also, incidentally, be fundamental in reaching a scientific assessment of the dangers of the presence and persistence of rinderpest virus in the meat and offals from cattle, which might be slaughtered in the preclinical or clinical phases of the disease. There is little evidence that the need for such data is properly appreciated at present, or furthermore that the potential value of tissue culture techniques, in providing economical answers to these problems, has even begun to be recognized (see, for example, Provost, 1960).

Finally, it was hoped that an elucidation of the pathogenesis of rinderpest in cattle might be of comparative interest to those concerned with human measles and canine distemper. In this connexion, Downie (1963) has remarked on the advantages of the veterinary virologist in being able to study his diseases in the definitive host, and not in another experimental one which is regarded as more or less comparable.

MATERIALS AND METHODS

Experimental animals

A total of twenty-nine grade cattle was used. All of them were approximately 2 years old and twenty-two of them had, apparently, a large proportion of the blood of the highly susceptible Channel Islands breeds (Guernsey and Jersey). Their sera were found to be negative in screening tests for rinderpest-neutralizing antibody (Plowright & Ferris, 1961) and they were housed in isolation units which precluded the possibility of accidental infection. They were fed with hay only and provided with water *ad lib.*

Rectal temperatures were recorded every morning and any rise to 102·0° F. or above was regarded as abnormal. Daily clinical examinations were performed to ascertain the time to appearance of mouth lesions and diarrhoea or dysentery.

Virus strain

The origin of the RGK/1 strain of rinderpest virus has been described in a previous paper (Liess & Plowright, 1964). It was stored at −70° C. as untreated fragments of spleen tissue derived from an ox (no. 8994), which represented the fifth laboratory passage, three of which were in primary calf kidney monolayers, and two in cattle. Pieces of frozen spleen were thawed immediately before use and 10 % (w/v) suspensions were prepared in Ten Broeck grinders, using culture maintenance medium as a diluent.

Infection of cattle

All animals were infected by dropping 2 ml. of a 10 % spleen suspension into the nostrils, with the head held in such a position that the material flowed quickly over the turbinates towards the nasopharynx; thereafter the nostrils were compressed with the hand to produce a short period of noisy, forcible inspiration. Cattle did not cough as a result of this treatment and it was assumed that the inoculum did not enter the larynx. Care was taken that no mechanical injury to mucosae took place during the manipulations.

The inoculum was always prepared with culture maintenance medium as a diluent and samples were titrated in primary calf kidney cultures, by the method to be described later. The figures obtained were pooled to give an estimate of the reliability of the titration technique as well as to ascertain the size of the infecting dose for cattle. The mean and standard deviation for twelve separate titrations over a period of 87 days was $10^{5\cdot37\pm0\cdot19}$ TCD 50/g. Hence each animal received about $10^{4\cdot7}$ TCD 50 intranasally.

Collection of tissues

Animals were killed by shooting at intervals of 1–16 days after intranasal infection (Table 1). The neck vessels were severed and a sample of *blood* for viraemia study was collected into one-third the final volume of 1·5 % EDTA in 0·7 % sodium chloride (Plowright & Ferris, 1962). In fourteen instances (see Table 1) blood was also collected for the separation of serum. Cattle were allowed to bleed out as fully

as possible and then subjected to immediate systematic autopsy; in two cases, however (animals nos. 9287 and 9292; Table 1) death occurred spontaneously about 1 hr. before the post-mortem examination was begun.

Table 1. *Details of some cattle used in a study of rinderpest pathogenesis*

Animal no.	Incubation period*	Duration of pyrexia*	Day of max. temp.	Days to onset of		Day killed or died (D)	Titre† of serum
				Mouth lesions	Diarrhoea		
9284	4	N.D.	N.D.	N.D.	N.D.	4	N.D.
9296	N.D.	N.D.	N.D.	N.D.	N.D.	4	N.D.
9297	4	N.D.	N.D.	N.D.	N.D.	4	N.D.
9283	4	N.D.	N.D.	N.D.	N.D.	5	N.D.
9306	4	N.D.	N.D.	N.D.	N.D.	5	N.D.
9302	4	N.D.	N.D.	N.D.	N.D.	6	0·0
9305	4	N.D.	N.D.	N.D.	N.D.	6	N.D.
9308	4	N.D.	N.D.	N.D.	N.D.	6	N.D.
9304	4	N.D.	N.D.	7	N.D.	7	N.D.
9309	4	N.D.	N.D.	7	N.D.	7	N.D.
9303	5	N.D.	N.D.	8	N.D.	8	0·0
9307	4	N.D.	N.D.	6	8	8	0·0
9287	4	4	7	7	8	9 (D)	1·6
9301	4	N.D.	N.D.	7	9	9	0·6
9289	3	⩾ 8	8	7	9	10	0·8
9300	4	6	9	7	9	10	0·8
9292	4	8	8	7	9	12 (D)	0·8
9298	4	⩾ 9	8	8	8	12	2·2
9329	4	5	7	6	8	12	2·8
9326	5	6	7	8	8	14	2·6
9327	4	7	6	8	9	14	2·2
9288	5	5	8	7	9	16	1·8
9299	4	7	7	9	Absent	15	2·8
Range	3–5	4–9	6–9	6–9	8–9	—	—
Mean	4·1	6·5‡	7·5	7·3	8·5	—	—

* = Figures calculated from days with morning temperature ⩾ 102·0° F.
† = Log_{10} S.N.50 at time of death or destruction.
‡ = Median.
N.D. = Not determined.

One each of the following lymph nodes was excised intact with separate sterile instruments and placed in individual Petri dishes, viz. *submaxillary* (mandibular), *pharyngeal* (supra-pharyngeal), *prescapular* (posterior superficial cervical), *mesenteric* (jejuno-ileal) and *ileocolic* (colic). The designations in brackets are those advocated by Sisson & Grossman (1940). One or more *haemolymph* (haemal) nodes were dissected out cleanly from the surrounding tissues of the prescapular groove or axilla. *Spleen pulp* was excised from the centre of the organ, free from capsule and larger trabeculae.

After washing free surfaces carefully with running water, portions of the *palatal*

tonsil, free of surrounding muscle and connective tissue, were excised with a scalpel. Similarly, a strip of mucosa was carefully dissected from the *base of the tongue* in the region lying just behind the vallate papillae; this latter, together with the pharyngeal mucosa, is a predilection site for the necrosis and erosion which is such a characteristic feature of rinderpest. Every effort was made to exclude tongue muscle fibres from the mucosal strip but many lobules of yellowish orange, mucous glands were closely adherent to the deep side of the epithelium and could not be removed effectively.

Nasal mucosa was stripped from a part of the washed middle third of the dorsal turbinate bone; separation in this region occurred cleanly, as a result of traction following outlining of an area by a scalpel incision. *Lung* tissue was excised from the left cardiac lobe and *myocardium* from the region of the papillary muscles of the left ventricle, excluding the endocardium. *Liver* and *kidney* were taken free of capsular connective tissue and '*brain*' consisted of portions of a cerebral hemisphere from which the meninges had been removed.

The *abomasal* (*pyloric*) *mucosa* was dissected with scissors from the underlying connective tissue, within an area situated not more than 3 in. from the pyloric protuberance. The surface was first washed liberally with running water to remove as much as possible of the food material and secretions; this precaution was also applied to other mucosae of the alimentary tract.

Ileal, *caecal* amd *colonic mucosae* were scraped off with a scalpel when the gut wall was held tightly stretched to eliminate folding; by this means it was possible to avoid the inclusion of elements deep to the mucosa. In the ileum areas were chosen which did not include any Peyer's patch, in the caecum harvests were taken from the segment within 6 in. of the apex, while colonic mucosa was obtained from the region near the abrupt bend in the *ansa spiralis*, i.e. near the mid-point of the viscus.

Bone marrow was collected from a number of posterior sternebrae, freed so far as possible of the surrounding muscle and connective tissues.

Further treatment of tissues

The nasal mucosa was always washed with phosphate-buffered saline (P.B.S.) (Dulbecco & Vogt, 1954), to free it from adherent blood-clot; nothing else required washing. Quantities of *ca.* 0·5–1·5 g. of each solid tissue were weighed in Petri dishes, chopped with crossed scalpels or scissors and transferred to Ten Broeck grinders. Sufficient culture maintenance medium to make a 10 % (w/v) preparation was added and the materials were reduced to uniform suspensions by vigorous grinding, without the use of abrasives. The suspensions were poured off, leaving any trabecular or interstitial connective tissue trapped between the plunger and the cylinder of the grinders. Tongue and nasal mucosae, together with the lung, were the most difficult tissues to disperse adequately. The mucosae of the abomasum, ileum, caecum and colon were all processed within about 1 hr. of removal from the body. This was to reduce to a minimum any inactivation of virus by proteolytic enzymes such as pepsin and trypsin. Free rinderpest virus particles are very rapidly inactivated by the latter (Plowright, 1964).

Bone marrow cells were obtained from fragments of red marrow, excised with bone forceps from the bodies of the posterior sternebrae. These were shaken with about 40 ml. of P.B.S. in a rubber-stoppered tube and the resulting suspension was decanted through muslin into a centrifuge tube. After spinning at 1000 rev./min. for 5 min. the fat formed a compact, solid layer on top of the fluid and both these fractions were poured off, leaving the cells as a deposit. The latter was resuspended in a smaller quantity of P.B.S. and packed by centrifuging in a graduated tube for 5 min. at 1000 rev./min. The deposit was then redispersed in maintenance medium to give a 10% (v/v) suspension, which was found by weighing to be practically the same as a 10% (w/v) preparation.

Tenfold dilutions of the different tissue suspensions were prepared in culture maintenance medium, with vigorous pipetting at each stage to break up cell aggregates. The range of dilutions extended to a minimum of 10^{-3} and a maximum of 10^{-8} (w/v). Dilutions from 10^{-2} upwards could be introduced into tissue cultures without adverse affects; at least two and usually four to five dilutions were inoculated into calf kidney monolayers, of which five were employed per dilution, the inoculum being 2·0 ml. each.

Blood with EDTA was tested for the presence of viraemia by the separation of leucocyte fractions from 20 ml. samples (Plowright & Ferris, 1962). These fractions were resuspended in 10 ml. of maintenance medium and inoculated in 2 ml. quantities into each of five tube cultures. Titrations of blood were performed in the manner already described (Plowright & Ferris, 1962).

All materials were inoculated into tissue cultures within 3 hr. of harvesting from the animal and during any period of extended delay they were placed at 4° C.

Representative blocks, from all solid tissues which were titrated, were also fixed in Zenker-formol and embedded in paraffin-wax for later study of the histopathology.

Preparation and maintenance of cell cultures

Primary calf kidney monolayers were prepared as already described in tubes of 160 × 15 mm. dimensions (Plowright & Ferris, 1959). They were used for titrations when 4–10 days old, all medium being decanted before the introduction of a 2 ml. inoculum for each tube and transfer to drums rotating at about 8 rev./hr. All tissues from one animal were titrated in tube cultures of the same batch.

On the following day the inoculum was decanted off and each tube was washed twice with 2 ml. of P.B.S. (Dulbecco & Vogt, 1954). The maintenance medium was then replaced, using 1 ml. only per tube and the cultures were returned to roller drums. Subsequent changes of medium were carried out on the 3rd, 5th and 7th days after inoculation.

Maintenance medium was as already detailed (Plowright & Ferris, 1959), with the addition of the following antibiotics per ml.: sodium penicillin, 100 units; streptomycin sulphate, 100μg.; neomycin sulphate, 25μg. and kanamycin sulphate, 50μg. No trouble was experienced with bacterial or fungal contaminants, even in titrations of mucosae of the alimentary or respiratory tracts. No cytopathic agent, other than rinderpest virus, was detected in any tissue.

Microscopic examination for the typical cytopathic effects of virulent strains of rinderpest virus (Plowright & Ferris, 1959, 1962) were carried out at intervals from the 3rd to the 9th days, final readings being taken on the latter day. Titres, expressed as $\log_{10}$ TCD 50/g. of solid tissues or per ml. of blood, were calculated by the method of Thompson (1947).

Evaluation of the results

The tissue suspensions from which tenfold dilutions were made consisted of viable and dead cells, either singly or in clumps, together with tissue fluids and blood. They therefore contained free virus and 'infective centres' which may have contained or released many more than one infectious particle. Some evidence that ultrasonic treatment released many infective particles from some cells in tissue suspensions was mentioned by Plowright (1962).

It might have been expected that titration results would have been irregular but in practice this was not the case. With the exception of a few tissues taken from animals 12 days after infection, reference to which will be made again later, titrations were as regular in their outcome as those performed with centrifuged culture fluids. The accuracy of replicate titrations of a tissue in different batches of calf kidney cells was shown by the small standard deviation observed in titrations of the inoculum for cattle (see above).

Since the minimum dilution inoculated into cultures was 10^{-2} and of this five tubes received 2 ml. each, the usual weight of solid tissues tested for the presence of virus was 0·1 g. and a nil result meant that virus was not detected in this amount of material. Negative outcome in the tests for detection of viraemia implied that virus was not present in *ca.* 13 ml. of blood.

An advantage of the use of inocula containing viable cells, whether from blood or solid tissues, was that it allowed the detection of virus in animals which had probably possessed circulating antibody for several days. It is doubtful if this infectivity would have been detected in cell-free suspensions. The possibility must also be mentioned that some of the first recoveries of virus, as from the cephalic lymph nodes, might have been accounted for by infected cells which were still in the phase of viral eclipse. This infectivity would also not have been demonstrable in cell-free extracts.

Serum neutralization tests

As shown in Table 1, quantitative neutralization tests were carried out on the sera of fourteen animals killed on the 6th to 16th days after infection. The technique employed was that described by Plowright & Ferris (1961) and all sera were tested against a 10^{-2} dilution of the same virus stock, providing a mean of $10^{2\cdot4}$ TCD 50 per tube.

RESULTS

Clinical signs in infected cattle

Table 1 gives some details for twenty-three cattle which were killed or died after the onset of fever. It will be seen that the incubation period varied from 3 to 5 days but that the majority of animals (19/23) showed a temperature reaction on

the 4th morning. The peak of the pyrexia was reached on the 6th–9th days after inoculation and the duration of fever was 4–9 days (median 6·5) in the ten animals which survived long enough for observations to be completed.

Mouth lesions, taking the form of focal necrosis and erosion of the epithelia and similar to those already described (Liess & Plowright, 1964), appeared on the 6th–9th days (mean 7·3) after infection, i.e. on the 3rd–6th days of the disease. Diarrhoea or dysentery was somewhat more delayed to 8 or 9 days (mean 8·5) after infection; it was absent in only one of the twelve animals which survived for an adequate time. Only two of the animals died, one on the 9th, and the other on the 12th day (see Table 1).

On the basis of these findings and those reported previously (Liess & Plowright, 1964), it was decided to subdivide the clinical course of the infection into four phases, these being successively:

(*a*) The incubation period, lasting about 4 days from the time of intranasal infection, and terminated by the first rise of temperature to 102° F.

(*b*) The prodromal phase, normally ending on the 7th day after infection with the appearance of mouth lesions. Its duration was thus approximately 3 days and the temperature of individual animals usually reached its maximum towards the end of this time or at the beginning of the next period. Other clinical signs during this phase included sero-mucoid ocular and nasal discharges, becoming muco-purulent later. Animals normally continued to eat but often became dull and apathetic.

(*c*) The mucosal phase, extending from the 8th to the 12th days. It was characterized at first by the increasing extent of mouth lesions and a little later by the sudden onset of diarrhoea or dysentery. Rectal temperatures declined and deaths often occurred on the 9th to 12th days. Animals which survived the 12th day after infection usually recovered and virtually all their temperatures had returned to normal by this time (see Table 1 and Liess & Plowright, 1964). Towards the end of this phase diarrhoea had often ceased or become less severe and mouth lesions were beginning to heal.

(*d*) The early convalescent phase, not clearly delineated but for the present purpose assumed to extend from the 13th to the 16th days, inclusive. Diarrhoea ceased during this period and mouth lesions were obliterated by rapid reconstitution of the epithelia.

It is not intended to claim that the figures proposed here for the duration of these phases have any strict, general application; they are reasonably accurate, but only in relation to the particular strain of virus and type of cattle employed in these experiments. In the description which follows, virological events will be correlated where possible with the four clinical phases.

Incubation period

Detailed titration figures for nine cattle killed on the first 4 days after infection are given in Table 2 and mean values for various tissues are shown in Figs. 1–6. One of two animals killed after 24 hr. (no. 9137) had virus in small quantities in a pharyngeal lymph node, but otherwise no infectivity was detected in either of

them. At 48 hr. one ox (no. 9135) showed appreciable amounts of virus in its cephalic lymph nodes and tonsil, while generalization had already occurred, as shown by a very low viraemia and by the presence of virus in a prescapular lymph node and spleen. The other animal (no. 9290) had virus in a submaxillary lymph node only. The difference between the site of primary proliferation in cattle nos. 9137 and 9290 suggested that virus could be absorbed and transported from the nasal mucosa to either the pharyngeal or submaxillary nodes. Such a deduction would not conflict with the known sources of their afferent vessels, which include the posterior part of the nasal cavity for the pharyngeal and the anterior part of the turbinates and *septum nasi* for the submaxillary nodes (Sisson & Grossman, 1940).

Table 2. *Titrations of the tissues of cattle killed on the first 4 days after infection*

Time after infection ...	1 day		2 days		3 days		4 days		
Cattle no.	9137	9291	9135	9290	9295	9360	9296	9297	9284
Submaxillary lymph node	0·0	0·0	2·2	2·2	N.T.	4·0	⩾ 6·2	6·6	6·6
Pharyngeal lymph node	2·2*	0·0	4·0	0·0	4·4	3·2	⩾ 6·2	7·2	6·2
Tonsil	0·0	0·0	3·4	0·0	5·4	4·0	⩾ 6·2	6·8	7·2
Prescapular lymph node	0·0	0·0	2·4	0·0	3·8	1·6	4·4	4·8	4·4
Mesenteric lymph node	N.T.	0·0	N.T.	0·0	3·0	0·0	4·8	5·8	4·8
Ileocolic lymph node	N.T.	N.T.	N.T.	N.T.	3·2	0·0	⩾ 5·2	4·8	5·6
Prescapular haemo-lymph node	N.T.	N.T.	N.T.	0·0	N.T.	1·8	⩾ 5·2	4·4	5·8
Spleen	0·0	0·0	2·2	0·0	4·0	3·0	⩾ 5·2	4·4	4·8
Blood	0·0	0·0	Tr.†	0·0	Tr.	Tr.	1·6	0·6	⩾ 2·2
Bone marrow	N.T.	N.T.	N.T.	N.T.	Tr.	Tr.	2·4	Tr.	2·2
Liver	N.T.	N.T.	N.T.	0·0	0·0	0·0	2·0	0·0	2·6
Kidney	N.T.	N.T.	N.T.	0·0	0·0	0·0	0·0	Tr.	0·0
Myocardium	N.T.	N.T.	N.T.	N.T.	N.T.	0·0	N.T.	N.T.	0·0
Brain	N.T.	N.T.	N.T.	N.T.	N.T.	0·0	N.T.	N.T.	0·0
Nasal mucosa	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Lung	N.T.	N.T.	N.T.	N.T.	2·0	0·0	2·0	0·0	2·4
Tongue mucosa	0·0	0·0	0·0	0·0	0·0	0·0	⩾ 4·2	3·6	5·4
Pyloric mucosa	N.T.	N.T.	N.T.	N.T.	0·0	0·0	2·8	0·0	3·2
Ileal mucosa	N.T.	N.T.	N.T.	N.T.	0·0	0·0	Tr.	0·0	2·8
Caecal mucosa	N.T.	N.T.	N.T.	N.T.	2·6	1·6	⩾ 4·2	3·0	4·2
Colonic mucosa	N.T.	N.T.	N.T.	N.T.	N.T.	Tr.	N.T.	3·2	3·4

* Titre in $\log_{10}$ TCD 50/g. or per ml. (blood). Where 2 or more tubes were infected with a 10^{-2} dilution of solid tissues it was assumed that all cultures would have been infected with a 10^{-1} dilution.

† Tr. = Trace, i.e. one to three cultures were infected out of five inoculated with leucocyte fractions from blood, or one out of five inoculated with a 10^{-2} dilution of solid tissues.

N.T. = not tested.

On the 3rd day virus in considerable quantities was present in the cephalic nodes and tonsils of both animals killed. There was a low-level viraemia, only just

detectable in leucocyte fractions from about 13 ml. of blood, while virus had begun to proliferate in the spleen and prescapular lymph nodes. It was also present in minimal quantities in the bone marrow and a haemolymph node of ox no. 9360. It was surprising to find that virus was already detectable in the caecal mucosa of both animals and in the colonic mucosa of one; the relatively late onset of diarrhoea had erroneously led to the expectation that virus would probably not reach these tissues until a relatively late stage of the infection. Visceral lymph nodes were infected in ox no. 9295 only and this animal also showed localization in the lung.

At this point it became clear that virus was not detectable in the nasal or tongue mucosae during the first 3 days after infection. This was in spite of the demonstration of a low-level viraemia in three of the six animals and the use of a large initial inoculum, part of which might have persisted in the nasal mucosa. No macroscopic abnormalities were noted in the latter and a preliminary examination of fixed and stained sections of turbinate bone also failed to reveal significant changes.

Of three cattle killed on the 4th day, two were already febrile, whereas the other, no. 9296, had not yet reacted. All three animals showed large amounts of virus in the cephalic lymph nodes and tonsils, titres varying between $10^{6\cdot2}$ and $10^{7\cdot2}$ TCD 50/g. There were also considerable but lower levels of infectivity in the prescapular and visceral lymph nodes, the spleen and haemolymph nodes and in the mucosa of the base of the tongue. Since the latter contains moderate numbers of large, aggregated lymphoid follicles, it was impossible in this experiment to determine whether the increase of virus was due to proliferation in these structures and/or in the surface epithelium.

In two cattle the level of viraemia had increased markedly and, possibly associated with this, measurable quantities of virus appeared in the bone marrow, liver and lung. The third animal, no. 9297, had a lower viraemia and virus was either absent or present in trace quantities in these tissues; it also showed a minimal quantity of virus in the kidney. Virus was proliferating in the caecum, probably also in the colonic mucosa of all animals, while in two of them (excluding no. 9297) it had appeared for the first time in the pyloric and ileal mucosae. The nasal mucosa was still negative.

Comment and conclusions on virological events during the incubation period

Summarizing the results for the incubation period it can be stated that virus in the inoculum almost certainly passed through the nasal mucosa without proliferating and without causing a local lesion. It was transferred to the associated pharyngeal or submaxillary lymph nodes, where it began to multiply within 24–48 hr. Some virus, probably in the form of infected cells, spilled over into the efferent lymph stream and gave rise to a low-level viraemia on the 2nd to 3rd days after infection. Secondary foci of proliferation were quickly established in the superficial and visceral lymph nodes, in the spleen and haemolymph nodes and in mucosal lymphoid follicles, such as those of the tongue and probably Peyer's patches. Finally, virus began to multiply in the mucosae of the alimentary tract,

beginning with the large intestine. The onset of fever was probably associated with the simultaneous proliferation of virus at many scattered sites and with its release in moderate quantities into the blood stream.

The prodromal period (days 5–7 inclusive)

From the beginning to the end of this phase virus titres in the cephalic lymph nodes and tonsils maintained very high levels, usually between $10^{7\cdot0}$ and $10^{8\cdot0}$ TCD 50/g. (Fig. 1 and Table 3). Titres of virus in the prescapular and visceral nodes were commonly lower by 1–2 log units, so that they exceeded $10^{6\cdot6}$ in only three of twenty-one instances. This was reflected in the depression of mean values shown graphically in Figs. 2 and 3.

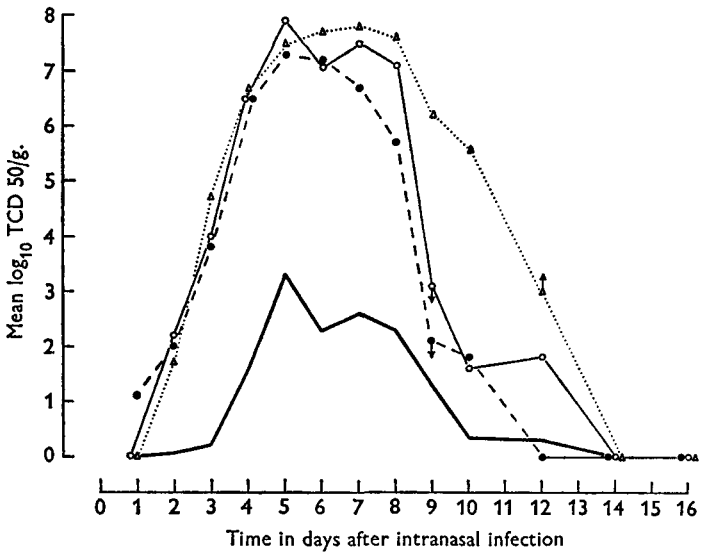

Fig. 1. Proliferation of virulent rinderpest virus, strain RGK/1, in lymphoid tissues of the head of cattle: correlation with viraemia. ○—○, Submaxillary lymph nodes; ●– –●, pharyngeal lymph nodes; △. . . . . △, palatal tonsil; ——, blood.

The level of virus in the spleen was lower still, a peak mean titre of $10^{6\cdot2}$ TCD 50/g. being attained on the 5th day after infection. The latter was also, incidentally, the day of peak viraemia (mean $10^{3\cdot3}$ TCD 50/ml., see Fig. 2). The haemolymph nodes developed considerably more virus than the spleen, to which they have many structural similarities (Trautmann & Fiebiger, 1952); titres on the 6th and 7th days were always $10^{7\cdot0}$ or higher. Fig. 2 shows quite clearly that virus proliferation commenced earlier in the spleen than in the haemolymph nodes and also began to decline sooner.

Mean figures for virus in the bone marrow (Fig. 2) did not exceed those for blood until the 6th day and the maximum was attained on the 7th day. Individual titres during this period varied widely from $10^{1\cdot8}$ to $10^{5\cdot2}$ TCD 50/g., being usually 1·2–2·2 log units lower than those for spleen. The tongue mucosa, with its associated lymphoid follicles, maintained a plateau of mean values around $10^{6\cdot0}$ during the

Table 3. *Titrations of the tissues of cattle killed on the 5th to 7th days after infection*

Time after infection	5 days		6 days			7 days	
Animal no.	9306	9283	9308	9305	9302	9309	9304
Submaxillary lymph node	⩾ 8·2*	7·6	6·6	7·6	7·2	8·0	7·0
Pharyngeal lymph node	7·2	7·4	7·4	7·0	7·2	7·0	6·4
Palatal tonsil	7·8	7·2	7·8	7·6	7·8	8·0	7·6
Prescapular lymph node	6·4	⩾ 8·2	5·4	6·2	6·6	6·4	6·8
Mesenteric lymph node	6·2	6·6	5·8	6·4	7·2	6·6	6·4
Ileocolic lymph node	6·8	6·2	5·9	5·8	5·6	7·2	6·2
Prescapular haemolymph node	6·7	6·9	7·0	7·2	7·6	7·8	7·6
Spleen	6·2	6·2	4·4	5·4	5·8	6·4	5·6
Blood	3·2	3·4	1·6	2·4	3·0	3·0	2·2
Bone marrow	1·8	4·8	2·5	4·0	3·6	5·2	3·6
Liver	3·4	4·0	0·0	3·2	4·6	4·0	3·2
Kidney	1·8	2·6	0·0	2·2	0·0	3·2	Tr.
Brain	N.T.	N.T.	N.T.	N.T.	0·0	1·8	0·0
Myocardium	N.T.	0·0	0·0	0·0	0·0	Tr.†	0·0
Nasal mucosa	2·4	2·6	0·0	5·0	1·6	5·0	2·6
Lung	⩾ 4·2	⩾ 4·8	< 2·4	6·0	5·6	5·4	5·4
Tongue mucosa	6·2	6·6	5·8	6·6	5·4	7·0	5·8
Pyloric mucosa	4·8	4·8	3·4	4·0	4·2	5·2	4·6
Ileal mucosa	4·0	4·8	< 2·4	4·8	5·6	4·6	4·2
Caecal mucosa	5·4	5·0	4·0	5·8	5·4	6·6	5·6
Colonic mucosa	4·6	4·6	⩽ 2·4	5·4	4·6	5·4	5·0

* Titre in $\log_{10}$ TCD 50/g. or per ml. (blood). Where two or more tubes were infected with a 10^{-2} dilution of solid tissues it was assumed that all cultures would have been infected with a 10^{-1} dilution.

† Tr. = Trace, i.e. one to three cultures were infected out of five inoculated with leucocyte fractions from blood, or one out of five inoculated with a 10^{-2} dilution of solid tissues.

N.T. = not tested.

whole of the prodromal phase (Fig. 4); in different animals titres ranged rather narrowly between $10^{5\cdot4}$ and $10^{7\cdot0}$.

All the mucosae of the gastro-intestinal tract supported a considerable multiplication of virus, with mean titres, except in the case of the pylorus, reaching a maximum towards the end of the period. There was an unexpected gradient in the amount of infectivity demonstrable in the intestinal mucosae; the caecum almost invariably showed the highest titres, followed by the colon and then by the ileum. This is clearly shown by the mean lines for these tissues, presented in Fig. 4. With one exception (ox no. 9308), individual figures for the caecum ranged from $10^{5\cdot0}$ to $10^{6\cdot6}$, for the colon from $10^{4\cdot6}$ to $10^{5\cdot4}$, for the ileum from $10^{4\cdot0}$ to $10^{5\cdot6}$ and for the pylorus from $10^{4\cdot0}$ to $10^{5\cdot2}$.

The exceptional animal, no. 9308, which was killed on the 6th day after infection, showed good average titres for its lymphoid tissues but very small quantities of virus in the pyloric, ileal and colonic mucosae. It also had a low-level viraemia and little or no infectivity in its liver, lung and nasal mucosa, whereas other animals killed during this period commonly showed considerable amounts of virus in these tissues. It is possible that this ox was one which would have rapidly controlled

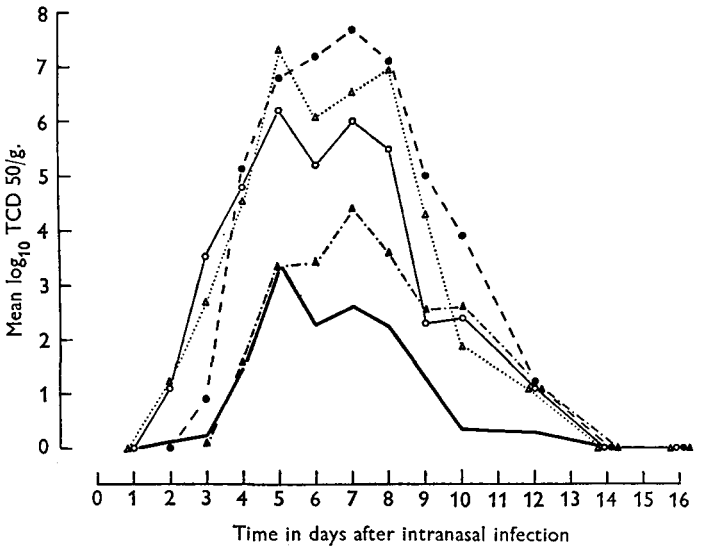

Fig. 2. Proliferation of virulent rinderpest virus, strain RGK/1, in the haemopoietic tissues of cattle: correlation with viraemia. ○—○, Spleen; ●– –●, prescapular haemolymph nodes; △. . . . . △, prescapular lymph nodes; ▲–·–▲, bone marrow; ——, blood.

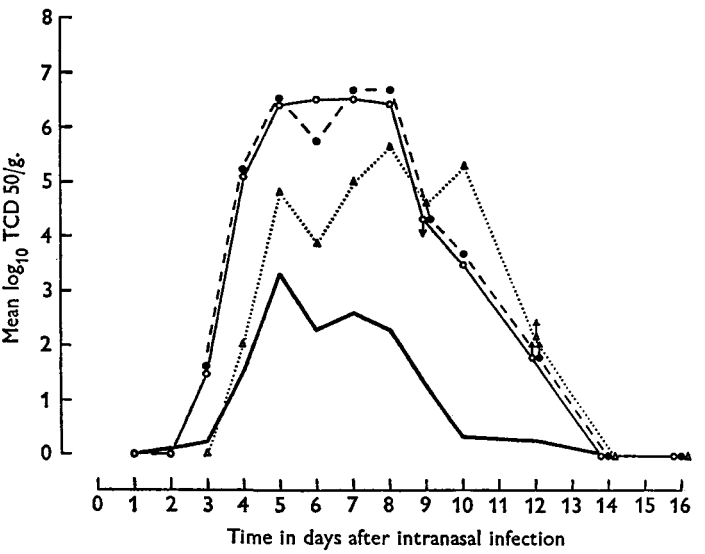

Fig. 3. Proliferation of virulent rinderpest virus, strain RGK/1, in the alimentary tract of cattle I: correlation with viraemia. ○—○, Mesenteric lymph nodes; ●– –●, ileocolic lymph nodes; ▲ . . . . ▲, pyloric mucosa; ——, blood.

virus proliferation by antibody production and thus recovered quickly after showing only mild clinical signs.

In the respiratory tract, virus first became detectable in the nasal mucosa on the 5th day. With the exception of ox no. 9308, already mentioned, all animals thereafter showed some virus but in greatly varying quantities. Thus individual titres ranged from $10^{2\cdot4}$ to $10^{5\cdot0}$ TCD 50/g.; these figures were surprisingly low and it was

only on the 7th day that the mean titre began to exceed that of the blood (Fig. 5). The lung tissue contained considerable quantities of virus in the absence of any macroscopic pathological changes. Titres on the 6th and 7th days, again with the exception of ox no. 9308, varied between $10^{5\cdot4}$ and $10^{6\cdot0}$, being always higher than those for the nasal mucosa, often by a wide margin (Fig. 5).

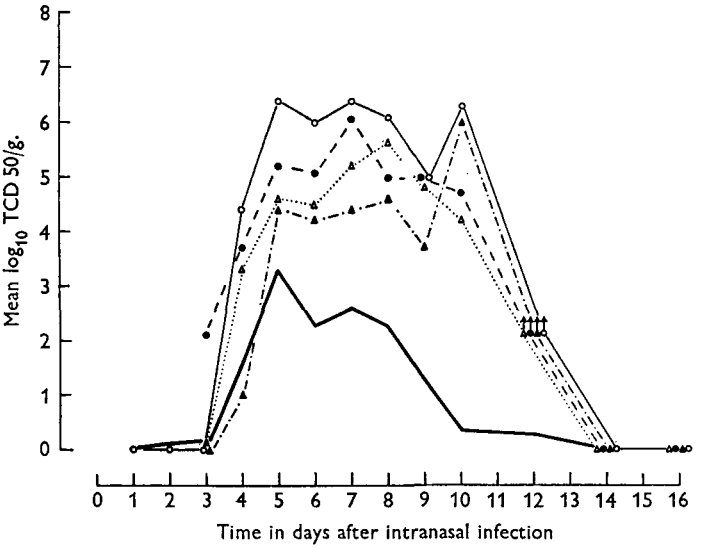

Fig. 4. Proliferation of virulent rinderpest virus, strain RGK/1, in the alimentary tract of cattle II: correlation with viraemia. ○—○, Mucosa of tongue; ●– –●, mucosa of caecum; △ . . . △, mucosa of colon; ▲–·–▲, mucosa of ileum; ——, blood.

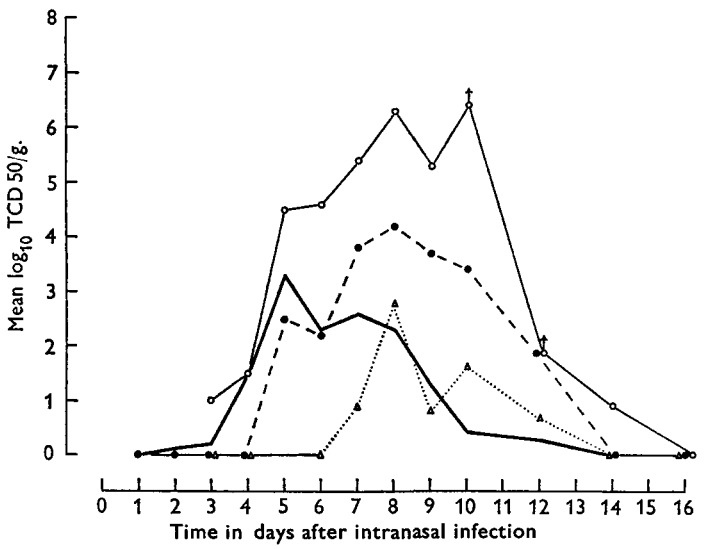

Fig. 5. Proliferation of virulent rinderpest virus, strain RGK/1, in the respiratory tract and brain of cattle: correlation with viraemia. ○—○, Lung; ●– –●, nasal mucosa; △ . . . . . △, brain; ——, blood.

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Virus in the liver of individual animals increased considerably during the prodromal phase and exceeded the blood level by 0·6 to 1·6 log units on the 6th and 7th days; individual titres ranged from $10^{3·2}$ to $10^{4·6}$ TCD 50/g. (again excluding ox no. 9308). In the kidney virus was not always present and titres were invariably low during this period (see Fig. 6). No infectivity was demonstrable in the myocardium, with the exception of a trace in one animal on the 7th day. The same state of affairs was also found in the brain (Fig. 5).

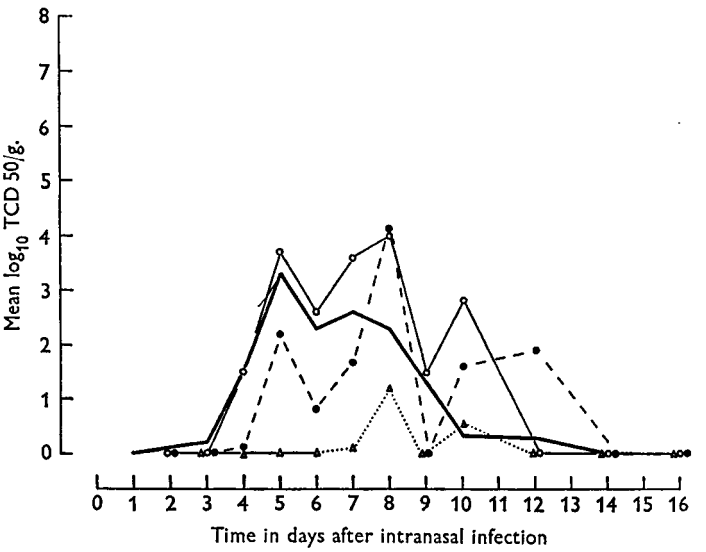

Fig. 6. Proliferation of virulent rinderpest virus, strain RGK/1, in the parenchymatous organs of cattle: correlation with viraemia. ○—○, Liver; ●– –●, kidney; △ . . . . . △, heart; ——, blood.

Comment and conclusions on virological events during the prodromal period

The prodromal period was, in general, characterized by a plateau of high virus titres in the lymphopoietic and lympho-epithelial tissues, with a gradient descending from the cephalic lymph nodes and tonsils to the superficial body nodes and haemolymph nodes, finally to the visceral lymph nodes and spleen. The level of viraemia was consistently high and reached a peak during this phase, while the bone marrow supported maximal virus proliferation towards its conclusion. Gastro-intestinal mucosae showed high virus contents throughout, peak values being reached towards the end of the period or early in the next phase; again there was a gradient, descending this time from caecum to colon, to ileum and pylorus.

Virus first appeared in the turbinate mucosa on the 5th day after infection and increased steadily in quantity during this period. Titres were, however, very variable and generally low. Virus in the lung increased slowly throughout and titres consistently exceeded those of the nasal mucosa. Although some local proliferation or accumulation of virus was probably demonstrated in the liver,

Table 4. *Titrations of the tissues of cattle harvested on the 8th to 12th days after infection*

Time after infection	8 days		9 days		10 days		12 days		
Cattle no. ...	9307	9303	9301	9287	9300	9289	9292	9298	9329
Submaxillary lymph node	7·0*	7·2	⩽ 3·2	3·0	⩽ 3·2	0·0	3·6†	0·0	1·8
Pharyngeal lymph node	4·0	7·4	⩽ 3·2	0·0	3·6	0·0	0·0	0·0	0·0
Palatal tonsil	7·0	⩾ 8·2	6·4	6·0	7·4	3·8	⩾ 6·2	2·8†	0·0
Prescapular lymph node	6·0	8·0	3·6	5·0	3·8	0·0	3·2†	0·0	Tr.†
Mesenteric lymph node	6·0	6·8	5·4	⩾ 3·2	7·0	0·0	⩾ 5·2	0·0	Tr.
Ileocolic lymph node	6·2	7·2	5·0	3·6	5·0	2·4	⩾ 5·2	0·0	0·0
Prescapular haemo-lymph node	6·4	7·8	4·2	5·8	7·8	0·0	0·0	3·6†	0·0
Spleen	4·8	6·2	2·4	2·2	4·8	0·0	3·2†	0·0	0·0
Blood	1·6	3·0	1·8	0·8	0·6	Tr.	0·8	0·0	0·0
Bone marrow	2·8	4·4	2·9	2·2	3·0	2·4	3·4	0·0	0·0
Liver	3·8	4·2	3·0	0·0	2·8	2·8	Tr.	0·0	0·0
Kidney	⩾ 4·2	4·0	0·0	0·0	3·2	0·0	2·8†	2·8	0·0
Myocardium	2·4	0·0	0·0	0·0	Tr.‡	0·0	0·0	0·0	0·0
Brain	2·8	2·8	1·6	0·0	3·2	Tr.	3·0	0·0	0·0
Nasal mucosa	4·0	4·4	4·2	3·2	3·2	3·6	3·8	1·8	0·0
Lung	6·4	6·2	6·6	4·0	⩾ 7·2	5·6	⩾ 5·2	0·0	Tr.†
Tongue mucosa	6·0	6·2	5·4	4·6	6·6	6·0	⩾ 6·2	0·0	0·0
Pyloric mucosa	5·2	6·0	5·0	4·2	6·6	4·0	⩾ 6·2	0·0	0·0
Ileal mucosa	4·8	4·4	4·8	2·6	7·0	4·9	⩾ 6·2	0·0	0·0
Caecal mucosa	5·8	6·2	5·8	4·2	6·4	3·0	⩾ 6·2	0·0	0·0
Colonic mucosa	5·2	6·0	5·2	4·4	5·2	3·2	⩾ 6·2	0·0	0·0

* Titre in $\log_{10}$ TCD 50/g. or per ml. (blood). Where two or more tubes were infected with a 10^{-2} dilution of solid tissues it was assumed that all cultures would have been infected with a 10^{-1} dilution.

† Irregular titration result, probably due to the simultaneous presence of virus and antibody.

‡ Tr. = Trace, i.e. one to three cultures were infected out of five inoculated with leucocyte fractions from blood, or one out of five inoculated with a 10^{-2} dilution of solid tissues.

there was no clear indication that it did so in the kidney during the prodromal period. The myocardium did not support virus multiplication.

The mucosal phase (days 8–12 inclusive)

This period of the disease, characterized clinically by the further development of mouth lesions and by the onset of diarrhoea and dysentery, began with very high levels of virus in all the major sites of proliferation, but these declined rapidly with the appearance of circulating antibody on the 9th day after infection (see Tables 1 and 4).

In the lymphopoietic tissues mean titres were always high on the 8th day (Figs. 1–3 inclusive) but, as shown in Table 4, a pharyngeal lymph node of ox no. 9307 already had a titre depressed to $10^{4\cdot0}$ TCD 50/g., whereas the submaxillary node

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and tonsil contained 1000-fold more infectious virus ($10^{7\cdot0}$). The tendency for irregularity in the virus content of lymphoid tissues became more marked on the 9th and 10th days after infection, when the infectivity demonstrable in the cephalic lymph nodes fell dramatically, while titres in the visceral nodes remained considerable in two of four instances (cattle nos. 9301 and 9300); appreciable amounts of virus were also recovered from the prescapular nodes in three of four animals killed on the same days. It appeared, therefore, that infectivity began to decline rapidly in different lymph nodes in roughly the same sequence as it made its appearance.

Table 5. *Some anomalous results for the titrations of tissues from ox no.* 9292, *killed on day* 12 *post-infection*

Tissue	Number of cultures with cytopathic effects at tissue dilution				Estimate of titre
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	
Submaxillary lymph node	1/5	5/5	2/5	0/5	$10^{3\cdot6}$/g.
Prescapular lymph node	2/5	3/5	2/5	0/5	$10^{3\cdot2}$/g.
Spleen	4/5	3/5	2/5	0/5	$10^{3\cdot2}$/g.

On the 12th day results were made irregular by a series of unexpectedly high figures in one animal (no. 9292) from which tissues were harvested immediately after its death. Otherwise, it will be seen from Table 4 that virus was only detectable in minimal quantities, if at all, in the lymphoid tissues and titration results for all three individuals were sometimes anomalous. Detailed results for some of the irregular titrations of tissues of ox no. 9292 are given in Table 5. The wide range of tenfold dilutions giving less than 100% infection was never observed for tissues harvested before the 12th day and unfortunately rendered accurate calculation of 50% end-points impossible. The figures in the final column of Table 5 were estimated from the proportion of tubes infected with 10^{-3} and 10^{-4} dilutions; it was assumed that all cultures inoculated with 10^{-2} dilutions would have yielded virus if it had not been for the effect of neutralizing antibody, or possibly interferon, either present at the time of inoculation or produced afterwards by viable cells included in the suspensions. The visceral lymph nodes of ox no. 9292 showed very high titres, probably in excess of $10^{7\cdot0}$ TCD50/g. and associated with comparable levels of virus in the intestinal mucosae (see below). The amount of virus in the tonsil was similarly very high.

Virus in the spleen had already begun to decline seriously on the 9th day, being absent from one of two animals killed on the 10th day, and from two of three on the 12th. Considerable amounts of infectivity persisted to the 9th day in the haemolymph nodes but had disappeared from one of two animals killed on each of the 10th and 12th days. Viraemia had declined to very low levels by the 10th and could not be demonstrated in two of three animals on the 12th day. As shown in Table 1, all animals killed or dying on the 9th day and later had measurable amounts of circulating antibody; hence, all infectivity in the blood must have been intracellular. Bone marrow titres declined steadily during this phase and virus

had disappeared from two of the three cattle killed on the 12th day. Its behaviour in this tissue was, therefore, very similar to that in the blood and spleen.

The behaviour of virus in the tonsil and tongue mucosa was somewhat different from that in the lymph nodes, since titres often remained high up to the 10th day. These tissues contain epithelial and lymphoid elements in both of which the virus produces cytopathic effects and therefore, presumably, multiplies. Predominantly epithelial tissues, such as the mucosae of the gastro-intestinal tract, also retained considerable amounts of infectious virus up to the 10th day. Excluding ox no. 9292, which died, virus had disappeared from the mucosae of the gastro-intestinal tract in two animals killed on the 12th day after infection. In one of the two, no. 9298, small amounts of infectivity were still recoverable from the lympho-epithelial tissue of the tonsil, whereas it had disappeared in the other. Ox no. 9292 had virus to extremely high titre in its intestinal mucosae; from the time to appearance of cytopathic effects in cultures inoculated with 10^{-6} (w/v) dilutions, it was estimated that titres for the ileal, caecal and colonic mucosae were probably of the order of $10^{7.5}$ to $10^{8.0}$ TCD 50/g.

In the nasal mucosa virus titres remained at moderately high levels ($10^{3.2}$ to $10^{4.4}$) up to and including the 10th day (Fig. 5). By the 12th, infectivity had disappeared from one of three animals, but was still fairly high ($10^{3.8}$) in ox no. 9292 and minimal ($10^{1.8}$) in the third (Table 3). Lung titres were consistently high, up to and including the 10th day; thereafter there was a rapid fall; only ox no. 9292 had appreciable amounts of virus in the lung on the 12th day, the titre probably exceeding $10^{6.0}$ TCD 50/g.

Liver and kidney both attained peak virus contents of about $10^{4.0}$ TCD 50/g. on the 8th day, but afterwards isolations were irregular and titres remained low, not exceeding $10^{3.0}$ (see Fig. 6). Two of three animals showed virus in the kidney on the 12th day. Recovery of infectivity from the myocardium was very irregular, the maximum titre being recorded on the 8th day ($10^{2.4}$ TCD 50/g. in ox no. 9307). All samples were negative on and after the 10th day (Fig. 6). In the brain a mean titre of $10^{2.8}$ was attained on the 8th day but thereafter recovery was irregular and the highest individual figure was $10^{3.2}$ TCD 50/g. in ox no. 9300 (day 10). One animal only (no. 9292) had virus in the brain on the 12th day.

Comment and conclusions on the virological events during the mucosal phase

On day 8, at the beginning of this stage of the disease, the virus content of all tissues was near maximal but within a very short time there were definite indications of a decline in titre, especially in those lymphopoietic tissues which had borne the brunt of the first virus attack. High titres were maintained longer—up to the 10th day—in tissues where the virus was probably proliferating in epithelial cells, e.g. the tonsils, tongue mucosa, lungs and gastro-intestinal mucosae. Nevertheless, by the 12th day considerable amounts of infectivity remained in only one animal of three examined and this had died with very severe clinical and pathological manifestations. The course of events in this ox (no. 9292) may have been determined by its slower development of neutralizing antibody, since the serum

titre ($\log_{10}$ S.N. 50) at death was only $10^{0.8}$ compared with $10^{2.2}$ and $10^{2.8}$ for cattle nos. 9298 and 9329, which were killed on the same day.

A factor which was probably of great significance in the decline of virus titres during this phase was the destruction of susceptible cells; thus, preliminary histological examination of lymphoid tissues showed an extreme degree of degeneration and depletion of cells of the lymphocytic series. Similar changes, but of less intensity, were seen in surface and glandular epithelia of the alimentary tract, including the tongue and palatal tonsil (Plowright, unpublished). The successive multiplication of the virus in the cephalic, followed by the body lymph nodes and spleen, then by the epithelia, would be likely to result in a similar sequence of exhaustion of cells capable of supporting virus proliferation.

In addition, the production and circulation of neutralizing antibody, perhaps also of interferon, could obviously have impeded the cell-to-cell transfer and even intracellular multiplication of the virus. The majority or all of the infectivity which was demonstrated on the 12th day was almost certainly intracellular and would not have been detected if cell-free extracts of tissues had been employed as inocula. It also seems logical to suppose that antibodies would have appeared first in significant concentration in those lympho-reticular structures which were first exposed to virus antigens. Since these were the cephalic lymph nodes it was not, therefore, surprising to find that an irregular but usually precipitous fall of virus titre occurred first in these situations.

The early convalescent period (*days* 13 *to* 16 *inclusive*)

Only four animals were killed during this period, two on the 14th and one each on the 15th and 16th days after infection. From a total of eighty-four tissues which were titrated, virus was recovered from only one, the lung of ox no. 9326. The titre was minimal, only a proportion of tubes being infected by 10^{-2} and 10^{-3} dilutions; the only lesion observed in the positive lung was an interstitial emphysema, which is common in animals autopsied at this period.

Failure to recover virus from the mucosae of the gastro-intestinal tract was somewhat surprising since no. 9326 still had severe, watery diarrhoea at the time of destruction and adherent necrotic deposits and/or unhealed erosions were still present in foci near the pylorus, on the Peyer's patches or in the caecum and colon of all 4 animals.

As shown in Table 1, all cattle had acquired high levels of circulating antibody by the 14th to 16th day after infection, log S.N. 50 titres ranging from $10^{1.8}$ to $10^{2.8}$.

Comment and conclusions on virological events during the early convalescent period

By the 14th day after infection virus had virtually disappeared from all the tissues examined. It is possible that small 'pockets' of virus do commonly persist to this time in situations where they are not exposed to antibody in significant quantities. In the lung, for example, this may include foci of tissue isolated by emphysematous bullae or virus-containing cells in aggregates within the bronchioli. In the gastro-intestinal tract, widespread necrosis and erosion of epithelia presum-

ably leads to the passage of dead and infected cells into the lumen, accompanied by fluid exudates containing antibody; hence virus would have little opportunity for prolonged persistence.

DISCUSSION

So far as is known to us, no systematic studies have ever been reported previously on the quantity of infectious rinderpest virus contained in different tissues of cattle at various intervals after infection. Such information as existed was usually fragmentary and acquired in the course of attempts to produce inactivated tissue vaccines of the greatest possible efficacy, consistent with economy.

Jacotot (1931), for example, killed cattle at the height of fever, 6–8 days after infection and titrated their tissues in calves and goats. He found that dilutions of abomasal mucosa were infective up to 1/300,000, whereas tracheal, buccal and vulvo-vaginal mucosae or skin were not usually infective beyond a 1/25,000 to 1/50,000 dilution; liver, kidney, blood, spleen, lymph nodes, thymus, tonsil and thyroid did not usually exceed 1/25,000 to 1/50,000, while lung failed to infect at a dilution of 1/50,000. Walker *et al.* (1946) stated that 1/10,000 dilutions of the lymph nodes, spleen and lungs of calves, killed on the 2nd or 3rd day of fever, were infective to cattle. Bergeon (1952) studied bone marrow, brain and spinal cord from cattle similar to those used by Jacotot (1931). He inoculated suspensions into cattle and found that brain had a low infectivity, not demonstrable in dilutions above 1/1000; no virus was detected in a 1/100 dilution of spinal cord but bone marrow was infective up to 1/20,000.

MacOwan (1956) stated that data had accumulated which showed that the titre of a virulent laboratory strain of virus was $10^{6\cdot0}$ in carcass lymph nodes, spleen and abomasal mucosa. The time of harvest was not mentioned but it was added 'Other tissues have considerably less virus'. Infectivity was demonstrable in the brains of cattle 4 days after infection, probably on the 1st to 2nd days of fever. The figures produced by MacOwan (1956) have apparently been accepted without comment by Provost (1960), who also asserted that bone marrow contained 10–100 infectious units of virus, probably per gramme.

It is quite evident, where details are provided, that the accuracy of these and many similar communications must be severely questioned, if only because they were based on the inoculation of relatively small numbers of cattle or goats, which may or may not have been susceptible and effectively isolated. In the case of each tissue it is usually found that one or, at best, a very few samples were titrated, at imprecisely determined or unstated intervals after infection. The details of preparation of the inocula, whether end-points were obtained, and the method of expressing the results, are matters often left in doubt. It is difficult, therefore, to compare the figures obtained with those presented here.

The Japanese workers Furuya and Fukusho were quoted by Mornet & Gilbert (1958) as having titrated rinderpest-infected cattle tissues by inoculating tenfold dilutions into rabbits, which were subsequently tested for immunity by challenge with the normally lethal lapinized rinderpest virus. This method gave figures which were said to be 10 to 100 times lower than those obtained by cattle inocu-

lations. Their titres were 10^{-4} to 10^{-6} (probably per gramme) for lymph nodes and ileum; 10^{-4} to 10^{-5} for caecal tonsil, spleen, tracheal mucosa and the posterior part of the nasal mucosa; 10^{-2} for the kidney, thyroid, submaxillary gland, oral and oesophageal mucosae; 10^{-1} for the adrenal, testis, skin, urinary bladder, gall-bladder, bone marrow, ruminal mucosa and C.S.F.; nil for the myocardium, voluntary muscles and spinal cord. These tissues were probably all taken at the height of the temperature reaction. With few exceptions the figures of Furuya and Fukusho would appear to fall within the ranges established for the same tissues in the present study, so long as 1 or 2 $\log_{10}$ units are added for the lower sensitivity of rabbits compared with cattle.

The primary phase of rinderpest infection was of comparative interest in that no evidence was obtained for virus proliferation at the presumed site of penetration, i.e. the nasal mucosa. As noted in the section on 'Materials and Methods', care was taken to wash off as much blood as possible from this mucosa and all samples were taken from the middle third of the dorsal turbinate bone. Hence primary sites of multiplication in the lower or upper thirds, of the same or other turbinates, may have been missed. However, rinderpest obviously differs from pox-virus infections, which have been the subject of many previous studies on the pathogenesis of generalized virus diseases and in which proliferation at the initial site of virus penetration is readily demonstrable. (See Fenner (1948*a*, *b*) for ectromelia; Fenner & Woodroofe (1953) for myxomatosis; Hahon & Wilson (1960) for monkey small-pox; Bedson & Duckworth (1963) for rabbit pox.)

Grist (1950), reviewing the position with respect to human measles, suggested that in this disease there may sometimes be a transient 'illness of infection', associated possibly with the production of a 'primary complex'. Robbins (1962) also concluded that measles virus probably invaded and proliferated in the respiratory epithelial cells. Neither of these opinions was supported by any experimental data for man, although Robbins quoted Sergiev, Ryazantseva & Shroit (1960) as having found considerable quantities of virus in the naso-pharyngeal mucosae of monkeys during the incubation period.

In a review of canine distemper, Gorham (1960) pointed out the discrepancy between different reports on the primary site of virus proliferation. Titration of ferret and mink tissues, at different times after infection, indicated that virus was present in the nasal tissues on the second day after exposure but this may have been due to the inclusion of viraemic blood (Crook, Gorham & McNutt, 1958). On the other hand, sequential studies in ferrets by the fluorescent antibody technique never revealed virus antigens in the epithelium covering the nasal turbinates, whereas the cervical lymph nodes showed specific fluorescence from the 2nd day onwards (Liu & Coffin, 1957). These experiments were extended to naturally infected dogs and it was found that the behaviour of this species was similar to that of ferrets, although nasal smears from dogs clinically sick with distemper were said to contain specific antigens (Coffin & Liu, 1957).

It is generally accepted that in prodromal measles there is a systemic involvement of the lympho-reticular tissues, beginning even in the incubation period (Grist, 1950; Robbins, 1962). The evidence on which this belief is based is largely

histopathological, particularly the presence of 'mesenchymal' or 'reticulo-endothelial' giant cells, especially in the upper respiratory tract, the lungs and parts of the alimentary tract. Grist (1950) considered it likely that the mesenchymal and epithelial reactions were manifestations of successive phases of viral attack, the onset of pyrexial illness coinciding with a flooding of the circulation by virus and products of cellular damage, which occurs towards the end of the first or lympho-reticular phase. The same reviewer concluded that widespread dissemination of virus occurred before symptoms appeared.

In distemper-infected ferrets, the work of Crook *et al.* (1958) showed that viraemia and generalization of virus to the spleen, liver and brain occurred before the onset of pyrexia. However, virus titres in these organs, as well as in the nasal tissue, lung and blood, continued to increase until the 5th to 7th day of estimated pyrexia; the course of events in mink was found, by the same authors, to be similar though somewhat slower. Liu & Coffin (1957) found specific fluorescence in the spleen of distemper-infected ferrets before the onset of fever (4th day after exposure) but they could not demonstrate viral antigens in the gastro-intestinal, respiratory and urinary tracts until the 6th or 7th days after infection.

Curasson (1932), in his monograph on rinderpest, remarked as follows: 'Pendant la période d'incubation, il semble que le virus cultive et se multiplie dans les tissus jusqu'à ce que en quantité suffisante, il puisse envahir la masse sanguine, la maladie explosant brusquement.' The results described in the present communication demonstrated unequivocally that generalization of rinderpest virus in cattle occurred during the late incubation and early prodromal phases. This process was presumably a sequel to the low-level viraemia which became demonstrable by the 2nd or 3rd days after infection, originating in the cephalic lymph nodes; it involved the lymph nodes of the body, the spleen, bone marrow, lung and alimentary mucosae. It can be seen, therefore, that generalization in measles, distemper and rinderpest infections occurs before the onset of clinical signs and, as pointed out by Fenner (1948*b*), the latter cannot be due to virus 'invasion' from a primary focus. In fact pox-virus titres are near-maximal at the time of the first rise of temperature but this was not true of rinderpest.

It was not possible, on the evidence obtained, to subdivide generalization in rinderpest into lympho-reticular and epithelial phases, as suggested by Grist (1950) for measles, since localization in the mucosae of the alimentary tract and in the lung occurred at about the same time as virus appeared in the lymphoid tissues of the body. A final decision on this question can be given only as a result of sequential studies with fluorescent antibody. In the tongue, as also in the gastro-intestinal mucosae, there was a distinct possibility that the first cells involved were local, nodular aggregations of lympho-reticular cells. The early appearance (3rd day) of virus in the lung could have been due to proliferation in either peribronchiolar lymphoid nodules or in epithelia. In the nasal mucosa there were diffusely scattered lymphoid cells and these could have supported the virus proliferation first detected on the 5th day; nevertheless, primary multiplication in this tissue may have been in the epithelium, especially as superficial necrosis and erosion became visible at this time near the nares (Liess & Plowright, 1964).

One of the most interesting aspects of the results described in rinderpest-infected cattle was the light which they threw on the phase of virus decline. There were clear-cut indications that titres began to fall first in those lymphoid tissues which supported initial virus multiplication. They were maintained longest in lympho-epithelial structures such as the tonsils, base of the tongue, the gastro-intestinal mucosae and the lung. An explanation of these findings would require further investigation, but it seems reasonable to suppose that the irregularity of lymph node titres, from the 8th day onwards, was due to local production of antibody and the exhaustion of cells capable of supporting virus proliferation. From the practical point of view it should be evident that tissues such as the tonsil or lung are more suitable than spleen or lymph nodes for virus recovery after the onset of diarrhoea or dysentery. Before this time cephalic lymph nodes are more likely to give positive results than are visceral or superficial nodes and spleen.

Nasal excretion of rinderpest virus was found, by Liess & Plowright (1964), to occur as early as 1–2 days preceding the onset of pyrexia in cattle, although most previous work had suggested that the nasal secretions became infective on the 2nd or 3rd days of thermal reaction. No virus was recovered by these authors from nasal swabs collected during the first 48 hr. following intranasal inoculation and this finding supports the contention that local multiplication of virus did not occur in the nasal tissues before infection of the local lymph nodes. Since no infectivity was detected in the nasal mucosa until the 5th day after infection, it is obvious that virus in nasal swabs taken before that time must have come from elsewhere.

Two alternative origins can be postulated, these being the palatal tonsils and the lungs. Virus in the tonsillar tissue reached moderately high titres by the 3rd day and some of this might have escaped into the epithelial crypts and thence to the nasopharynx; lung tissue was irregularly infective on the 3rd and 4th days after infection, titres being low. The quantity of virus found in nasal swabs taken during the later course of the disease (often $> 10^{4\cdot0}$, sometimes $> 10^{5\cdot0}$ TCD 50 (Liess & Plowright, 1964)) also militated against its exclusive origin from the nasal mucosa, where titres only occasionally exceeded $10^{4\cdot0}$ TCD 50/g. The disappearance of virus from nasal excretions collected on the 9th day of pyrexia, corresponding with the 11th to 13th days after infection (Liess & Plowright, 1964), agrees fairly well with the data obtained in this study, where two animals killed on the 12th day retained very little virus in their tissues.

The first urinary excretion of virus was noted on the 1st day of pyrexia and the rate increased to affect a maximum of 62·5 % of the animals on the 5th to 7th days of the disease (Liess & Plowright, 1964). These observations correlate very well with the irregular figures for virus in kidney tissue, as do also the variable and generally low titres for virus in the urine. It is possible, of course, that some urinary infectivity was derived from the epithelium of the lower urinary tract but there would not appear to have been any necessity for this, so long as virus was able to pass into the glomerular filtrate.

No virus was demonstrable in the faeces of cattle before the 3rd day of pyrexia, corresponding approximately to the 6th day after infection. The faecal excretion rate rose to a maximum of 40 % on the equivalent of the 10th day after infection

and titres were very variable (Liess & Plowright, 1964). It must be concluded that the consistent presence of moderately high-titre virus in the gastro-intestinal mucosae, lasting from the 5th to the 10th or 12th days after infection, did not automatically lead to appreciable levels of excretion in the faeces. The longevity of infectious virus liberated into the lumen of the alimentary tract would obviously depend on the presence and concentration of bile salts and enzymes capable of inactivating it. Free virus would also be killed rapidly at body temperature, especially in locations where pH values deviated widely from neutrality and where putrefactive bacteria were multiplying rapidly. The cessation of faecal excretion in animals surviving to the 9th day of the disease (Liess & Plowright, 1964) correlates well with the disappearance of virus from the alimentary tract of two animals killed on the 12th day after infection. Ox no. 9292, however, was probably excreting large quantities of virus in its faeces, when it died on the 12th day, since titres in all the gastro-intestinal mucosae were extremely high (see Table 4).

SUMMARY

The pathogenesis of rinderpest virus was studied in twenty-nine grade cattle, which were infected by the intranasal route with a virulent strain of virus recently isolated in East Africa (RGK/1). These animals were killed or died at intervals of 1–16 days after infection and a number of tissues from each of them (usually 21) was titrated for virus infectivity in monolayer cultures of primary bovine kidney cells.

Temperature reactions were first detected on the 3rd to 5th days (mean 4·1), mouth lesions on the 6th to 9th and diarrhoea on the 8th or 9th days, following infection. The course of the disease was divided into four phases, viz. incubation (days 1–4), prodromal (days 5–7), mucosal (days 8–12) and early convalescence (days 13–16). Virus proliferation in different tissues was related to these clinical phases, detailed results being presented in tabular and graphical form.

No primary multiplication was detected in the nasal mucosa but virus was demonstrable within 48 hr. in its associated lymph nodes. Low-level viraemia began on the 2nd or 3rd days after infection and generalization had occurred by the end of the incubation period. The virus had established itself at this time throughout the alimentary tract and, sometimes, in the lungs.

The prodromal phase was characterized by plateaux of high virus titres in the lymphopoietic and lympho-epithelial tissues; there was a descending gradient from the cephalic nodes to the superficial lymph and haemolymph nodes of the body, to the visceral lymph nodes and spleen. There was a similar gradient in the titre of virus in the mucosae of the gastro-intestinal tract—from caecum to colon, to ileum and pylorus. Virus first appeared in the turbinate mucosa on the 5th day, post-infection and lung titres were high towards the end of this period. Some virus proliferation may have occurred in the liver, but none was certainly demonstrable in the kidney, myocardium or brain.

The mucosal phase began with continuing high titres of virus in all the major sites of proliferation, but a decline set in from the 9th day onwards. This at first

involved the cephalic lymph nodes but soon extended to the spleen and other lymphopoietic tissues. It was most delayed in lympho-epithelial structures such as the tonsil, lung and gastro-intestinal mucosae. It was suggested that the decline in virus titres was due to the destruction of susceptible cells, accompanied by the local production and later circulation of antibody. Neutralizing antibody was present in the serum on and after the 9th day.

During the early convalescent period virus had disappeared from four animals, with the exception of one recovery from the lung tissue. Antibody titres were high during this time.

These results were discussed with reference to previously existing information on the distribution of rinderpest virus in infected cattle. An attempt was made to correlate them with published information on the pathogenesis of human measles and canine distemper. The data were also used to explain some previously reported observations on the excretion of rinderpest virus by experimentally infected cattle.

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