Studies on the pathogenesis of rinderpest in experimental cattle

I. Correlation of clinical signs, viraemia and virus excretion by various routes

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Very little information is available on the pathogenesis of rinderpest in any domestic animal or on the routes and duration of virus excretion; such data as exist (see Curasson, 1932, 1942) are usually fragmentary and qualitative. The recent development of tissue-culture techniques, for the quantitative recovery of virulent strains of rinderpest virus from animal tissues, has made it possible to re-investigate these problems in a systematic, though economical, manner. Studies of this type have a practical significance in indicating the optimal time for collection of materials for diagnostic purposes. They also assist in reaching sound conclusions on the epizootiology of the disease and have a comparative value for those interested in infections caused by the closely-related agents of human measles and canine distemper.

The first paper of this series describes the results of experiments designed to elucidate the relationships between viraemia, clinical signs and virus excretion in cattle infected with a single field isolate of moderately high virulence.

MATERIALS AND METHODS

(1) *Virus*

The virus strain, RGK/1, was isolated in primary calf kidney monolayers from the spleen and lymph nodes of a reticulated giraffe (*Giraffa reticulata*; de Winton) which was shot in the Northern Frontier District of Kenya in January 1962. This animal was one of many of the same species which succumbed to the infection, after showing typical clinical signs of rinderpest; cattle in the same area had been immunized by vaccination and did not have the disease but warthogs (*Phacochoerus aethiopicus* Pallas) died in large numbers, presumably from the same infection (W. Plowright, unpublished).

After a second passage in calf kidney cells the virus was inoculated into cattle to confirm its identity, re-isolated in tissue culture and then returned to three cattle. The spleen of one of these animals (no. 8186) was harvested on the 3rd day of fever and small pieces of the pulp were stored frozen at $-70°$ C. In a cattle

titration of one sample of this material, sixteen of nineteen reacting cattle, i.e. 84 %, died. For use in these experiments a 10^{-1} or 10^{-2} (w/v) suspension of frozen spleen was prepared in culture maintenance medium and titrated by the technique described by Plowright & Ferris (1962), using primary calf kidney cells as the assay system. A few cattle were infected with virus in stored spleen fragments derived from another animal (no. 8994), which had been inoculated with spleen from ox no. 8186 and killed on the 4th day of pyrexia. The dose of virus administered to all animals was calculated from the results of simultaneous titrations in tissue cultures (see Table 1).

Table 1

Expt. no.	Ox no.	Route of infection	Dose of virus (TCD 50)	Incubation period (days)*	Duration of pyrexia*	Day of death
I	8361	Intranasal	$10^{5 \cdot 1}$	4	6	Recovered
	8362	Intranasal	$10^{5 \cdot 1}$	4	5	Recovered
	8363	Contact	N.D.†	8	6	Recovered
II	8918	Subcutaneous	$10^{3 \cdot 1}$	4	6	6/7
	8826	Subcutaneous	$10^{3 \cdot 1}$	4	5	6/7
III	8960	Intranasal	$10^{3 \cdot 0}$	4	6	Recovered
	8961	Intranasal	$10^{3 \cdot 0}$	4	5	7/8
	8920	Contact	N.D.	8	8	8/9
IV	8923	Subcutaneous	$10^{2 \cdot 1}$	5	4	Recovered
	8922	Contact	N.D.	9	9	11/12
	8985	Contact	N.D.	11	6	Recovered
V	8995	Subcutaneous	$10^{4 \cdot 7}$	3	6	Recovered
	8996	Subcutaneous	$10^{4 \cdot 7}$	3	5	8/9
	8997	Contact	N.D.	9	6	7/8
VI	9123	Subcutaneous	$10^{5 \cdot 1}$	3	4	6/7
	9124	Contact	N.D.	8	6	Recovered
VII	9153	Subcutaneous	$10^{5 \cdot 3}$	3	5	Recovered
	9154	Subcutaneous	$10^{5 \cdot 3}$	3	5	Recovered
	9134	Contact	N.D.	8	8	9/10
	9136	Contact	N.D.	8	6	7/8
VIII	9159	Intranasal	$10^{4 \cdot 9}$	3	6	6/7
	9162	Intranasal	$10^{4 \cdot 9}$	3	5	6/7
	9156	Contact	N.D.	8	24	Recovered
	9163	Contact	N.D.	9	6	Recovered
	9165	Contact	N.D.	9	9	Recovered

* Calculated from the day with first morning temperature $> 102°$ F. For contact cases estimated from the time of first exposure to a viraemic donor.

† N.D. = Not determined.

(2) *Experimental animals and their infection*

The majority of the twenty-five cattle used were 2-year-old grade steers, with a varying though usually high percentage of the blood of such European breeds as the Ayrshire, Jersey, Guernsey and Friesian. All were housed in isolation units, which effectively prevented any transfer of infection from stall to stall. They were

fed on hay only, with water provided *ad lib.* No therapeutic treatment was administered except when *Babesia bigemina,* a common protozoal parasite, was detected in blood smears.

The sera of all cattle were screened for rinderpest-neutralizing antibody and found to be negative before they were placed on experiment (Plowright & Ferris, 1961). Rectal temperatures were recorded every morning before 9 a.m., beginning several days before virus exposure.

Eight separate experiments were performed, each being conducted in a single stall and utilizing two to five cattle (Table 1). Of twenty-five animals, eight were inoculated subcutaneously on the side of the neck, whilst six received 2 ml. of spleen suspension by the intranasal route. In the latter case the inoculum was dropped slowly into both nostrils with the head held in such a manner that the material flowed quickly over the turbinates towards the nasopharynx; great care was taken to avoid mechanical injury to any of the mucosae. The majority of cattle which were infected by stall contact with inoculated animals (Table 1), were first introduced 2 days after the latter had received virus. In this way accidental infection, by ingestion or inhalation of spleen virus, was avoided. In Expt. VIII, cattle nos. 9163 and 9165 acquired the disease by contact with the reacting ox, no. 9156; they therefore represented the 2nd contact passage.

All cattle were subjected to a careful clinical examination every day from at least the time of onset of pyrexia. Particular attention was paid to the oral and nasal mucosae, where necrosis and/or erosion are such characteristic features of rinderpest.

(3) *Collection of materials from cattle*

Collections of blood, nasal secretion, urine and faeces were made almost daily from all animals before, during and for some days after the time when it was expected that virus would be present. Blood was taken from the jugular vein into one-third the final volume of a 1·5 % solution of EDTA (ethylene-diamine-tetra-acetic acid: disodium salt) in 0·7 % NaCl (see Plowright & Ferris, 1962). Serum for antibody determinations was also collected at intervals of 1–4 days, depending on the stage of the disease; these samples were stored frozen at about $-20°$ C. and the results of their examination will be described in a later communication.

Nasal secretion was obtained by inserting one sterile cotton swab about 7–8 cm. into each nostril; the amount of material adhering to the swab naturally varied with the type of discharge in various phases of the disease. The two swabs from each animal were broken off immediately into a single 1 oz. screw-cap bottle, containing 2 ml. of culture maintenance medium, in which antibiotics had been incorporated at the following concentrations per ml.: sodium penicillin, 1000 i.u.; streptomycin sulphate, 1000 μg.; nystatin, 500 units; neomycin sulphate, 500 units, later replaced by kanamycin sulphate, 500 μg.

Urine was collected, as voided by the animal, into an aluminium vessel about 20 cm. in diameter. Sometimes, in advanced stages of the disease, cattle became recumbent and severely dehydrated; in these cases urine could not be obtained easily and no sample was examined.

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Faeces were also collected by the attendants into similar aluminium vessels, from which 0·5 to 1·5 g. was removed by a sterile tongue-depressor for transfer to weighed, empty screw-cap containers.

(4) *Further treatment of materials*

Samples of 10 ml. of blood-anticoagulant mixture were processed in the manner already described (Plowright & Ferris, 1962) to produce the same volume of a crude leucocyte suspension in maintenance medium. This was regarded as equivalent to undiluted blood and was distributed in a dose of 2 ml. to each of five tubes of primary calf kidney (BK) cells. Tenfold dilutions of blood-anticoagulant mixtures were prepared directly in maintenance medium and these were also inoculated in a dose of 2 ml. to each of five tubes.

All the excretions were treated by techniques designed to produce suspensions in which: (a) any toxicity for cultured cells had been eliminated or reduced, (b) bacterial, fungal or yeast contamination had been reduced to a minimum, and (c) the maximum possible quantities of virus were retained in a uniformly distributed state.

Nasal swabs were exposed for half an hour at room temperature to the concentrated antibiotic solution and then expressed with sterile forceps to yield about 1·5 ml. of crude extract. To this was added 10·5 ml. of normal maintenance medium, after which the mixture was pipetted vigorously and treated for 5 min. in an ultrasonic bath* containing iced water. This served to break up floccules of mucus and any desquamated cells. The resulting suspension was arbitrarily designated as a 10^{-1} (v/v) dilution of nasal secretion but the true dilution factor was probably considerably higher.

Of the urine samples, 1·2 ml. volumes were mixed with an equal volume of concentrated antibiotic solution, which contained phenol red and served to give an approximate indication of the pH of the mixture. Wide divergencies from neutrality were then corrected by the drop-wise addition of 1 N-NaOH or 1 N-HCl. Not infrequently two to three drops of acid were necessary to neutralize the normal pre-reaction alkalinity of urine and two to three drops of alkali to counteract the acidity observed during the middle to late course of the disease. The change of pH of the urine in rinderpest-infected cattle has been known for a very long time (Marcet, 1866; Curasson, 1932).

Faeces samples were suspended in maintenance medium with concentrated antibiotics to give a concentration of 10^{-1} (w/v). After vigorous shaking the suspensions were left for 0·5–1 hr. at room temperature and then subjected to 5 min. ultrasonic treatment, as already described for nasal secretions. Large particles were allowed to sediment for several minutes on the bench and the supernatant was finally diluted tenfold in normal maintenance medium to give a 10^{-2} (w/v) suspension of faeces. This was the most concentrated preparation which was ordinarily found to be of low toxicity for BK cells. It is important to note

* 'Soniclean' Transducer Tank, Type 1160/T 24, 41.23 KC/S, Dawe Instruments, Ltd., London.

that centrifugation procedures could not be employed readily for faecal virus isolation, in view of the large size of the virus (Plowright, Cruickshank & Waterson, 1962) and its probable association with particles of mucus, blood or desquamated mucosal cells.

Further tenfold dilutions of the various excretions were prepared as necessary from the basic 10^{-1} or 10^{-2} suspensions and all were inoculated in a dose of 2 ml. into each of five tubes of BK cells from which all the previous medium had been discarded.

(5) *Preparation and maintenance of cell cultures*

Primary calf kidney cultures were prepared in tubes of 160×15 mm. dimensions. Details of the technique, the growth and maintenance media and the frequency of medium changes have already been given (Plowright & Ferris, 1962). The normal maintenance medium contained one-tenth the concentration of antibiotics mentioned in subsection (3); Kanamycin was found to be particularly useful in controlling the growth of otherwise resistant bacteria.

Cultures were inoculated at 4–11 days after seeding, the best results being obtained with cells not more than 1 week old. After inoculation all tubes were transferred to rollers, rotating at about 8 rev./hr. Debris from the inocula was removed after 24 or 48 hr. by three washes with 2–3 ml. of prewarmed P.B.S. (Dulbecco & Vogt, 1954). The shorter period of exposure was found to be an advantage, in that cytotoxicity and microbial contamination was thereby reduced in tubes inoculated with nasal secretion and faeces.

Microscopic examination was carried out at frequent intervals from the 3rd day post-inoculation and all cultures showing cytopathic changes typical for rinderpest (Plowright & Ferris, 1959, 1962) were discarded. Final observations were made on the 10th or 11th days and 50 % end-point titres were calculated by the method of Thompson (1947). In the case of blood these were expressed as $\log_{10}$ TCD 50 per 2 ml., in all other instances as $\log_{10}$ TCD 50 per ml. or per g.

(6) *Evaluation of the results*

Ultrasonic treatment probably had little or no effect on virus in the excretions, since it was known that culture virus, under identical conditions of exposure, had a half-life of 7·5 min. (Plowright, 1963*a*).

So far as completely negative results are concerned, it can be calculated in these instances that the virus was not detectable in: (*a*) 10 ml. of blood-EDTA mixture equivalent to 6·6 ml. of undiluted blood; (*b*) in 10 ml. of the basic dilution of nasal secretion arbitrarily designated as 10^{-1}; (*c*) in 1 ml. of urine, and (*d*) in 0·1 g. of faeces.

Where only one or two tubes became positive, out of five inoculated with the basic dilution of an excretion, the result was expressed as a 'trace'. This was also related to the same quantities of material as detailed above for completely negative results. Where three or more tubes were infected out of five used for the basic dilution, a titre was calculated on the assumption that all tubes would have become positive in the next lowest of a tenfold dilution series.

To minimize the effect of different incubation periods all viraemia and virus excretion data were related to the onset of pyrexia in the individual animal; for this purpose a morning temperature > 102° F. was regarded as abnormal.

RESULTS

Clinical signs

The incubation period, as determined by the first morning temperature of > 102° F., was 3–4 days in all cattle which were inoculated with $10^{3.0}$ to $10^{5.1}$ TCD 50 of virus; ox 8923, which received only $10^{2.1}$ TCD 50, reacted on the 5th day (Table 1).
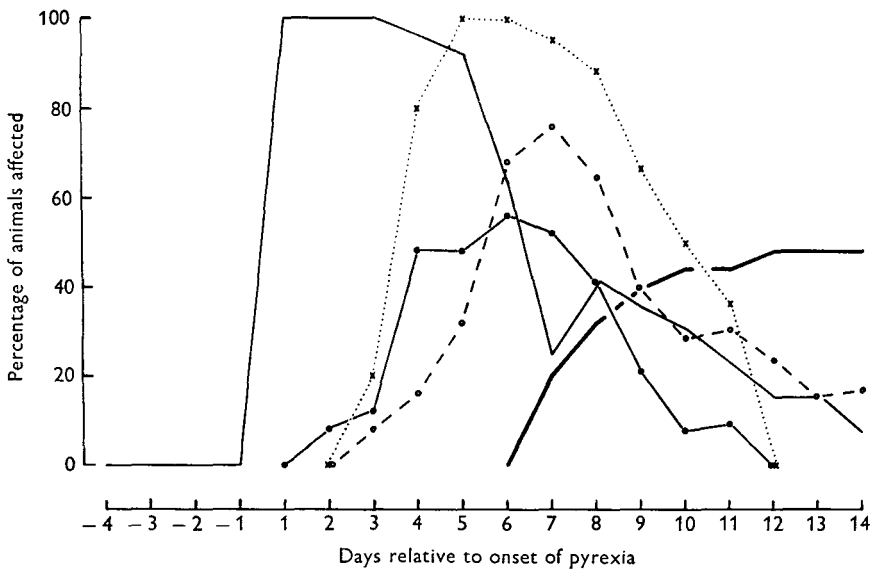
Fig. 1. Clinical signs in cattle infected experimentally with virulent rinderpest virus (strain RGK/1). ——-, Pyrexia (> 102·0° F.); × · · · ×, mouth lesions; ●—●, nasal lesions; ○– – –○, diarrhoea; ━━━, mortality.

The incubation periods for cattle infected by contact were calculated by assuming that the inoculated animals did not excrete virus before they were viraemic and that the time of exposure therefore began with the appearance of viraemia in any one of the donors. This assumption was justified by our data on virus excretion, to be described later; in fact, virus shedding was seldom detected before the first day of fever and the calculated incubation periods for contact infection can probably be regarded as maximal. As shown in Table 1 they ranged from 8 to 11 days, with a mean for eleven animals of 8·6 days.

The minimum duration of pyrexia was 4 days, the percentage of animals with fever falling rapidly after the 5th day (Fig. 1); the mean rectal temperature reached a maximum of 104·8° F. on the 5th, falling to 102·8° F. on the 6th day. By the 7th day, eleven of thirteen cattle which eventually recovered had temperatures below 102° F. Nevertheless two of these eleven animals showed mild

relapses on the 8th and 9th days, and one had uninterrupted pyrexia for 24 days.

Nasal and ocular congestion with discharges of a serous or seromucoid type were commonly observed on the 2nd and 3rd days of the reaction, but these later became mucoid or mucopurulent. Necrosis and erosion of the visible parts of the nasal mucosae was first found on the 2nd day and the incidence of these lesions reached 50 % on the 4th day of pyrexia (Fig. 1 and Table 2). From the 7th day onwards regeneration of the epithelia occurred rapidly in cattle which survived and only one of thirteen animals showed nasal abnormalities as late as the 10th day of the disease. In general, therefore, nasal lesions appeared slightly before or simultaneously with those of the mouth cavity, but their incidence was reduced to about one-half and they disappeared more quickly.

Mouth lesions were usually preceded by 1 or 2 days of local or generalized hyperaemia of the mucosae. Characteristic raised, whitish foci of necrosis or small reddened erosions were first detected on the 3rd day and were present in all animals by the 5th day of the disease (Fig. 1). Predilection sites were the lips, gums and buccal papillae, especially near the corners of the mouth, together with the under-surface of the free part of the tongue; lesions of the hard palate often appeared 1–2 days later. Extensive confluent or semi-confluent necrosis was seen in twelve animals, beginning on the 6th day at the earliest. It is of interest to record here that seven of twelve cattle which died of the disease had numerous, rounded foci of yellowish papillary necrosis on the dorsal surface of the tongue. Such lesions were also beautifully depicted in the 1866 Cattle Plague report, and yet some recent publications (Maurer, Jones, Easterday & DeTray, 1955; Smith & Jones, 1957; Scott & Brown, 1961) state categorically that the anterior part of the dorsum of the tongue is never involved. Complete reconstitution of the mouth epithelia had occurred in all survivor cattle by the 12th day following onset of the disease.

'Diarrhoea' for the purpose of this paper was taken to mean the passage of fluid faeces containing varying quantities of mucus, blood or portions of de-squamated intestinal mucosa. As shown in Fig. 1, this clinical sign was first recorded in a few animals on the 3rd or 4th days of pyrexia, but the proportion affected rose sharply on the 5th and 6th days, to reach 68 %. All the twelve cattle which died developed persistent diarrhoea, resulting in dehydration and, in the majority of cases, severe prostration during the 1 or 2 days before death.

In thirteen individuals diarrhoea developed 1–3 days (mean 1·5 days) after the first detection of oral lesions, but in one case it appeared on the day preceding and in three cases on the same day as oral necrosis and erosion. Hence, generally speaking, diarrhoea was the last of the major clinical signs of rinderpest to make its appearance (Fig. 1).

The first and heaviest mortality occurred on the 7th day but the peak figure of 48 % was reached on the 12th day following onset of the disease. There was no apparent relationship between the mortality rate and the route of infection; death occurred in five out of eleven animals infected by contact and in seven out of fourteen which were inoculated intranasally or subcutaneously.

Viraemia

Virus was first demonstrated in the blood of two animals on the 2nd day preceding the onset of fever. The time from inoculation to the detection of virus in the blood was 1–3 days (mean 2·5) in fourteen animals, whereas after contact exposure viraemia did not develop for 8–13 days (mean 9·2). On the day preceding first pyrexia, 76 % of the cattle had viraemia, with a mean titre of $10^{0·3}$ TCD 50 per ml. There was some indication that viraemia appeared later in animals infected by contact; thus, whereas all cattle infected by inoculation had viraemia on the 1st day of fever, four of eleven which acquired the disease naturally were found to be non-viraemic at this time. One further ox (no. 9124) did not have detectable quantities of virus in its blood until the 4th day of reaction.

As shown in Fig. 3, the peak mean titre for viraemia was $10^{2·3}$ TCD 50 per ml., attained on the 3rd day of the disease. It is necessary to point out, however, that the mean line, shown in Fig. 3, was depressed by the inclusion of results from many titrations in which, for various reasons, the end-point was not obtained. The highest blood titres recorded in individual animals were about $10^{4·0}$ TCD 50 per ml. (Fig. 3).

Of twelve animals which died, eight were viraemic until the day of death, i.e. 7–9 days after the onset of pyrexia; in three of them viraemia ceased 1, 2 and 5 days respectively prior to death, whilst in the remaining animal the test failed on the day preceding the fatal termination. In ten cattle which recovered and for which complete data were obtained, viraemia lasted 2–8 days, with a mean of 6·6 days.

Nasal excretion of virus

Virus was never recovered from nasal swabs taken on the day following inoculation, even in those animals which were infected by the intranasal route. Two animals showed viral excretion on the 2nd day preceding pyrexia, i.e. as early as circulating virus was detected, but the subsequent rate of development of nasal excretion was slower than the rate of increase of viraemia (Fig. 2). The nasal excretion rate rose to a maximum of 87·5 % on the 4th day and thereafter declined, steadily at first but later abruptly, so that by the 10th day virus was no longer demonstrable (Fig. 2). This latter time corresponded very well with the disappearance of viraemia, which was complete on the 9th day. It also recalled the disappearance of nasal erosion and necrosis, in all but one animal, by the 10th day. The exceptional animal was one which never excreted virus by the nasal route.

Fig. 4 shows that the greatest quantities of virus were present in nasal secretions on the 3rd to 7th days of the disease. Many swabs collected during this period yielded virus with a titre of $> 10^{4·0}$ TCD 50, and a few even exceeded $10^{5·0}$ TCD 50. The rapid decline in the nasal excretion rate after the 7th day was probably attributable, at least partially, to the death of severely affected animals. Thus, six of eight cattle which died on the 7th and 8th days, had a mean nasal-swab titre of $\geqslant 10^{4·7}$ on the day preceding death; but among fifteen of the seventeen survivors, which were successfully tested on the 8th day, only three showed nasal excretion.
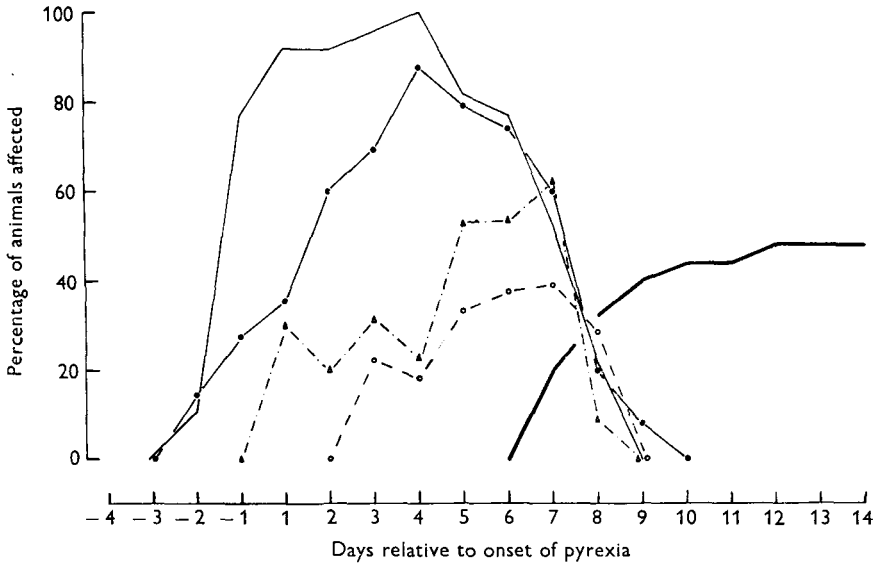
Fig. 2. Correlation of viraemia and virus excretion in cattle infected experimentally with virulent rinderpest virus (strain RGK/1). ——, Viraemia; ●—●, nasal excretion of virus; △—△, urinary excretion of virus; ○ – – ○, faecal excretion of virus; ▬▬, mortality.
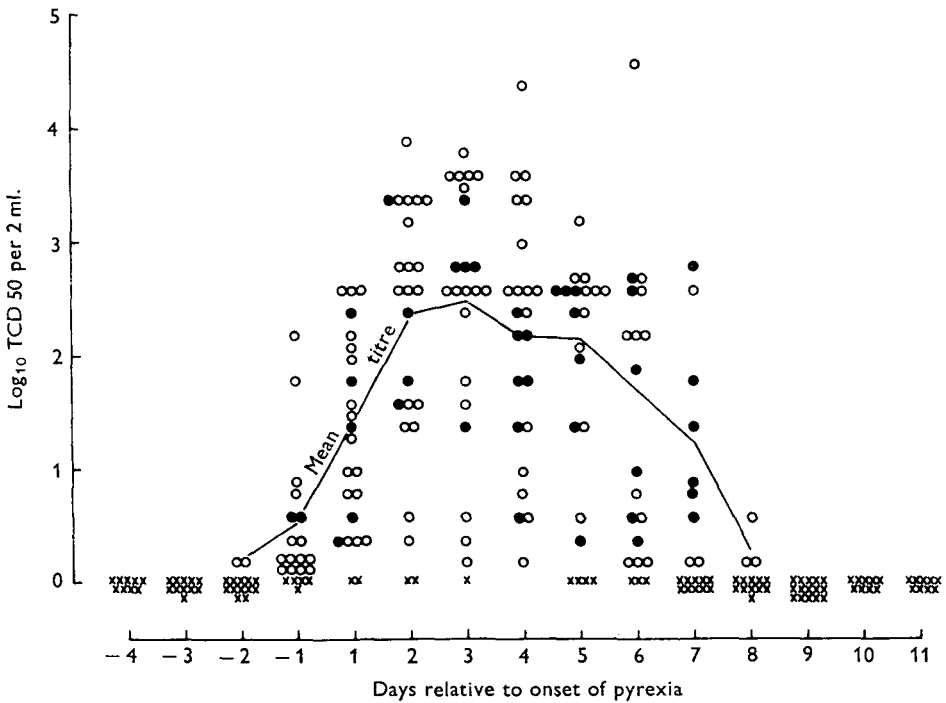

Fig. 3. Viraemia in cattle infected experimentally with virulent rinderpest virus (strain RGK/1). ●, End-point not obtained.

All but two of twenty-four animals (no. 8918 excluded) excreted virus at one time or another during the course of the infection; in four animals this was demonstrated on 1 day only, in eighteen animals on 2 or more days. Repeated sampling in the other two cattle (nos. 8922 and 9124) failed to reveal nasal excretion; both of these animals developed viraemia of short duration and low titre, although pyrexia was not less marked than in the remainder. One of them (no. 8922) died later, having developed diarrhoea and mouth lesions, the other (no. 9124) recovered after a mild disease course.
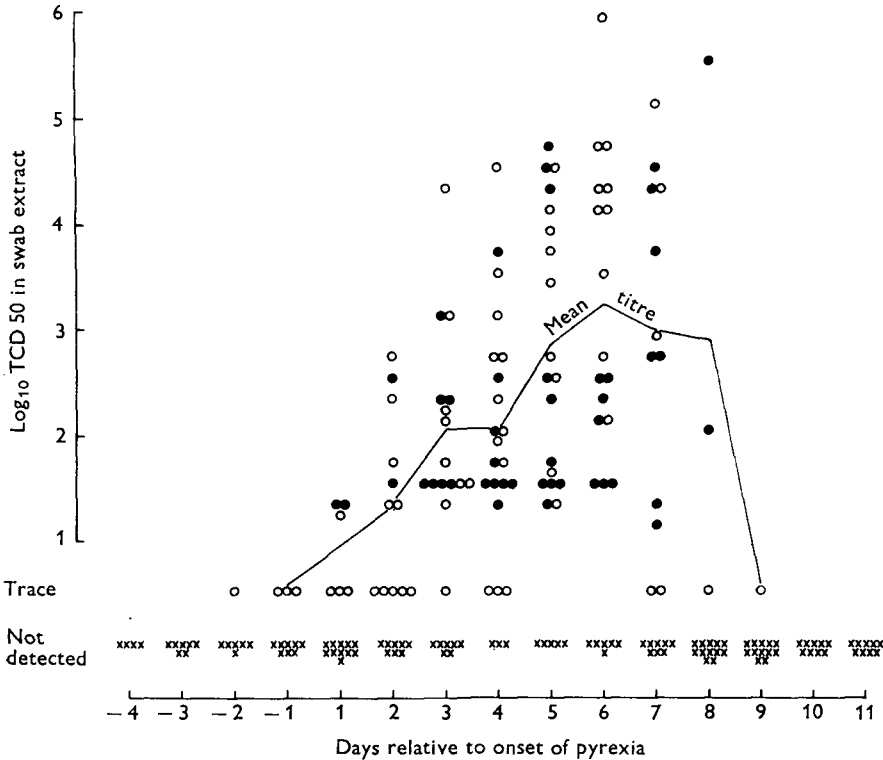

Fig. 4. Nasal excretion of virus by cattle infected with virulent rinderpest virus (strain RGK/1). ●, End-point not obtained.

Excretion of virus in urine

Traces of virus were first detected in the urine of two animals on the first day of fever, hence excretion by this route commenced 2 days after viraemia first became demonstrable. In this connexion, however, it is necessary to remember that the technique adopted utilized the equivalent of only 0·2 ml. of undiluted urine, whereas the method used to demonstrate virus in blood made use of leucocytes from 6·6 ml. of blood (see Materials and Methods).

The urinary excretion rate was in the region of 20–30 % during the first 4 days of the disease, during which time the mean titre did not change greatly (Figs. 2 and 5). On the 5th day, however, at a time when the amount of virus in the circulating blood was already beginning to decline, there was a sudden increase in

the proportion of animals (53 %) shedding virus in the urine and, on the 7th day of the illness, the excretion rate rose still further, to a peak of 62·5 %. The highest mean titre, of $10^{1·7}$ TCD 50 per ml., was attained on the 6th day. The abrupt decline of urinary excretion on the 8th day coincided with the rapidly mounting figures for mortality and the decline in those for viraemia and nasal excretion.

Of twenty-four animals for which satisfactory results were obtained, twelve shed virus in the urine on 2 or more days; in four animals virus was only detected on one day, while in the remaining eight cattle it was not demonstrated at all. To some extent this may have been due to the difficulties, already mentioned, in collecting urine from very sick, recumbent animals. Nevertheless, three animals which were tested regularly during the critical period and all of which recovered (nos. 8923, 8985 and 8995) were never found to excrete virus by this route; one of them (no. 8995) showed a moderately severe clinical course, including extensive mouth and nasal lesions together with diarrhoea.
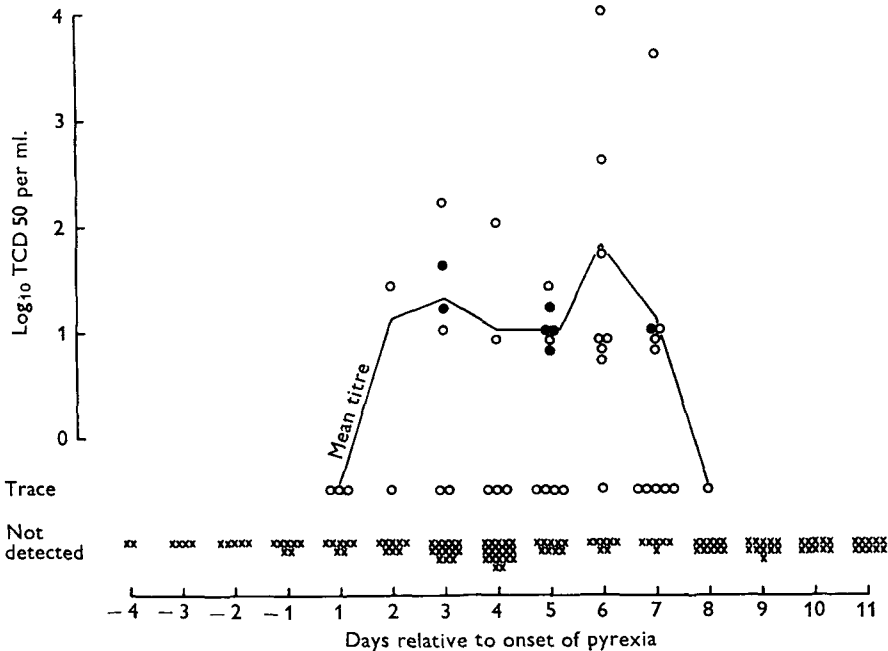

Fig. 5. Urinary excretion of virus by cattle infected with virulent rinderpest virus (strain RGK/1). ●, End-point not obtained.

Excretion of virus in faeces

Virus was first detected in faeces on the 3rd day of fever in two of nine cattle (nos. 8362 and 8363). Neither of these animals had diarrhoea at the time, although one of them (no. 8362) did develop it 2 days later. As shown in Fig. 1, diarrhoea was first seen in two animals on the 3rd day of pyrexia; in one of them (no. 8826) no virus was demonstrated in faeces taken on the same day, while in the other case (ox, no. 9123) cultures became contaminated. The latter animal did, however, excrete considerable quantities of virus in its faeces on the following day ($\geqslant 10^{3·2}$

TCD 50 per g.) and the high level was maintained until death on the 6th–7th days of the disease. Similarly, virus was found in the faeces of ox no. 8826, on the 5th, 6th and 7th days, the titre shortly after its death being $\geqslant 10^{4 \cdot 2}$ TCD 50/g.

The faecal excretion rate rose to a maximum of 40 % (7/18) on the 7th day of the disease but this was still considerably less than the maximal proportion (76%) of animals with diarrhoea (Fig. 1). The rapid decline on the 8th day in both the number of animals with diarrhoea, as also those showing faecal excretion of virus, was undoubtedly associated with the death at this time of many severely affected animals. On the 9th day four survivors still had diarrhoea but virus was no longer detectable; it was not recovered later from animals with prolonged enteric signs, which were tested continuously for as long as 16 days.
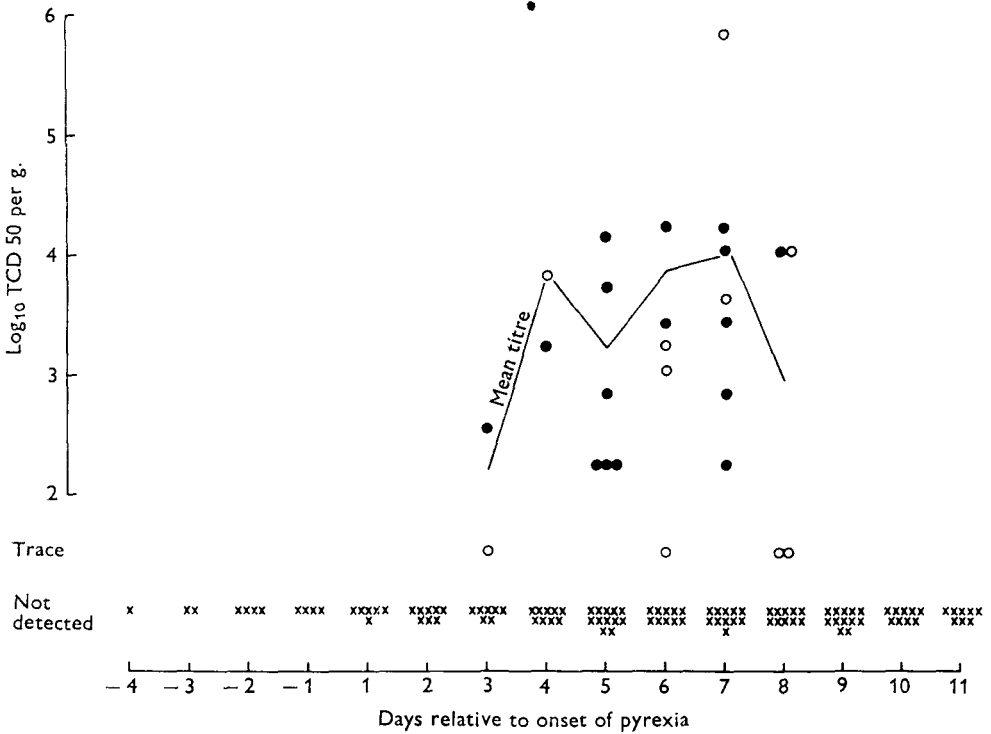

Fig. 6. Faecal excretion of virus by cattle infected with virulent rinderpest virus (strain RGK/1). ●, End-point not obtained.

Of nineteen cattle which exhibited diarrhoea fourteen were shown to excrete virus in the faeces at some time or another between the 3rd and 8th days of the disease. The results for many animals were irregular, since cytotoxicity of faeces or microbial contamination of the cultures terminated many faecal tests prematurely; in addition, the total quantity of faeces tested (0·1 g.) was perhaps too small to exclude low-level excretion. Nevertheless, several animals which developed diarrhoea were successfully tested virtually every day during the time when the clinical sign persisted and yet no virus was recoverable. It must be concluded

that, in these individuals, the total amount of virus excreted in the faeces could not have been great. The behaviour of these animals should be compared with others for which faecal titres were recorded of $> 10^4$ TCD 50, rising to $10^{6\cdot2}$ TCD 50/g. (Fig. 6).

<center>DISCUSSION</center>

Much of the information on the disease course and clinical signs of rinderpest was acquired several decades or even more than a century ago. It has often been repeated in text-books without any reference to the original sources or the experimental methods employed. In the past, the necessity for using cattle as the only satisfactory virus-susceptible species, carried with it the danger of prior immunity in some of the experimental animals, and also the risk of contact infections amongst those which had been inoculated. Furthermore, the vast majority of these older observations were usually qualitative and at best semi-quantitative, whereas tissue culture techniques allow a quantitative approach throughout.

The virus strain used in our study can probably be regarded as typical of the more virulent field strains at present current in East Africa. It had undergone a maximum of two culture and three cattle passages following first isolation and had been given, therefore, very little opportunity for modification. Its virulence for East African grade cattle was considerably greater than that of many of the strains isolated in this laboratory during the past 3–4 years, from cattle or game animals (Plowright, 1963*b*; Plowright, unpublished observations). The difference between the 84 % mortality, observed in a titration of the spleen of ox no. 8186 (see Materials and Methods) and the 48 % death-rate in these experiments, may be attributable to variations in the genetic resistance of the comparatively small groups of animals which were employed. It was, in fact, fortunate that the clinical picture varied so markedly, since we were thereby enabled to study cases of different degrees of severity, ranging from very mild to rapidly fatal.

The use of three different routes of infection—i.e. subcutaneous and intranasal inoculation, or close contact with infected animals—made it possible to compare the course of events in the disease acquired by artificial or natural means. Although numbers were small the figures for mortality and death time did not suggest that there was any material difference between infection by inoculation and by contact. There was simply an elongation of the incubation period from a mean of 3·57 to 8·63 days, which may have been due to a smaller virus uptake on susceptible mucosae.

It is commonly asserted in the literature that natural infection of cattle usually takes place by the oral route (Edmonds & Walker, 1929; Curasson, 1932; Hutyra, Marek & Manninger, 1946; Hagan & Bruner, 1957) and yet there are numerous reports that the virus easily invades the body from the nasal mucosae (see, for example, Hornby, 1926; Hall, 1933) whilst drenching with virulent material frequently fails to set up the disease (Schein & Jacotot, 1925; Hornby, 1926). Our results with intranasal installation certainly agree with those of Hornby and Hall and suggest that this is the common natural route of infection with rinderpest.

It has been known for nearly 100 years that rinderpest virus appears in the blood at the time of the first rise of temperature to 102° F. or above (*Third Cattle Plague Report*, 1866, p. iv) and it is commonly held to persist throughout the period of early clinical reaction and diarrhoea. Plowright (1963*b*), in a study of recently isolated strains of low cattle pathogenicity, found that viraemia was detectable from the 2nd day before the onset of fever to the 3rd day after its subsidence, but in the majority of animals (82 %) virus was no longer demonstrable in the blood on the 2nd day of normal temperature. These results were essentially in agreement with those for survivor cattle in the experiments here described, but differ some-what from those of Hall (1933) who found that viraemia in some animals persisted until the 4th or 5th days of normal temperature. They also conflict with the data provided by MacOwan (1956); he reported that the eclipse phase following a large parenteral dose of virulent, Kabete 'O' virus was only 1 day and that the duration of this phase was inversely proportional to the dose. The duration of viraemia was 13–14 days and independent of the size of the inoculum. Some of the apparent discrepancies may be related to the strains of virus employed and to the sensitivity of the techniques adopted for the detection of virus. Hall (1933), for example, inoculated 2 ml. quantities of blood subcutaneously into cattle; this is, quite possibly, a more sensitive technique than cultural isolation.

Curasson (1932), in a discussion of some earlier reports on the possibility of prolonged retention of virus by convalescent cattle, described how he had infected an ox with the blood of an animal whose temperature had returned to normal 37 days previously. This and other incidents he regarded as evidence for the occasional existence of virus 'carriers'. We obtained no indications from our viraemia or excretion studies that such a carrier state was established with the RGK/1 strain of virus in East African grade cattle.

Hornby (1926) stated that nasal discharge was infective as early as the second day of fever and within 5 days of inoculation; he further noted that a muco-purulent discharge was generally obvious at that time and suggested that the leucocytes contained therein were carriers of the virus. In addition, he reported that infectivity was no longer detectable 2–3 days after complete remission of the fever. Hall (1933) asserted that the nasal secretions were nearly always virulent on either the 2nd or 3rd days of thermal reaction and continued to be so until the end of pyrexia or even afterwards in severe clinical cases. He also found that the infectivity of nasal discharges could exceed that of blood collected simultaneously—in one experiment, for example, $10^{-2.7}$ ml. of blood was infective compared with $10^{-3.7}$ ml. of nasal exudate.

These observations are in close agreement with our own, although in a few animals we were able to recover virus from nasal swabs taken on the 1st and 2nd days preceding pyrexia. The constant disappearance of virus from the nasal secretions on the 2 days following its intranasal instillation, suggested that there was no primary local proliferation but that it was rapidly removed to the regional lymphoid tissues. The hypothesis has received support from further studies in this laboratory on the pathogenesis of rinderpest in the tissues of cattle (Plowright & Liess—to be published); it also does not conflict with the facts that nasal excretion

always followed viraemia, accompanied the emergence of nasal abnormalities (in the form of mucosal congestion, necrosis or erosion) and finally, in our experience, ceased with the disappearance of nasal lesions.

Although Hornby (1926) stated that urine in small quantities was not infective before the 3rd day and had lost its virulence on the 3rd to 5th days following the subsidence of fever, many text-books still emphasize the importance of urine in disseminating the infection (Hutyra *et al.* 1946; Hagan & Bruner, 1957). Our experiments showed that virus became detectable on the first day of pyrexia and was demonstrable up to the 9th day following its onset. It is of interest to note here that Curasson (1932) established the limits of urinary excretion as the day preceding fever to the 9th day following, whereas Hall (1933) smeared urine in the nostrils of susceptible cattle and established by this means that viruria did not appear until the 2nd day of pyrexia and was no longer demonstrable 2 days after return to normal temperature.

The infrequency with which urinary excretion was detected in many animals, considered together with the low titres recorded, cannot be regarded as lending support to the idea of the importance of urinary contamination in transmitting the RGK/1 strain of rinderpest virus. The very high nasal excretion rate and frequent elevated titres for nasal discharges leave little room for doubt about the relative significance of these two routes of excretion.

The origin of the virus in the urine is not at present established; we failed to find in fatal cases any evidence for an acute cystitis such as was mentioned by Hornby (1926), neither were there macroscopic signs of a nephritis.

According to the data reported here, faecal excretion began, at the earliest, on the 3rd day of temperature reaction and, on occasion, somewhat before the onset of diarrhoea (e.g. ox no. 8362) or in the absence of dysenteric signs (e.g. ox no. 8363). The limits of faecal excretion were given by Hornby (1926) as the 6th to the 12th days following onset of fever, but he accepted that animals with severe diarrhoea might shed virus for 'several days' longer. Curasson (1932) asserted that virus was present in the faeces from the first day of pyrexia and for 2–3 days after its subsidence in cattle with diarrhoea. Neither of these authors gave details of their experimental protocols. Hall (1933) found that the faeces became infective after the appearance of diarrhoea and at some time between the 2nd and 7th days of temperature reaction. Faecal excretion, in cases with severe diarrhoea, was not demonstrable 4 days after the subsidence of fever, whilst in other instances no virus was detected on the 2nd or 3rd days after the end of pyrexia.

From our accumulated data on rinderpest virus excretion it is now possible to answer the problem posed in the 1866 Cattle Plague Report, viz. 'how soon after the poison is put into the blood the animal becomes capable of giving the disease by natural infection to other animals'. Virus may be excreted from the time of onset of viraemia, at least by the nasal route; infected cattle may, therefore, contaminate their environment 2 days before the first clinical manifestation of disease, i.e. fever, and at least 5 days before diagnostic lesions become detectable in the mouth cavity. These factors have an obvious significance in formulating disease-control regulations for enzootic or rinderpest-free countries.

This discussion would not be complete without some comparison of the rinderpest data with those available for canine distemper and human measles. In distemper-infected dogs, Bindrich (1954) established that virus circulated in the blood on the first day of the temperature reaction, whilst Rockborn (1957 *a*, *b*) detected viraemia on the 4th and 6th days following experimental or contact exposure. After intramuscular inoculation of virus, viraemia was not detectable after the 6th day, whereas in dogs infected by contact it persisted for at least 14–19 days (Rockborn, 1957 *b*, 1958). Liu & Coffin (1957) demonstrated by the immunofluorescent technique that virus-specific antigens appeared in the circulating leucocytes of ferrets 3–4 days after inoculation of virulent distemper virus and before the onset of pyrexia.

Clinically recognizable changes occur in the nasal mucosae of infected dogs as early as the 2nd day of fever; they are accompanied by a serous discharge (Bindrich, 1950). It has been known for several decades that nasal exudates are infectious and it has been reported that mink, infected by aerosols, excreted virus in the nasal discharges from the 5th to 46th day post-exposure (see Gorham (1960) for a review).

So far as urinary excretion of distemper virus is concerned, Bindrich (1950) stated that he had demonstrated viruria in experimentally-infected dogs from the 3rd to the 17th days following temperature reaction and thereafter irregularly to the 8th week post-inoculation. Hence urinary excretion began at least 2 days after the appearance of viraemia. Bindrich unfortunately restricted his observations on the first 2 days of fever to only 4 dogs; if he had been able to investigate a larger number of cases, his results may well have been comparable to ours with rinderpest, in which three of ten cattle had viruria on the first day of fever.

Mucoid or blood-streaked diarrhoea is common in the later course of virulent distemper infection (Hutyra *et al.* 1946) but data on faecal viral excretion have not been published either for the dog, the ferret or the mink. Gorham & Brandley (1953), however, failed to find virus in the colon contents of infected ferrets.

Systematic investigations of measles viraemia and virus excretion in the natural host present considerable difficulties; nevertheless, some information has recently become available, particularly through the use of tissue culture techniques. The natural incubation period of 10–11 days (Robbins, 1962) or 5–9 days (Rake, 1959) corresponds quite closely with the contact incubation periods in rinderpest. It is followed by a prodromal phase of 1–5 days (Rake, 1959) or 2–4 days (Robbins, 1962) which is characterized by fever, conjunctivitis, rhinitis and tracheobronchitis. Koplik's spots appear during this stage on the mucosae of the cheeks, soft palate and lower lips. The prodromal period is terminated by the appearance of the rash, at first a focal, macular or maculopapular exanthem, which may later become confluent (Rake, 1959; Robbins, 1962). Virus is probably present in the blood and nasopharyngeal secretions during the whole of the prodromal period and up to 32 hr. after the appearance of the rash (Ruckle & Rogers, 1957). According to Papp (1937) it could also be demonstrated in the blood during the incubation period, by the subinoculation of leucocytes into susceptible children; Enders,

Katz & Medearis (1959) found that monkeys developed measles viraemia 5–7 days after inoculation and 3–4 days before the first clinical signs.

There are obvious similarities between the course of the infection in rinderpest and measles. In both of them viraemia becomes detectable during the incubation period; the latter is followed by a prodromal phase of 3–5 days duration, accompanied by non-specific, general clinical signs, with continuous viraemia. In rinderpest the prodromal period is terminated by the appearance of a pathognomonic enanthem, after which the viraemia declines rapidly. In measles the oral enanthem appears *during* the phase which is designated prodromal, and this phase is ended by the appearance of the skin exanthem. Stated in this manner the differences between measles and rinderpest are quite artificial, especially when it is known that the skin eruption of rinderpest, if it occurs, appears on the 4th or 5th days of clinical reaction (Burdon-Sanderson, 1866) at the same time as diarrhoea and stomatitis (Curasson, 1932). Although Robbins (1962) claimed that there was no direct evidence that Koplik's spots are the primary site of viral proliferation in the mucous membrane, multinucleate syncytia have been observed in the buccal or tongue epithelia and these may well indicate localization of virus in these situations (for example, Masugi & Minami, 1938; Semsroth, 1939; Roberts & Bain, 1958). Similarly, Grist (1950) stated: 'The thinness of buccal mucosa may explain why Koplik's spots are visible before the skin rash, though both types of lesion are initiated at the same time.'

While measles in many countries is now a relatively mild disease, in which enteric signs are often not considered worthy of mention (Robbins, 1962) or receive very brief consideration (Rake, 1959), this is certainly not true of certain African countries or of nineteenth-century England and France (Morley, Woodland & Martin, 1963). In Nigeria, the last-named authors found that diarrhoea or dysentery was 'second only in importance to bronchopneumonia as a feature of measles'. It most commonly appeared after the rash but sometimes began during the prodromal period or at the end of the latter; the weight loss and dehydration associated with it was a major cause of the mortality, which was estimated to reach an over-all 5 % in Nigeria. Thus measles virus may, in some highly susceptible populations, cause a clinical syndrome which is comparable in all important respects to rinderpest in cattle.

Little is known about the urinary and faecal excretion of the measles agent, but Gresser & Katz (1960) were able to isolate virus from the urine of eight out of eleven patients tested; in one instance virus was still present 4 days after appearance of the rash. Enders (1962) regarded the question of faecal excretion of measles virus as still unanswered, while Ruckle & Rogers (1957) could not recover virus from the faeces of patients.

SUMMARY

A total of twenty-five grade cattle were infected experimentally with a strain of rinderpest virus of moderately high virulence (RGK/1). Three methods of introduction of the virus were employed, i.e. subcutaneous or intranasal inoculation (14 animals) and housing in contact with reacting cattle (11 animals).

A quantitative study of the viraemia and of virus excretion by the nasal, urinary and faecal routes, was made by the inoculation of primary calf kidney cultures. All virological data were related to the onset of pyrexia, the incubation period in inoculated animals being 3–5 days, while in those infected by contact it was 8–11 days. Viraemia preceded the first rise of temperature by as much as 2 days in inoculated animals and 1 day in the contact cases. All animals had viraemia by the 4th day of the disease, but thereafter the percentage of positives declined rapidly, reaching nil by the 9th day.

The case mortality rate was 48 % with the majority of animals dying on the 7th to 10th days after reaction.

Virus was detected in the nasal secretions of some animals on the 2nd day preceding pyrexia. The proportion of nasal excretors rose slowly to reach a maximum of 87·5 % on the 4th day and no positives were detected after the 9th day of fever. The titre of virus in nasal discharges reached high levels, often probably in the region of $10^{5·0}$ to $10^{6·0}$ TCD 50/ml.

Urinary excretion of virus began on the 1st day of fever in about 25 % of animals; reaching a maximum of 62·5 % on the 7th day. No virus was recovered from the urine after the 8th day of the disease, the rapid decline in the excretor rate being only partially attributable to the death of severe cases. Urinary titres were relatively low; they seldom exceeded $10^{2·0}$ TCD 50/ml.

Virus was first recovered from the faeces on the 3rd day of pyrexia. Excretion by this route was not constantly associated with the development of diarrhoea, but the rapid decline which occurred in the excretor rate on the 8th and 9th days was undoubtedly attributable to the death of diarrhoeic individuals which took place at that time. The majority of faecal titres were in the approximate range of $10^{3·0}$ to $10^{4·0}$ TCD 50/g. but occasional samples attained *ca.* $10^{6·0}$ TCD 50/g.

It was calculated, from the clinical and virological data that cattle can excrete virus, at least by the nasal route, as much as 6 days before the appearance of pathognomonic clinical signs, i.e. oral necrosis and erosion.

Our findings for rinderpest were compared with those of other investigators of this disease and also with the available information on canine distemper and human measles.

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