STUDIES OF VIRAL INTERFERENCE INDUCED BY RINDERPEST VIRUS

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DECLARATION

This thesis entitled STUDIES ON VIRAL INTERFERENCE INDUCED BY RINDERPEST VIRUS is my original work and has not been presented for a degree in any other University.

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CERTIFICATE

This is to certify that the thesis entitled STUDIES ON VIRAL INTERFERENCE INDUCED BY RINDERPEST VIRUS is bonafide work of Mr. Fida Hussain, Assistant Research Officer, Veterinary Research Institute, Lahore, Pakistan; and that the part of work completed in Lahore was carried out under my supervision, and that this thesis has been submitted for examination with my approval as External Supervisor.

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SUMMARY

The impetus for research on "studies on viral interference induced by rinderpest virus" has been the recognition of protection afforded by live attenuated tissue culture rinderpest vaccine (TCRV) virus against virulent infection before the development of specific antibodies.

Review of literature has revealed that rinderpest is still enzootic in certain parts of the world including Pakistan. Attenuated rinderpest virus vaccines are being mostly used to control and eradicate the disease. These vaccines produce protection against virulent infection before the development of antibodies through interference. Viral interference both *in vitro* and *in vivo* with other viruses has been shown to be mediated by interferon production. Where as, bovine cells both *in vitro* and *in vivo*, have also been shown to have the potential to produce interferon following stimulation with viruses.

TCRV on exposure to U-V irradiation and 56°C heat treatment was inactivated in an exponential fashion. Infectivity of the virus was stabilized by the addition of serum or vaccine additive.

TCRV was found to interfere with both homologous as well as heterologous viruses and viral interference was observed to be mediated by interferon produced by bovine cells. Fully attenuated rinderpest vaccine virus strain induced more interferon production *in vitro* as compared to virulent strains. Between the virulent strains, non-contagious strain stimulated bovine cells to produce more interferon than the virulent contagious strain of rinderpest virus. The increased interferon inducing character of a virus strain may then be used as a marker of virus modification.

Buffaloes inoculated intravenously with $10^{6.0}$ $TCID_{50}$ of TCRV developed detectable levels of circulating interferon as early as 48 hours post infection. Interferon titres, however, paralleled viraemia. Circulating neutralizing antibodies were detectable as early as day 6 p.i. and their increase

effected decline in titre of interferon and viraemia.

Animals challenged at 48 hours p.i. succumbed to virulent virus infection in the same manner as the controls, but those challenged 72 to 96 hours p.i. were protected and showed a transient mild reaction. Animals challenged on day 6, 10 and 14 were solidly immune.

It is concluded that early protection afforded by TCRV virus to buffalo was mediated through endogenous interferon.

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CHAPTER I

I N T R O D U C T I O N

1.1. HISTORY AND INCIDENCE OF RINDERPEST

Rinderpest (Synonyms: Peste bovine, Peste bovina, Contagious typus and Cattle plague) appears to have originated in Asia (Merchant and Packer, 1967). The earliest recognizable disease descriptions were written in the 4th Century A.D. (Barton, 1956). Thereafter the disease was recorded as inevitable sequel to every major military compaign in Europe. In the 18th Century alone it is estimated that about 200 million cattle in Europe fell victim to rinderpest (Curasson, 1932). With the development of international trade in live cattle in the 19th Century, incidence of this disease increased manifold and led to such destructive outbreaks, as the 1865 epizootic in Great Britain, introduced with cattle transported from Revel (Tallinn - Seaport of Estonian Soviet Socialist Republic of U.S.S.R.). In this epizootic 500,000 cattle died of rinderpest only in Great Britain in two years. In Africa infection gained repeated access to the Nile Valley from Europe in 1805, 1828 and 1865. According to Curasson (1932, 1942) it spread as far as Sudan and West Africa.

The most severe epizootic of recent times resulted with the introduction of Indian cattle in Somaliland during the Italian invasion of Ethiopia in

1889 (Lugard, 1893 and Hutcheon, 1902). It spread across Kenya, Uganda by 1890 and as far south as Lake Nyasa by 1892. In Rhodesia, it was reported in 1896 and by 1897 spread throughout South Africa, Angola and S.W. Africa. Cattle losses in Rhodesia were 1.5 million, in South Africa 2.3 million and 1.0 million in Botswana (Curasson, 1932, 1942).

In Asia rinderpest has continued to persist particularly in the South and South East as well as in the Indian sub-continent. For example, in 1959 there were about 8,000 outbreaks in India alone (Anon, 1965). The major epizootic of rinderpest in Pakistan appeared in 1958 and spread almost in all the Central region and many areas in the North West as well as in the South. The losses occured during the period 1958-1962 were estimated at 300,000 heads, and the disease was finally brought under control in 1962 (Akhtar, 1968).

With the exception of isolated reports of cases of fever and diarrhoea from North, South and Southeast regions, such as Loralie "1965-1966", Tharparker "1966-1967", Bahawalpur "1967-1968", Sukur "1968-1969" (Anon, 1970) and Karachi "1975" (Ismail, 1975) no active rinderpest outbreak has been recorded in any part of Pakistan since 1962. At present the country is reportedly free from the disease. It may, however, be pointed out that the reporting system of outbreaks is not very reliable and it is possible that there may be some unrecognized foci of infection in the reportedly free areas.

A control and eradication programme was conducted during 1958-1962 on a country wide basis (Akhtar, 1968) and vaccination was carried out by mobile teams. Systematic vaccination with goat adapted rinderpest virus has kept the disease under control for the last 14 years.

From June 1969 till June 1970, an epizootic of rinderpest occured in Iran, when about 20,000 cattle died from this disease (Hessami, 1972).

North America and New Zealand have remained free of rinderpest, while it was eradicated from Britain in (1877), South Africa (1903), Formosa (1920), Western Europe (1929), Phillipines (1933), Ceylon and Turkey (1934), Ireland (1950) and South India in 1960 (Dalling, Robertson and Boddie, 1966).

1.2. CLINICAL AND PATHOLOGICAL FEATURES OF RINDERPEST

Curasson (1932) who worked in Europe and Africa described seven clinical variations of the natural disease. Scott (1967a) summarized these seven forms into clinical, abortive and inapparent reactions. Incidence of the various forms appear to be related to the innate resistance of the infected stock and to the virulence of the virus. Henning (1949) postulated that resistant races were evolved by the process of natural selection from ancestors, which had survived previous visitation of the disease. The influence of age and innate resistance does not appear to have any effect on the disease epizootiology (Schein, 1917; Curasson, 1932;

Watson, 1950, 1951; Plowright, 1957 and Brown, 1958).

1.2.1. Incubation Period:

The usual incubation period of rinderpest in natural cases is 6 to 9 days (Blood and Henderson, 1963), but Plowright, (1963a) reported an incubation period of 15 days duration in crossbred Zebu cattle of East Africa. In experimental infections the incubation period is considerably shorter (Curasson, 1932; Liess and Plowright, 1964), even though it is influenced by host adaptation of the virus (Scott, 1959a), virus dose (Wolley, 1906; Scott and Macleod, 1955; Piercy, Scott and Witcomb, 1958), the route of infection (Scott and Rampton, 1962) and innate resistance of the host (Lall, 1947). Jezierski, Scott, Wiktor and De Zutter, (1957) have reported that innate resistance of the host did not affect the incubation period. Curasson, (1932) observed that the response of young and adult animals was the same to rinderpest infection, but Plowright, (1957) noticed that the incubation period of rinderpest infection in white Fulani calves was shorter than in adult Fulani cattle.

1.2.2. Clinical Features:

A sharp fever marks the onset of the disease but it is frequently missed, except in the case of lactating cows whose milk yield falls. Within 24 hours the animal becomes ill, and restless. The body coat stares, breathing becomes shallow and rapid. The muzzle

is dry, visible mucous membranes are congested, appetite is impaired and the animal is constipated. Fever reaches its peak in two or three days and falls with the onset of diarrhoea. Mild leukocytosis present at the beginning of illness is replaced by a leukopenia that persists until death or for ~~seven~~ several weeks if the animal survives (Refik-Bey, 1902). Leukopenia particularly involves the lymphocytes and 2 to 3 days after its onset, there is a sudden shift to predominance of immature neutrophils (Maurer, Jones, Easterday and DeTray, 1956). Simultaneously there is an eosinopenia (Refik-Bey, 1902).

When fever is at its maximum, marked depression results. Nasal and lacrimal discharge commence as clear serous exudate which later become mucopurulent. The characteristic lesions appear in the mucosa of the mouth, nose and genital tract as greyish-white pin-heads that enlarge and coalesce. Their necrotic centres readily fall off leaving irregularly demarcated shallow erosions with raw red floor. Predilection sites are the lips, gums, buccal papillae and undersurface of the tongue. Similar lesions develop later on the hard palate. The irritation stimulates salivation and the breath becomes foetid. The eyes become shallow with bright red conjunctivae and mucopurulent lacrimal discharge is profuse (Henning, 1956).

Diarrhoea persists for about a week or so. The faeces are watery dark and emit characteristic odour. The animal strains frequently. In fatal cases diarrhoea becomes progressively worse and contains specks of free

blood, mucus and shreds of epithelium. There is rapid dehydration, which leads to enaciation, prostration and death. Most of the clinical cases die within 6-12 days after the onset of illness (Curasson, 1932). Pregnant cows which survive mostly abort. Convalescence is prolonged and animals take several weeks to regain good bodily condition.

In peracute cases which are often seen in virgin epizootics animals die suddenly before any of the clinical signs become evident.

In enzootic areas subclinical or mild infections are frequently encountered and may be difficult to diagnose (Eggebrecht, 1910; Boynton, 1928 and Jacotot, 1929), but recent advances in serological techniques have facilitated their detection (Plowright, 1962a).

1.2.3. Pathological Findings:

Intensity of the lesions varies with the innate resistance of the animal (Thiery, 1956a). Rinderpest virus has a major tropism for lymphoid tissues, causing marked destruction of lymphocytes (Maurer, Jones, Easterday and DeTray, 1955, 1956; Thiery, 1956b,c and Khera, 1958a,b,c). Macroscopically the lymph nodes are swollen, oedematous and congested. The lymph nodes in animals that die late are shrunken, greyish and show radial streaks in the cortex.

Microscopically there is massive destruction of lymphocytes, particularly in the germinal centres of the lymphoid follicles, which are replaced by an

eosinophilic matrix (Maurer, Jones, Easterday and DeTray, 1956). Multinucleated syncytia in the affected lymph nodes are scattered throughout the cortex (Thiery, 1956b; and Khera, 1958a). The Peyer's patches are usually swollen, haemorrhagic and necrotic. They are often covered by diffuse fibrinonecrotic deposits. Occasionally the whole patch sloughs off. The caecal-tonsil at the junction of the caecum and colon is similarly and invariably affected, but the degree of congestion is greater.

Other characteristic lesions are found in the alimentary tract. Petechial erosions and necrotic encrustations of the lower lip, adjacent gums, the cheeks, underneath the tongue and the posterior hard palate, are common. The lesions may extend to the pharynx and œsophagus. The muzzle and nasal nopic are congested, haemorrhagic and often ulcerated. The erosions are covered by putrid greyish yellow necrotic crusts.

Histologically necrosis begins on the stratified squamous epithelial cells of the prickle cell layer. Their nuclei become pyknotic and fragmented, syncytia are frequently seen in this region (Thiery, 1956b; Khera, 1958b; Plowright and Ferris, 1959a). Necrotic lesions seldom penetrate below the stratum germinativum of the epithelium.

Lesions are rare in the forestomachs, if present, they are sited on the pillars of the rumen and omasal leaves. The abomasal folds appear congested

and oedematous. Erosions are linear and lie along the margins of the folds. The pyloric region of the abomasum is more commonly attacked and necrosis of the epithelium produces grey round patches, that slough off to leave sharply marginated erosions. The underlying tissue is edematous, the congested capillary base is haemorrhagic and the ulcers often contain blood clots. The lesions in the small intestines are of the same general character, but less intense. Those in the large intestine are characterised by haemorrhagic stripes which may extend from the blind end of the caecum to the anus. The ileo-caecal valve is often congested. In peracute fatal cases the heart demonstrates variable degree of sub-endo-cardial haemorrhages in the left ventricle and sub-epicardial petechiae at the base and along the coronary grooves.

The mucous membrane of the bladder is usually irregularly congested. The vaginal mucosa often demonstrates lesions similar to those which develop in the oral mucosa.

1. 3. PATHOGENESIS:

All the domestic cattle and water buffalo are susceptible to natural infection with rinderpest virus and these species constitute the most important natural hosts. In recent years considerable attention has been paid to the possibility of wide spread infections in small domestic ruminants as an important factor in the maintenance of rinderpest virus in enzootic areas. The

literature on this subject has been reviewed by Curasson, (1932, 1942), Bhanda and Manjrekar, (1952) and Scott, (1955). Rinderpest has long been known to affect domestic swine in Asia (Carre and Fraimbault, 1898; Boynton, 1916). Inapparent form of rinderpest has also been observed in European type pigs by feeding infected materials or by contact with infected cattle (Scott, Defray and White, 1959, 1962); Plowright, (1963a) made a serological survey which has clearly indicated that game population taken as a whole was capable of maintenance of the virus. Strains of virus vary also in their virulence, for example, Robson, Arnold, Plowright and Scott, (1959) isolated a strain from an eland, which was avirulent for cattle. Seven other strains isolated in East Africa have proved likewise to be nonlethal for cattle (Plowright, 1962b). There as a strain isolated from the epizootic of 1960 in game of Kenya proved more virulent for cattle than the laboratory strain (Macowan, 1961). Similarly peste des petits ruminents virus killed sheep and goats but was avirulent for cattle (Mornet, Gilbert, Orue, Thiery and Mamadou, 1956).

1. 4. CHARACTERISTICS OF RINDERPEST VIRUS:

1.4.1. Classification:

Rinderpest virus is now classified as a member of the paramyxovirus group (Wildy, 1971; Andrewes and Pereira, 1972). On the basis of cytopathogenicity in vitro, Plowright and Ferris, (1957) /and/ suggested classification of the virus in the group III of Ender's (1954) among viruses causing large syncytial aggregates. About the same time the immunological relationship between rinderpest and canine distemper viruses (Adams and Imagawa, 1957; Carlstrom, 1957); rinderpest and measles viruses (Plowright and Ferris, 1959a) was demonstrated. This prompted Imagawa, Goret and Adams, (1960) to propose that measles, canine distemper and rinderpest viruses constituted a closely related group. But their classification remained obscure. Cooper, (1961) even classified them together with herpes viruses. Electron microscopy rendered this hypothesis untenable. The morphology of measles (Waterson, Cruickshank, Laurence and Kanarek, 1961), rinderpest (Plowright, Cruickshank, and Waterson, 1962) and distemper (Cruickshank, Waterson, Kanarek and Berry, 1962) viruses had a structure similar to that of Newcastle disease virus and other "larger myxoviruses" (Waterson, 1962). The virion is a spherical structure enclosed in an envelope; the nucleocapsid is helical. The ribonucleic acid nature of the genome was demonstrated by Wild and others (cited by Wild, Underwood and Brown, 1974). Due to

the close immunological relationship between measles, canine distemper and rinderpest virus, these viruses are generally known as the morbipest triad of the paramyxovirus group (Melnick and McCombs, 1966).

1.4.2. Morphology:

Carmichael and Hughes estimated the size of the virus particles to be 84-126 millimicron by passing bovine tissue extracts through collodion membrane (Simmons, 1941). With the aid of the electron microscope, the diameter of the virion has been shown to be 120-300 millimicron (Plowright, Cruickshank and Waterson, 1962; Breese and De Boer, 1963).

Plowright, Cruickshank and Waterson, (1962) showed that the RBOK strain at the 91st calf kidney passage had a definite outer membrane, often fringed with short projections. Internally there was a tightly coiled component approximately 18 millimicron in diameter and characterized by easily resolved serrations along the length. The periodicity of the serrations was 5-6 millimicron. Waterson, (1963) found that the limiting membrane contained both viral and cellular proteins, as well as lipids. He considered the internal structure to be ribonucleic acid coated along its coiled length with regularly arranged molecules of protein.

1.4.3. Nucleic acid and other biochemical properties:

The characteristics of the virus and its RNA depended on the passage history of the virus. Undiluted passage virus had a density greater than 1.21g/ml in potassium tartrate gradients, while virus obtained by dilute passage had a density of 0.124g/ml. Both diluted and undiluted passage viruses contained polypeptides with molecular weights of 98, 79, 75, 66, 45, 43 and 37 x 10^3. The molecular weights of the major polypeptides are similar to those of polypeptides of measles and canine distemper viruses (~~Hall~~, Underwood and Brown, 1974).

1.4.4. Resistance to physico-chemical agents:

Rinderpest virus has long been known to be highly labile in tissues of dead animals (Theiler, 1897a; Shilston, 1917; Daubney, 1931 and Piercy, 1956). Scott, (1959b) studied the heat stability of Kabete "O" and caprinized strains in lymphoid tissues and found the half life periods to be 2.3 days at 7°C; 6.4 hours at 25°C; 105 minutes at 37°C and 5 minutes at 56°C. Inactivation in heparinized blood at 25° and 37°C was significantly slower than in the other tissue suspensions. Scott, (1964) suggested that this was due to protection of virus either by plasma proteins or by leukocytes. Ox serum was also found to have a protective capacity on heat inactivation of cell culture virus (Plowright and Ferris, 1961).

The virus is sensitive to repeated cycles of freezing and thawing (Scott, 1959b; Grieff, Rightsel and Schuler, 1964; Anon, 1966), but such losses may be reduced by the addition of 2% dimethyl sulphoxide to the suspending medium. However, frozen stocks are stable at -25°C and -70°C for upto six months (Plowright and Ferris, 1961). During freeze drying there is upto 80% loss in virus titre (Daubney, 1951; Andrianne, Scott and Viktor, 1957; Nguyen-Ba-Luong, Nguyen-Van-Liem, Nguyen-Ngoc-Minh and Vu-Thien-Thai, 1959 and Johnson, 1962a). Such losses may be minimised by the addition of preservatives to the suspending medium, like defibrinated or citrated blood (Cheng and Fishman, 1949; Scott, 1954), peptone water (Nguyen-Ba-Luong, Nguyen-Van-Liem, Nguyen-Ngoc-Minh and Vu-Thien-Thai,1959) or 2.5% lactalbumin hydrolysate and 5% sucrose (Plowright, Rampton, Taylor and Herniman, 1970). Freeze dried virus is stable at -20°C (Johnson, 1962b; Plowright, Herniman and Rampton, 1971).

Rinderpest virus is labile to sunlight and ultraviolet light (Theiler,1927b; Macowan, 1956; Plowright, Herniman and Rampton, 1971).

Putrifaction of infected carcases rapidly destroys the virus (Edwards, 1925; Curasson, 1932). Rinderpest virus is pH labile, and is best stable over the pH range 7.2 - 8.0 (Daubney, 1928; Lecan and Plowright, 1963a). It is ether sensitive (Plowright, 1962b; Waterson, 1962). Glycerine, phenol, formalin and chloroform readily destroy the infectivity but not

the antigenicity of the virus. Consequently these agents have been used in the past to prepare inactivated vaccines (Bruner and Gillespie, 1961).

1.4.5. Antigenic structure and relationships:

Like other members of the MED group, strains of rinderpest virus show an immunological homogeneity. Complement fixation and gel diffusion tests have been used to study antigenic components of rinderpest virus. The complement fixing antigen is smaller than and distinct from the infectious particles. It is stable to heating, freezing and dessication (Sasaki, 1931; Kakizaki, 1934; Nakamura and Goto, 1941; Nakamura and Kishi, 1952; and Nakamura, 1956).

In ouchterlony double diffusion tests two precipitinogens may be observed viz - a heat labile and heat resistant antigen (White, 1958a,b; Plowright, 1962b; Scott and Brown, 1961; Stone, 1960). White and Cowan, (1962) considered the heat labile precipitinogen to be protein in nature but different from infectious particles. Scott and Brown, (1961) and White, (1962) suggested that the complement fixing and the precipitating antigens were similar but Plowright, (1962b) regarded them to be different.

There is only one immunological type of rinderpest virus (Jacotot, 1931; MacOwan, 1961; Robson, Arnold, Plowright and Scott, 1959; Plowright, 1962b). In West Africa a disease known as "peste des petits ruminants" has been reported in sheep and goats, and / a

is clinically, virologically and serologically identical to rinderpest (Mornet, Orue and Gilbert, 1956; Mornet, Orue, Gilbert, Thiery and Mamadou, 1956; Gilbert and Monnier, 1962). Cattle are not infected by contact with sick sheep or goats. It appears that the actiological agent is a strain of rinderpest virus which has lost its capacity to infect cattle by the natural route.

Recently Imagawa, Goret and Adams, (1960) showed that samples of rinderpest antiserum uniformly contained neutralizing antibodies to measles and distemper viruses. The homologous titre was higher than the heterologous titres. Delay, Stone, Karzon, Katz and Enders, (1965), reported immunological reaction with measles, distemper and rinderpest viruses. Monkeys inoculated with distemper virus had antibody response to distemper and rinderpest viruses, but none to measles virus. On the other hand, cattle inoculated with distemper virus showed no response to rinderpest or measles virus. Likewise, dogs inoculated with rinderpest virus showed antibody response to rinderpest and measles virus but not to canine distemper virus. However, dogs were refractory to challenge with virulent canine distemper virus. Dogs inoculated with measles virus had low antibody titre to rinderpest and none to distemper virus. All dogs, however, were refractory to distemper challenge and showed marked increase in antibody level to all the three viruses after challenge with the three viruses. Measles is the only member of this group which has the property of agglutinating certain red blood cells (Weaies and Chany, 1960).

1.4.6. In vitro cultivation:

Until relatively recently when Plowright and Ferris, (1957) demonstrated the adaptation of Kabete "O" strain to growth in calf kidney monolayer cultures, the only practical method of cultivating rinderpest virus was by animal inoculation, mainly goats (Edwards, 1930) and rabbits (Nakamura, Wagatuma and Fukusho, 1933). Most authors have used calf kidney monolayer cultures for the propagation and assay of rinderpest virus (Plowright and Ferris, 1957; DeBoer, 1961; Provost and Villemot, 1961; Johnson, 1962a, Plowright and Ferris, 1962b; Plowright, 1963b; Plowright, Herniman and Rampton, 1969). The virus also replicates in calf testis cells (Huygelen, 1960), lamb, pig and dog kidney monolayer cell cultures (Plowright and Ferris, 1957; Plowright and Ferris, 1959a; Plowright, 1962c), Hela cells (Lieus and Plowright, 1963b) and Vero cells (Rweyemamu, personal communication), but not in rabbit kidney or lymph node cells (Plowright and Ferris, 1959a; Takematsu and Morimoto, 1954). Nakamura, Motohashi and Kishi, (1958), grew the LA strain in suspended fragment cultures of fowl embryos.

CPE and virus yield are optimal in rapidly growing calf kidney monolayers, hence Plowright and Ferris, (1957), Plowright, Herniman and Rampton, (1969) preferred inoculation of virus into freshly trypsin dispersed cells and incubating cell cultures were rolled throughout the virus growth cycle. Cytopathogenic

changes were seen as early as the 3rd day post inoculation (Plowright and Ferris, 1959a). The CPE of rinderpest virus is characterised by the formation of multinucleated syncytia with long fine anastomosing processes. Infected cells frequently contain eosinophilic cytoplasmic and intranuclear inclusions, depending on the virus strain, the cell type and the stage of infection. These syncytia may vary from small angular to rounded refractile structures containing 2 to 3 or more nuclei with ill-defined edges of the common cytoplasm.

In BK cultures infected at seeding with large inocula of the RBOK strain of virus two cycles of syncytium formation occur. The first beginning on the 4th or 5th day and reaching its maximum on the 6th to 10th day. It is characterised by large numbers of relatively small, heavily vacuolated syncytia containing a central, granular mass including nuclei. The second cycle reaches its peak 14th to 18th day. It consists largely of the fusion of small, vacuolated syncytia and single cells. These expand and form glossy sheets containing one or more centrally located clumps of nuclei with large homologous cytoplasmic inclusions surrounding them. The centre of the nuclear ring is often occupied by amorphous granular material (Plowright and Ferris, 1959a; Johnson, 1962a). Small spindle-shaped syncytia are found in BK cultures infected with recent field isolates of low cattle virulence (Plowright, 1962a, 1963c).

Masses of deeply eosinophilic material are seen in the cytoplasm of syncytia which gradually increase with aging. The earliest inclusions were small, granular and outlined by a narrow clear zone but later they fused and became homogenous, forming continuous masses surrounding the nuclei of syncytia. Single nuclei usually contained one or two eosinophilic bodies, the larger ones showed an irregularity of structure, suggesting vacuolation, but the smaller ones were homogenous (Plowright and Ferris, 1957; 1959a).

1. 5. IMMUNITY AND IMMUNIZATION IN RINDERPEST:

Immunity to rinderpest has been studied for many years and several immunizing methods have been tried. Recovery from infection is followed by a lifelong immunity.

Artificial active immunity was first produced by the injection of immune serum and virus simultaneously (Kolle and Turner, 1897). Although this method was successful, it was abandoned because of the high cost of serum, and the use of live virus which spread infection to new areas.

Later, chemically inactivated vaccines were tried; such as Boynton's tissue vaccine, prepared by the addition of phenol and glycerin to lymphoid tissues of rinderpest infected cattle. The mixture was heated to 40°C and held for 3 hours to accomplish inactivation. The final concentration of phenol was 0.5% (cited by Boynton, 1928).

Chloroform inactivated vaccine prepared according to Kelser's (1929) method cited by Bruner and Gillespie, (1961) was also found to be useful in producing active immunity. Cattle inoculated with virulent rinderpest virus were sacrificed during the acute stage of the disease and chloroform was added to make 0.75% concentration to the lymphoid tissue emulsion. After 48 hours the vaccine was ready to use. These methods of vaccination were superseded by the use of live virus attenuated by passage in goats (Edwards, 1930), rabbits (Nakamura, Wagatuma and Fukusho, 1938), chicken embryos (Jenkin and Shope, 1946) and tissue culture (Plowright and Ferris, 1959b).

When the virulent Kabete strain was passaged in monolayers of calf kidney cells there was at first an exaltation of its virulence, followed after the 10th passage by a phase of increasing attenuation for cattle (Plowright and Ferris, 1959a). No clinical rinderpest disease signs were observed in animals immunized by virus of the 21st to 45th passage levels except in a few and they only had mild transient fever. The attenuated strain was supplied to Nigeria in its 65th passage and was used there as a vaccine at the 66th to 70th passage levels (Johnson, 1962b). In East Africa the virus was used as a vaccine at the 90th to 96th passage levels (Plowright, 1963b). Johnson and Smith, (1962), and Plowright, (1963b) published their methods for the production of cell culture vaccine. Their

techniques were essentially similar. Seed culture adapted virus was mixed with calf kidney cells suspended in growth medium, dispensed into flat bottles and incubated at 36-37°C. The optimal dose of virus inoculation was $10^{3.7}$ to $10^{4.6}$ $TCID_{50}$ per millilitre of the cell suspension. The fluids were harvested 5-7 days when cytopathic effects were well defined. In Nigeria the harvest was mixed with an equal volume of mist desiccans, containing 2% lactalbumin hydrolysate alone. In East Africa protective additive was 2.5% lactalbumin hydrolysate and 5% sucrose. The vaccine was freeze dried in ampules. Potency and safety tests were carried out in cattle, guinea pigs and cell cultures. Each field dose contained 50 to 100 $TCID_{50}$.

Stable characteristics of cell culture vaccine virus are its high degree of attenuation for cattle (Plowright and Ferris, 1962b) and its inability to spread by contact (Plowright and Ferris, 1959b; Provost, 1961; Johnson, 1962b) and inapparent reactions in vaccinated East African cattle (Plowright, 1962c). In Nigeria, however, mild fever was observed in most nonresistant cattle and even in 34% of vaccinated Zebus. There were no other clinical signs (Johnson and Smith, 1962). Cattle with trypanosomiasis were not adversely affected (Provost, 1961). Seven back passages of the culture vaccine in cattle did not alter its level of attenuation (Plowright and Ferris, 1962b; and Johnson, 1962b).

Cattle inoculated with cell culture vaccine resisted challenge with virulent virus 14 days later (Plowright and Ferris, 1959b; Johnson, 1962b). Early protection to challenge was observed within 3 to 5 days of vaccination although serum neutralizing antibody was not detected until 7 days post vaccination (Plowright and Ferris, 1959b; Taylor and Plowright, 1965).

Plowright and Ferris, (1962b) observed that following higher dose inoculation of the vaccine, neutralizing antibody could be detected earlier than animals which were immunized by low dosage. Taylor and Plowright, (1965) reported generalization of virus within 96 hours of subcutaneous inoculation with $10^{4.6}$ TCRV but serum neutralizing antibody remained undetectable until the 7th day of virus infection. Recently Okuna and Rweyemamu, (1974) detected rinderpest neutralising antibody on the 7th day following subcutaneous inoculation of cattle with $10^{4.7}$ $TCID_{50}$ of TCRV.

The duration of immunity according to Johnson and Smith, (1962) and Plowright, (1963b) was over two years. Recently, Rweyemamu, Reed and Okuna, (1974) reported that animals inoculated with TCRV, 6 to 11 years previously resisted ~~to~~ intranasal challenge confirming the earlier suggestions by Plowright and Taylor, (1967) that TCRV confered life long immunity.

1. 6. OBJECTIVES FOR A STUDY OF VIRAL INTERFERENCE IN RINDERPEST INFECTION:

Despite the extensive studies carried out on rinderpest vaccination and more particularly on the tissue culture rinderpest vaccine, there has been little attention paid to the production of interference either *in vitro* or *in vivo* as a result of rinderpest virus infection. However, other viruses of the paramyxovirus group have been shown to induce interferon production. Rosenquist and Loan, (1969) demonstrated low levels of circulating interferon in calves inoculated with Newcastle disease virus. Measles virus, a member of the midpest triad (Melnick and McCombs, 1966), has been shown to produce interferon both *in vitro* and *in vivo* by De-Maeyer and Enders, (1965); Petralli, Merigan and Wilbur, (1965a).

That interference might play a part in protection from rinderpest infection has been a matter of conjecture for a long time. Pfaff, (1938) observed that cattle and buffalo resisted challenge with virulent rinderpest virus 48 hours after inoculation with 20-40 50% minimum infective dose (MID_{50}) of caprinised rinderpest virus before antibodies were detected. This observation was further confirmed by Wild and Scott, (1961). Similarly Brotherston, (1951a) and Simpson, (1954) reported that lapinised rinderpest virus (Nakamura-III strain) protected vaccinated animals 84-108 hours post infection; while avianized rinderpest

virus (Gross Isle strain) afforded protection 96 hours after infection, although antibody did not appear until the 8th day (Hale, Walker, Maurer, Baker and Jenkins, 1946). Using tissue culture attenuated rinderpest virus at a dose of $10^{4.5}$ 50% tissue culture infective dose ($TCID_{50}$), Plowright and Finter, (1959b) noticed protection against a challenge dose of 10^{5} to 10^{6} cattle ID_{50} of virulent noncontagious rinderpest virus, 72 hours after vaccination, although neutralising antibody was not detected until the 7th day at the earliest (Taylor and Plowright, 1965) and they attributed this early protection to viral interference.

Plowright and Finter (cited by Plowright, 1968) were able to detect a slight depression in rinderpest virus growth in calf kidney cell cultures which had been pre-inoculated with Sindbis virus. They suggested that this depression was due to interferon. Recently Fujisaki, Ishi and Watanabe, (1968) described an interferon-like substance produced in rabbits inoculated with modified lapinised rinderpest virus.

In the absence of authenticated reports, it was decided to study viral interference in rinderpest and to try and evaluate its role in early protection of buffalo afforded by the tissue culture vaccine.

CHAPTER 2

REVIEW OF THE LITERATURE ON INTERFERENCE

2. 1. Viral interference:

The observation that attenuated rinderpest vaccines induce protection long before antibody can be detected in the sera raised speculation that protection was mediated through interferon. This constitutes the central objective for the study in this thesis. In order to provide a background to this study the following paragraphs summarize the important aspects of viral interference.

Interference was first described by Tobie (1935), who noticed that neurotropic strain of yellow fever virus protected monkeys against infection with lethal viscerotropic strain. Subsequent reports with interference between strains of one virus differing in their biological properties such as, tissue affinity and adaptation to experimental hosts (Doerr and Seidenberg, 1937; Andrewes, 1942; Duffy, 1944; Green and Stulberg, 1937; Wellman, 1937; Doerr and Seidenberg, 1937; Wellman, 1937; Andrewes, 1942; Duffy, 1944; Green and Stulberg, 1938; Wilde and Scott, 1961), the Nakamura III strain of lapinized and virulent strains of rinderpest virus (Brotherston, 1951a, Ullerstein and Garret, 1954; 1954), Grosse Isle strain of aviatised and the mother strain of rinderpest virus (Jenkins and Scott, 1961)

Dale, Walker, Maurer, Baker and Jenkins, 1948), the Hatete "O" culture attenuated and the parent strain (Blowright and Ferris, 1959b; Johnson, 1962b). Finally naturally occuring variants of one virus with low and high pathogenicity were employed to demonstrate interference between strains of Newcastle disease viruses (Bang, 1949).

These examples of interference are between agents immunologically closely related, if not identical. Such close kinship is not a prerequisite for the occurence of interference as it has also been observed to occur, between more distantly related agents, such as between ectromelia and vaccinia viruses (Andrewes, Elford and Niven, 1948), between members of the psittacosis - lymphogranuloma venereum group agents (Golub and Wagner, 1948a,b) and between influenza "A". Swine influenza viruses (Sigel, 1944; Eichler and Horsfall, 1944) and between immunologically unrelated viruses such as Columbia SK and EM with poliomyelitis viruses (Jungeblut and Sanders, 1940; Jungeblut and Sanders, 1942; Sanders and Jungeblut, 1942; Jungeblut, 1945), foot and mouth disease and rabies viruses (Levaditi and Koury, 1943), Western equine encephalomyelitis and Newcastle disease viruses (Gildemeister and Herzberg, 1925; Gildemeister and Haln, 1932) influenza "A" and Newcastle disease viruses (Florman, 1948; Bang, 1949), Newcastle disease and poliomyelitis viruses (Reagan, Lillie, Hauser, Poelma and Buechner, 1947), rabbit papiloma and sheep dermatitis viruses

(Selbie, 1946), vaccinia and rabies viruses (Levaditi and Nicolau, 1925) and vaccinia with foot and mouth disease viruses (Gildemeister and Helm, 1932).

It was noted first in the case of bacteriophage by Luria and Delbruck, (1942) that the interfering property of a virus may also be retained upon inactivation of the agent to such an extent that it was no longer able to propagate. Similar observations were made between inactivated and active animal viruses such as Eastern equine encephalomyelitis with mumps (Bang, 1949), fowl pox with herpes simplex (Anderson, 1942), herpes simplex with rabies (Anderson, 1942), influenza "A" with rabies (Vilches and Hirst, 1947), lymphocytic choriomeningitis with distemper (Dalldorf and Douglass, 1938), mumps with Newcastle disease and vice-versa (Bang, 1949), poliomyelitis with rabies and vice-versa (Levaditi, 1943), yellow fever with Venezuelan equine encephalomyelitis (Lennette and Koprowski, 1945).

In vivo interference is best demonstrated when infection of an interfering virus is given a few days before challenge virus (Jungeblut and Sanders, 1942; Jungeblut, 1945).

In the case of live viruses the assumption is that the interfering virus has to multiply sufficiently before interference can be observed (Henle, 1950). In agreement with this interpretation is the fact that the interval required is shorter, when the dose of the primary administered virus is larger (Ziegler

and Horsfall, 1944; Golub and Wagner, 1948a). On the other hand if the interval between interfering and challenge infections is too long, interference may no longer be demonstrable (Jungeblut and Sanders, 1942; Dalldrof and Whitney, 1943; Vilches and Hirst, 1947). This limitation is for immunologically unrelated agents.

In the case of immunologically related viruses the state of interference run into immunity (Hallauer, 1937; Doerr and Kon, 1937; Doerr and Seidenberg, 1937; Green and Stulberg, 1946; Golub and Wagner, 1948b) and the appearance of antibodies in serum is preceeded by antibody formation in the affected organ (Morgan, 1947; Schlesinger, 1949). The first demonstrable appearance of antibodies in the serum of experimental animals however depend upon the sensitivity of methods used for the assay of antibodies.

In the case of superinfected virus, antibody formation precedes the interfering virus, but several factors determine failure or success. Such as the interfering virus has to be markedly in excess of the one to be excluded (Andrewes, 1942; Jungeblut and Sanders, 1942; Sanders and Jungeblut, 1942; Sigel, 1944; Ziegler and Horsfall, 1944; Vilches and Hirst, 1947; Golub and Wagner, 1948a), or has to be repeated (Jungeblut and Sanders, 1942; Jungeblut, 1945) in order to attain the desired results, or the interfering virus must multiply at a faster rate than the agent to be inhibited (Findlay and Maccallum, 1937; Dalldorf,

Douglass, 1938; Ziegler and Horsfall, 1944; Andrewes, Elford and Niven, 1948) and finally the interfering virus may have to be given by a route which permits it to reach susceptible organs or tissues in advance of the virus to be excluded which is injected by a different route (Green and Stulberg, 1946). In every instance, an interfering effect may be obtained when blocking virus is administered within a relatively short period of time after the virus to be excluded (Hoskins, 1935; Findlay and Maccallum, 1937; Dalldorf and Douglass, 1938; Jungeblut and Sanders, 1942; Ziegler and Horsfall, 1944; Jungeblut, 1945; Green and Stulberg, 1946; Vilches and Hirst, 1947; Bang, 1949). The interval however, depends to a large extent upon the rate of propagation of the primary infecting virus (Henle, 1950).

Demonstration of interference in the chick embryo (Henle and Henle, 1943), in tissue explants and in tissue culture (Huang, 1943; Lennette and Koprowski, 1945; Schlesinger, 1951) which are lacking / immune mechanism afforded further interest in its understanding but the mechanism was poorly understood until the discovery of interferon by Isaacs and Lindenmann, (1957).

2. 2. Interferon:

Interferon is a cellular protein produced in response to, and acting to prevent replication, of an infecting virus within the invaded cell. Interferon can be produced in cells both in tissue culture and in the intact animal (Kleinschmidt, 1972). De Maeyer, DeMaeyer-Guignard and Jullein, (1969), have obtained evidence from radioisotope tracer studies that lymphocytes are the primary source of interferon induced by myxoviruses. In animals, interferon produced by cells of the reticuloendothelial system and circulating in the blood is taken up by other cells.

Interferon was originally described as a product formed by incubation of heat inactivated (1 hour at 56°C) influenza virus with isolated pieces of the chick chorioallantoic membrane (Isaacs and Lindenmann, 1957). Subsequent investigations showed that interferon was indeed distinct from particles of inactivated virus, that is, it was produced by chorioallantoic membrane cells, after they had been in contact with inactivated virus, but not in the absence of incubation with virus. Studies of physicochemical properties revealed that interferon was stable on dialysis against buffers ranging from pH 1-10. It was precipitated by ammonium sulphate, its activity was sensitive to the action of trypsin but resistant to ribonuclease and deoxyribonuclease, and it was not sedimented by centrifugation for 10 minutes at 100,000 x g. It was also shown that, interferon did

not inactivate virus directly, but rendered cells resistant to virus (Isaacs, Lindenmann and Valentine, 1957; Lindenmann, Burke and Isaacs, 1957).

It was soon realized that the procedure used for the inactivation of virus had a profound effect on the amount of interferon produced. Ultra-violet (UV) inactivated influenza virus was found to be a better inducer of interferon synthesis than heat inactivated virus (Lindenmann, Burke and Isaacs, 1957; Burke and Isaacs, 1958a). Virus inactivated by heating for 1 hour at 60°C did not induce interferon synthesis and caused hardly any detectable degree of interference (Isaacs and Lindenmann, 1957). Increasing the dose of UV-irradiation also decreased, and finally abolished, the interferon inducing capacity of influenza virus (Lindenmann, Burke and Isaacs, 1957; Burke and Isaacs, 1958a). All these results suggested that the ability of inactivated influenza virus to induce interferon synthesis and interference were closely linked and led to the belief that interferon was responsible for viral interference (Burke and Isaacs, 1960).

Burke and Isaacs, (1958b) showed that other myxoviruses, inactivated by UV-irradiation, elicited interferon synthesis in chorioallantoic membrane cells. Henle, Henle, Dienhardt and Bergs, (1959) demonstrated that interferon was induced by active or inactivated myxoviruses in stable cell lines. Tyrrell, (1959) found that interferon was formed during the growth of

live influenza virus both in isolated pieces of chicken chorioallantoic membrane and in cultures of calf kidney cells. Using cells infected with a live attenuated strain (RMC) of polio virus, Ho and Enders, (1959a), found the synthesis of a virus inhibitory substance which turned out to be interferon. Ho, (1966) reported that interferon could be induced by papova, herpes, pox, picorna, myxo and arbo viruses. Induction of interferon by adeno viruses was shown by Beladi and Pusztai, (1967). However the efficiency with which interferon synthesis is elicited varied greatly from one virus-cell system to another (Ho and Kohler, 1967). By the same token interferon has been shown to inhibit the multiplication of virtually all viruses. But again the degree of inhibition is very variable and depends on the sensitivity of the particular virus and cells to the action of interferon. Parkman, Buescher, Arlenstein, McCowan, Mundon and Druzd, (1964) and Vilcek, (1964), observed that cells which were more sensitive to the action of interferon, were more resistant to viruses.

Baluda, (1957 and 1959) observed interference between inactivated and active Newcastle disease virus which was not mediated by interferon. Instead, the inactivated virus acted by destroying the viral receptor sites on the surface of sensitive cells. Depending on the dose of interfering virus, Baluda, (1957) found that maximum resistance was reached on 0.1 to 6 minutes.

The cells reverted to their original sensitivity after 36-60 hours, which appeared to be the time required for the regeneration of cell receptors. A similar mechanism of interference was demonstrated in some coxsackie-viruses (Crowell and Syverton, 1961).

Henderson and Taylor, (1961) studied interference between Mayaro and Sindbis viruses in chick embryo cell cultures. Live Mayaro interfered with the multiplication of Sindbis virus. The multiplication of Sindbis virus was completely inhibited when it was inoculated as early as 1 hour after infection with a high multiplicity of Mayaro virus. Since interferon did not appear in Mayaro infected cultures until much later, the authors called this early interference "infection interference" as opposed to the interferon mediated interference which developed later. But they did not show whether Sindbis virus was adsorbed on Mayaro infected cells or not. Infection of cells, with undiluted vesicular stomatitis virus provides lower yields than infection with diluted virus (Cooper and Bellett, 1959). Huang and Wagner, (1966) in a series of experiments showed that: (1) this lower yield was due to interference of defective T particles with infectious B particles present in the same virus stock. (2) Purified T particles completely inhibited the growth of homotypic virus. (3) The growth of B particles was inhibited if T virus was added as late as 30-120 minutes after B. (4) Short exposure to UV-light destroyed the

interfering capacity of T particles, while their adsorption was still impaired. This finding indicated the importance of the ribonucleic acid (RNA) of T particles for the interference. (5) Interferon was not involved because it was not induced by T and also because no interference was demonstrated with interferon sensitive heterologous EMC virus. This type of interference then, represented a highly specific homologous (Homotypic) interference between defective and complete virus particles of the same serotype, such as between incomplete and complete influenza virus described by Magnus, (1954). Another type of viral interference not mediated by interferon was described by Marcus and Carver, (1965). It was revealed, when green monkey kidney cell cultures after inoculation with rubella virus were challenged 3-4 days later with Newcastle disease virus and tested for haemadsorption after a further 15 hours incubation. Infection of the cells with the latter virus was detected by adsorption of red blood cells added to the culture. But cells infected with rubella virus failed to support the multiplication of Newcastle disease virus as foci of nonhaemadsorbing cells were seen. The unique feature of this type of interference was the specificity against challenge virus. Because fully refractory to latter virus, rubella infected cells did not show resistance to ECHO 11, polio type 1, vaccinia, influenza B and some other viruses. So interferon was not responsible for

this interference. Moreover, the action of [illegible] did not influence the viral interference (Marcus and Carver, 1967). Similarly rubella virus induced interference was also not due to the destruction of receptors for Newcastle disease virus, since adsorption of the latter virus on infected cells was not impaired. Marcus and Carver, (1967), called this type of interference "intrinsic interference". Sindbis, West Nile and Polio viruses have also been shown to induce intrinsic interference against Newcastle disease virus. The mechanism underlying this type of interference is not understood, but it is thought to depend upon the synthesis of viral protein (Marcus and Carver, 1967).

An interesting aspect of intrinsic interference is the fact that rubella virus is also known to induce interferon in human amnion and bovine embryo cells (Neva and Weller, 1964).

Thus four distinct types of viral interference could be distinguished that is (1) interference mediated by interferon, (2) interference due to destruction of receptors by interfering virus, (3) homologous interference between complete and incomplete virus and (4) intrinsic interference.

2. 3. Induction of interferon by viruses:

It seems that even before Isaacs and Lindenmann, (1957) detected and named interferon, other investigators (Orskove and Andersen, 1938; Gard, 1944; Lennette and Koprowski, 1945; Nagano and Kojima, 1954), had encountered effects most likely due to interferon but were not fully aware of the importance of their observations (reviewed by Isaacs, 1963). The production of interferon was demonstrated in cell culture inoculated with a wide variety of live infectious viruses, including influenza "A" (Tyrrell, 1959) mumps (Henel, Henel, Dienhardt and Bergs, 1959), attenuated polio virus (Ho and Enders, 1959a,b), parainfluenza-3 virus (Chany, 1960), foot and mouth disease (Dinter, 1960), tickborne encephalitis (Vileck, 1960) and rabies virus (Kaplan, Wecker, Forsek, and Koprowski, 1960). Wagner, (1960) reported the accumulation of interferon in the allantoic fluid of chick embryo inoculated with influenza virus. Isaacs and Hitchcock, (1960) found interferon in the lungs of mice infected with type A influenza virus. Nagano and Kojima, (1958) detected antiviral activity, probably due to interferon, in the extract of rabbit skin lesions which had been produced by vaccinia virus. The list of various virus cell systems in which interferon synthesis had been demonstrated, was published by Ho, (1962). Ho, (1966) reviewed the induction of interferon synthesis by members of all major groups of viruses. He concluded that myxo- and arbo-viruses most readily induce interferon synthesis in most systems.

Arbitrarily cell-virus interactions can be distinguished in three types which result in interferon synthesis, (Blaskovic, 1963). First, after contact of the cells with U-V or heat inactivated or defective virions which are unable to reproduce. Secondly, by live virions in cells, which do not allow the complete replication of another invading virus and thirdly, following infection with live virus undergoing complete replicative cycle. The following viruses have been shown to act as interferon inducers after gentle UV-inactivation or heating at 37°C or 56°C; different types of influenza, Newcastle disease, fowl plague, mumps and Sendai viruses (Burke and Isaacs, 1958a,b; Henle, Henle, Deinhardt and Bergs, 1959; Cantell, 1961), vaccinia (Glasgow and Habel, 1962). Inactivated viruses induced better and earlier interferon synthesis than when used without any treatment (Burke and Isaacs, 1958a; Wagner, 1960; Ho, 1964a).

There are not only great variations in the efficiency of interferon induction synthesis among members of different groups of viruses, but also among different types and even strains of same virus. Wagner, Levy, Snijder, Ratcliff and Hyatt, (1963), studied the induction of interferon synthesis with two variants of the Indiana type of vesicular stomatitis virus. One produced large plaques, multiplied well and led to no detectable synthesis of interferon in L cells. The other one formed smaller plaques, multiplied to lower titres and produced some interferon in the same type

of cells. There was also a marked difference in the sensitivity of the two variants to the action of interferon, the variant producing large plaques being less sensitive than the small plaque variant. Likewise Lockart, (1963) studied the synthesis of interferon with two variants of Western equine encephalomyelitis virus in different lines of mouse L cells. He found that one virus variant which grew to higher titres and produced cytopathic effect, also consistently induced more interferon synthesis than another variant of the same virus which multiplied at a lower rate and was less cytocidal.

Viruses which readily inhibit host cell biosynthesis upon infection not only fail to induce interferon synthesis, but can inhibit the production of interferon induced by another virus. This effect was demonstrated by Wagner and Huang, (1966), working with suspended cultures of Krebs-2 carcinoma cells. Interferon synthesis was rapidly induced in these cells with an avirulent strain of Newcastle disease virus, but not by vesicular stomatitis virus.

Aurelian and Roizman, (1965) compared the behaviour of two strains of herpes simplex virus in cultures of dog kidney cells. One strain multiplied in these cultures and produced no detectable interferon. The other did not multiply but induced interferon when inoculated at a multiplicity of 10 PFU/cell. However, no interferon was detected when the latter strain was

inoculated at multiplicity of 100 PFU/cell. The first strain and the higher multiplicity of the latter strain caused a depression in cellular RNA synthesis and this effect apparently prevented interferon synthesis. Thus interferon inducer virus must not shut off cellular RNA synthesis to a degree which would interfere with the synthesis of the interferon molecule.

2. 4. Induction of interferon by non-viral agents:

Interferon may also be induced by some bacteria and bacterial toxins (Youngner and Stinebring, 1964; Ho, 1964b; Michaels, Weinberger and Ho, 1965; DeSomer and Billiau, 1966; Lukas and Hruskova, 1967; Smith and Wagner, 1967; Nagano, Kojima, Arakawa and Kanashiro, 1966), chlamydia, rickettsia and mycoplasmata (Hoppe, Kohno, Kohno and Smadel, 1964; Kazar, 1966; Merigan, Hanna, 1966; Jenkin and Lu, 1966; Rytel and Jones, 1966; Freshman, Merigan, Remington and Brownlee, 1966; Yersho and Zhdanov, 1965), statolon and helenine (Kleinschmidt, Cline and Murphy, 1964; Kleinschmidt and Murphy, 1965, 1967; Shope, 1948, 1953; Rytel, Shope and Kilbourne, 1966), phytohaemagglutins (Wheelock, 1965) by anionic polymers (Regelson, 1967; Merigan, 1967; Merigan, Frinkelstein, 1968) and nucleic acid (Isaacs, 1961a,b; Rotem, Cox and Isaacs, 1963; Kohlhage, Falke, 1964; Jensen, Neal, Owens and Warren, 1963).

CHAPTER 3

INACTIVATION OF TISSUE CULTURE RINDERPEST VACCINE VIRUS BY ULTRAVIOLET IRRADIATION AND BY HEAT TREATMENT AT 56°C.

3. 1. INTRODUCTION:

The use of noninfective virus is preferred to the use of live virus in interferon studies (Vilcek, 1969). Literature has revealed only scanty information on the rate of inactivation of rinderpest virus by heat (56°C) or ultraviolet (U-V) irradiation. It was therefore considered imperative as the first step to establish conditions for adequate inactivation of tissue culture rinderpest vaccine virus at 56°C and by U-V irradiation.

3. 2. MATERIALS AND METHODS:

Animals:

Bovine calves (cross bred) 1- [illegible] yrs old were used for preparing kidney cultures.

Media and solutions:

Hank's and Wallace (1949) balanced salt solution containing 0.5% lactalbumin hydrolysate (LAH), 0.1% yeastiolate (YE) and 10% bovine serum (BS) was used as growth medium. Maintenance medium comprised Earle's (1943) balanced solution supplemented with 0.5% LAH,

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All the chemicals, LAH, YE and 1:250 Trypsin were obtained from Fisher or Difco Laboratories U.S.A.

0.1% YE and 5% donkey serum (DS). Trypsin solution (0.25%) was prepared in phosphate buffered saline (Dulbecco and Vogt, 1954).

Lactalbumin hydrolysate sucrose vaccine additive:

500 gm of LAH and 1,000 gm of sucrose were dissolved in 10 litres of distilled water. The solution was sterilized by filtration and stored at room temperature.

Virus:

Kabete "O" tissue culture rinderpest vaccine virus strain (TCRV) adapted to culture growth in lamb (Plowright and Ferris, 1957), at 96th passage level in primary bovine kidney cultures was obtained from Dr. Rweyemamu, Head Division of Virus Diseases, East African Veterinary Research Organization, Muguga/Kenya (EAVRO).

Cell culture:

Primary cultures were prepared by the method described by Plowright, Hernimann and Rampton, (1960).

Preparation of virus stock:

Freshly trypsinized calf kidney cells were dispersed in growth medium to a concentration of 1:200 (V/V). 1 ml of were seeded with virus at the rate of log $10^{2.0}$ per ml of cell suspension. 75 ml quantity of cell-virus suspension was then dispensed in each of "Bellco roll bottles." These bottles were initially incubated at 37°C for three days and rolled from 2 hours before the first medium change. After replacing growth medium with

maintenance medium, bottles were replaced on the roller apparatus (rolled at 8 revolutions per minute). On the 5th day post infection (p.i.), medium was replaced again by fresh maintenance medium. On day 7 p.i. more than 80% of the cell population showed cytopathic changes. Bottles were shaken and the cell culture fluid was harvested. This was subjected to light centrifugation (4,000 r.p.m. for 60 minutes at 4°C). Clear supernatant fluid constituted the stock virus. This was dispensed into 15ml aliquots and stored at -70°C till used. At the time of experimentation one bottle containing stock virus suspension was thawed under cold tap water. This was further subdivided into three portions. To the first portion an equal volume of maintenance medium was added. The second portion was mixed with an equal volume of DS, while the third portion was diluted in an equal amount of vaccine additive before inactivation.

Heat inactivation:

Virus diluent was equilibrated to 56°C and at time zero 5ml. virus suspension was pipetted into 5 ml. of prewarmed diluent in bijou bottles (14 ml. capacity) and immersed in a water bath maintained at 56°C temperature (thermostatically controlled and fixed with lid and stirrer). Samples were removed from the water bath at 10 minute intervals. These were cooled rapidly in a bath of ice water and stored at 4°C from 6 to 8 hours.

All the virus titrations were performed in a single batch of bovine kidney primary culture system.

Ultraviolet rays inactivation:

90 mm open Pyrex petri dishes each containing 10 ml. of virus suspension were exposed to U-V rays released from Philips TGC 30 watt germicidal tube connected in series with a constant volt transformer regulated to 220 volts. The virus suspension was placed at a distance of 10 centimeters from the ultraviolet tube where the dose was found to be 1014800 A° in uw/cm^2/second. To ensure adequate exposure virus preparations were shaken manually and the ultraviolet tube was allowed to warm 30 minutes prior to use. During the inactivation process, control virus suspension was kept cool in a bath of ice water and all irradiated samples were stored at $4^{\circ}C$ from 6 to 8 hours. All the virus titrations were performed in a single batch of bovine kidney primary culture system.

Assay of virus infectivity:

10 fold serial virus dilutions were prepared in the cooled maintenance medium and 0.1 ml. of each virus dilution was inoculated into each of a set of five tubes of 48 hours old primary bovine kidney monolayers. The cultures were examined daily microscopically for evidence of cytopathic effect (CPE) upto 12 days p.i. On each occasion, cultures showing definite CPE were discarded. Maintenance medium was changed every 48 hours. Infectivity titres were calculated by the method of Spearman and Karber (Lennett, 1964).

3. 3. RESULTS:

Heat inactivation of the virus:

Results of a series of experiments on inactivation of virus at 56°C suspended in maintenance medium, donkey serum, and vaccine additive respectively are presented in tables 1 to 3. The means of virus survival for this experiment are plotted in figure 1. It is evident from these studies that virus inactivation at 56°C proceeded very rapidly during the first 10 minutes. The half life was 2.5, 3.35 and 4.0 minutes for virus suspended in maintenance medium, 50% donkey serum and vaccine additive respectively. In all the experiments performed with infectious culture fluid diluted in maintenance medium, a small quantity of infective virus survived up to 60 minutes. In the presence of donkey serum a small quantity of residual infective virus was detected after 70 minutes exposure at 56°C and in virus preparations containing vaccine additive some infective virus survived upto 80 minutes at 56°C. The influence of donkey serum and vaccine additive on the rate of virus inactivation at 56°C heat is shown in table 4.

Inactivation by ultraviolet irradiation:

The rates of virus inactivation by ultraviolet rays are shown in table 5 to 7 and in figure 2. It is evident from these studies that inactivation of cell culture rinderpest virus by ultraviolet irradiation proceeded exponentially. A small quantity of infectious

virus was detectable after 110 seconds of ultraviolet irradiation of virus diluted in the maintenance medium and after 250 and 300 seconds of exposure to preparations containing donkey serum and vaccine additive respectively. The respective half life periods were 5, 12 and 16 seconds. A comparison of the effect of donkey serum and vaccine additive is presented in table 8. The vaccine additive stabilized the virus against ultraviolet irradiation better than serum or maintenance medium.

TABLE 1

LOSS OF INFECTIVITY AT 56°C OF CELL CULTURE VIRUS FLUID CONTAINING EQUAL VOLUME OF MAINTENANCE MEDIUM

TIME OF EXPOSURE IN MINUTES	TITRE OF VIRUS (BK/97) $\log_{10}$ $TCID_{50}$/ml					AVERAGE VIRUS TITRE $\log_{10}$ $TCID_{50}$/ml.
	EXPERIMENT					
	1	2	3	4	5	
0	5.8	6.1	5.3	5.6	5.7	5.7
10	3.4	4.1	3.2	3.7	3.1	3.5
20	2.3	2.9	2.4	2.6	2.3	2.5
30	1.5	1.7	1.4	1.6	1.8	1.6
40	0.9	1.2	0.7	0.8	0.9	0.9
50	$1/5^{+}$at 10^{0}	$4/5^{+}$at 10^{0}	-	$2/5^{+}$at 10^{0}	$3/5^{+}$at 10^{0}	$2/5^{+}$at 10^{0}
60	$1/5^{+}$at 10^{0} $1/5^{+}$at 10^{1}	-	$2/5^{+}$at 10^{0}	-	$2/5^{+}$at 10^{0}	$1/5^{+}$at 10^{0}
70	-	$2/5^{+}$at 10^{0} $1/5^{+}$at 10^{1}	$1/5^{+}$at 10^{0}	-	$1/5^{+}$at 10^{0}	$1/5^{+}$at 10^{0}
80	-	-	-	-	-	-
90	-	-	-	-	-	-
Half Life = 2.5 minutes						

Key: A/B^{+} at X = A tubes positive out of a total of B tubes at log X virus dilution.

TABLE 2

LOSS OF INFECTIVITY AT 56°C OF CELL CULTURE VIRUS FLUID CONTAINING 50% DONKEY SERUM

TIME OF EXPOSURE IN MINUTES	TITRE OF VIRUS (BK/97) $\log_{10}$ $TCID_{50}$/ml					AVERAGE VIRUS TITRE $\log_{10}$ $TCID_{50}$/ml.
	E X P E R I M E N T					
	1	2	3	4	5	
0	5.7	5.3	5.6	5.4	5.5	5.5
10	4.5	4.1	3.9	4.3	4.2	4.2
20	3.5	3.3	3.1	3.4	3.2	3.3
30	2.6	2.5	2.3	2.3	2.8	2.5
40	1.9	1.6	2.2	1.9	1.8	1.9
50	1.2	1.3	1.7	1.2	1.1	1.3
60	$1/5^{+}$ at 10^{o}	$3/5^{+}$ at 10^{o}	$4./5^{+}$ at 10^{o}	$1/5^{+}$ at 10^{o}	$1/5^{+}$ at 10^{o}	$2/5^{+}$ at 10^{o}
70	-	$2/5^{+}$ at 10^{o}	$2/5^{+}$ at 10^{o}	-	$1/5^{+}$ at 10^{o}	$1/5^{+}$ at 10^{o}
80	-	-	-	-	-	-
90	-	-	-	-	-	-
100	-	-	-	-	-	-
110	-	-	-	-	-	-
120	-	-	-	-	-	-
Half life = 3.35 minutes						

Key: $1/5^{+}$ at 10^{-1} = 1 tube positive out of total of 5 tubes at 10^{-1} virus dilution.

TABLE 3

LOSS OF INFECTIVITY AT 56°C OF CELL CULTURE VIRUS FLUID CONTAINING AN EQUAL VOLUME OF VACCINE ADDITIVE

TIME OF EXPOSURE IN MINUTES	TITRE OF VIRUS (BK/97) $\log_{10}$ $TCID_{50}$/ml					AVERAGE VIRUS TITRE $\log_{10}$ $TCID_{50}$/ml.
	EXPERIMENT 1	2	3	4	5	
0	5.7	5.5	5.6	5.9	5.8	5.7
10	4.3	4.5	4.8	4.9	4.5	4.6
20	3.3	3.6	3.7	3.8	3.6	3.6
30	2.4	3.0	2.9	3.1	3.0	2.9
40	1.9	2.7	2.5	2.6	2.3	2.4
50	1.9	2.1	2.0	2.0	1.9	2.0
60	1.6	1.9	1.7	1.5	1.4	1.6
70	$1/5^+$ at 10^0	$5/5^+$ at 10^0 $3/5^+$ at 10^1	$4/5^+$ at 10^0 $1/5^+$ at 10^1	$3/5^+$ at 10^0 $1/5^+$ at 10^1	$2/5^+$ at 10^0	$3/5^+$ at 10^0 $1/5^+$ at 10^1
80	-	$1/5^+$ at 10^0	$1/5^+$ at 10^0	-	-	-
90	-	-	-	-	-	-
100	-	-	-	-	-	-
110	-	-	-	-	-	-
120	-	-	-	-	-	-
Half life = 4.0 minutes						

Key: A/B^+ at X = A tubes positive out of a total of B tubes at log X virus dilution.

TABLE 4

COMPARISON OF THE MEAN INACTIVATION RATES OF RINDERPEST VIRUS STRAIN KARETE "O" SUSPENDED IN EQUAL VOLUME OF MAINTENANCE MEDIUM, DONKEY SERUM AND VACCINE ADDITIVE

EXPOSURE TIME IN MINUTES	MEAN VIRUS TITRE (log_{10} $TCID_{50}$/ml) IN THE FOLLOWING SOLUTIONS		
	MAINTENANCE MEDIUM	DONKEY SERUM	VACCINE ADDITIVE
0	5.7	5.5	5.7
10	3.5	4.2	4.6
20	2.5	3.3	3.6
30	1.6	2.5	2.9
40	0.9	1.9	2.4
50	0.4	1.3	2.0
60	0.2	0.9	1.6
70	0.1	0.5	1.3
80	0.0	0.2	1.0
Half life in minutes	2.5	3.35	4.0

TABLE 5

LOSS OF INFECTIVITY BY ULTRAVIOLET IRRADIATION OF CELL CULTURE FLUID DILUTED IN EQUAL VOLUME OF MAINTENANCE MEDIUM

TIME OF EXPOSURE IN SECONDS	TITRE OF VIRUS (BK/97) $\log_{10}$ $TCID_{50}$/ml.					AVERAGE TITRE OF VIRUS $\log_{10}$ $TCID_{50}$/ml
	EXPERIMENT					
	1	2	3	4	5	
0	5.9	5.3	5.4	6.1	5.8	5.7
10	3.2	2.3	2.7	3.3	2.9	2.9
20	2.1	1.9	1.7	2.5	2.3	2.1
30	1.7	1.3	1.3	2.3	2.1	1.7
40	1.6	1.1	1.0	1.9	1.8	1.5
50	0.7	0.5	0.6	1.5	1.3	0.9
60	0.5	0.7	0.7	1.3	1.1	0.9
70	0.6	0.6	0.7	1.1	0.9	0.8
80	0.5	0.7	0.8	0.9	1.0	0.8
90	0.4	0.5	0.7	0.6	0.8	0.6
100	0.4	0.4	0.6	0.5	0.6	0.5

TABLE 5

	1	2	3	4	5	
110	0.2	0.4	0.5	0.7	0.6	0.5
120	-	$1/5^{+}$ at 10^{0}	$3/5^{+}$ at 10^{0}	$3/5^{+}$ at 10^{0}	$2/5^{+}$ at 10^{0}	$2/5^{+}$ at 10^{0}
130	$1/5^{+}$ at 10^{0}	$1/5^{+}$ at 10^{0}	$2/5^{+}$ at 10^{0}	$3/5^{+}$ at 10^{0}	$1/5^{+}$ at 10^{0}	$2/5^{+}$ at 10^{0}
140	-	-	$1/5^{+}$ at 10^{0}	$1/5^{+}$ at 10^{0}	-	-
150	-	$1/5^{+}$ at 10^{0}	$1/5^{+}$ at 10^{0}	$1/5^{+}$ at 10^{0}	-	$1/5^{+}$ at 10^{0}
160	-	-	-	-	-	-
170	-	-	-	-	-	-
180	-	-	-	-	-	-

Half life = 5 Seconds

Key: A/B^{+} at 1 = A tubes positive out of total of B tubes at log X virus dilution.

TABLE 6

LOSS OF INFECTIVITY BY ULTRAVIOLET IRRADIATION OF CELL CULTURE FLUID CONTAINING AN EQUAL VOLUME OF DONKEY SERUM

TIME OF EXPOSURE IN SECONDS	TITRE OF VIRUS (BK/97) $\log_{10}$ $TCID_{50}$/ml					AVERAGE TITRE OF VIRUS $\log_{10}$ $TCID_{50}$/ml.
	1	2	3	4	5	
0	5.6	5.7	5.3	5.9	5.5	5.6
10	4.7	4.8	4.3	4.9	4.6	4.7
20	3.9	4.3	3.9	4.3	3.7	4.0
30	3.3	3.8	3.5	3.9	3.0	3.5
40	3.1	3.3	3.2	3.5	2.8	3.2
50	2.7	2.8	3.1	3.0	2.5	2.8
60	2.3	2.5	3.0	2.8	2.7	2.7
70	2.3	2.1	2.9	2.6	2.3	2.4
80	2.0	1.7	2.6	2.3	2.1	2.1
90	2.1	1.9	2.5	2.0	2.1	2.1
100	1.3	1.7	2.1	2.1	2.4	2.0
110	1.3	1.5	2.0	1.9	2.1	1.9
120	1.3	1.3	2.3	1.9	1.7	1.7
130	1.3	1.3	1.9	1.5	1.3	1.5

TABLE 6

	1	2	3	4	5	
140	1.4	1.0	1.4	1.7	1.5	1.6
150	1.2	1.1	1.2	1.5	1.8	1.4
160	1.3	1.5	1.1	1.3	1.6	1.4
170	1.0	1.3	0.9	1.1	1.3	1.1
180	1.1	1.2	0.9	1.5	1.0	1.1
190	1.0	1.1	1.0	1.2	0.7	1.0
200	0.6	0.7	0.8	0.9	0.5	0.7
210	0.9	0.8	0.5	0.7	0.6	0.7
220	0.7	0.9	0.7	0.3	0.8	0.7
230	0.6	1.0	0.5	0.4	0.4	0.6
240	0.5	0.7	0.3	0.5	0.3	0.5
250	0.3	0.7	0.2	0.7	0.3	0.5
260	-	-	-	-	-	-
270	-	-	-	-	-	-

Half life = 12 seconds

TABLE 7

LOSS OF INFECTIVITY BY ULTRAVIOLET IRRADIATION OF CELL CULTURE FLUID CONTAINING 50% VACCINE ADDITIVE

TIME OF EXPOSURE IN SECONDS	TITRE OF VIRUS (BK/97) $\log_{10}$ $TCID_{50}$/ml.					AVERAGE TITRE OF VIRUS $\log_{10}TCID_{50}$/ml.
	1	2	3	4	5	
0	5.6	5.9	5.7	5.3	6.0	5.7
10	4.3	4.9	5.2	4.9	5.7	5.0
20	4.1	4.2	4.9	4.3	5.3	4.6
30	3.9	3.9	4.3	4.1	4.7	4.2
40	3.3	3.3	3.9	3.8	4.3	3.8
50	2.9	3.0	3.4	3.1	3.6	3.2
60	2.7	3.2	3.3	3.8	3.2	3.2
70	2.5	2.9	3.3	3.3	2.9	3.0
80	2.1	2.7	3.0	3.5	2.5	2.8
90	2.3	2.7	3.2	3.3	2.3	2.8
100	1.9	2.3	2.7	2.7	1.9	2.3
110	2.0	2.3	3.3	2.7	2.6	2.6
120	1.9	2.1	2.5	2.4	2.2	2.2

TABLE 7

	1	2	3	4	5	
130	2.3	2.1	2.4	2.3	2.2	2.3
140	2.3	2.0	2.3	2.1	1.9	2.1
150	2.0	1.9	2.3	2.1	-	2.1
160	2.1	1.9	2.1	1.9	2.3	2.1
170	1.9	1.3	1.9	1.7	2.1	1.8
180	1.7	1.3	1.7	1.7	2.0	1.7
190	1.3	1.7	1.5	1.5	1.3	1.5
200	1.5	1.6	1.5	1.3	1.7	1.5
210	1.3	1.5	1.4	1.5	1.3	1.4
220	1.3	1.5	1.5	1.3	1.6	1.4
230	1.3	1.2	1.3	1.3	1.5	1.3
240	1.0	1.3	1.1	1.1	1.3	1.2

TABLE 7

	1	2	3	4	5	
250	0.7	1.0	1.3	0.9	1.1	1.0
260	1.1	0.9	1.3	1.2	1.1	1.2
270	1.0	1.0	1.5	1.2	1.7	1.2
280	1.1	1.0	1.3	0.9	0.9	1.0
290	1.0	1.0	1.2	1.1	1.3	1.1
300	$1/5^{+}$ at 10^{0}	$1/5^{+}$ at 10^{0}	$2/5^{+}$ at 10^{0}	$2/5^{+}$ at 10^{0}	$3/5^{+}$ at 10^{0}	$2/5^{+}$ at 10^{0}
310	-	-	-	-	-	-
320	-	-	-	-	-	-
Half life = 16 Seconds						

Key: A/B^{+} at X = A tubes positive out of a total of B tubes at log X virus dilution.

TABLE 8

COMPARISON OF THE MEAN INACTIVATION RATES OF CELL CULTURE FLUID SUSPENDED IN EQUAL VOLUMES OF MAINTENANCE MEDIUM, DONKEY SERUM AND VACCINE ADDITIVE.

TIME OF EXPOSURE IN SECONDS	MEAN VIRUS TITRE (log_{10} $TCID_{50}$/ml) IN THE FOLLOWING SOLUTIONS		
	MAINTENANCE MEDIUM	DONKEY SERUM	VACCINE ADDITIVE
0	5.7	5.6	5.7
10	2.9	4.7	5.0
20	2.1	4.0	4.6
30	1.7	3.5	4.2
40	1.5	3.2	3.8
50	0.9	2.8	3.2
60	0.9	2.7	3.2
70	0.8	2.4	3.0
80	0.8	2.1	2.8
90	0.6	2.1	2.8
100	0.5	2.0	2.3
Half life in Seconds	5	12	16

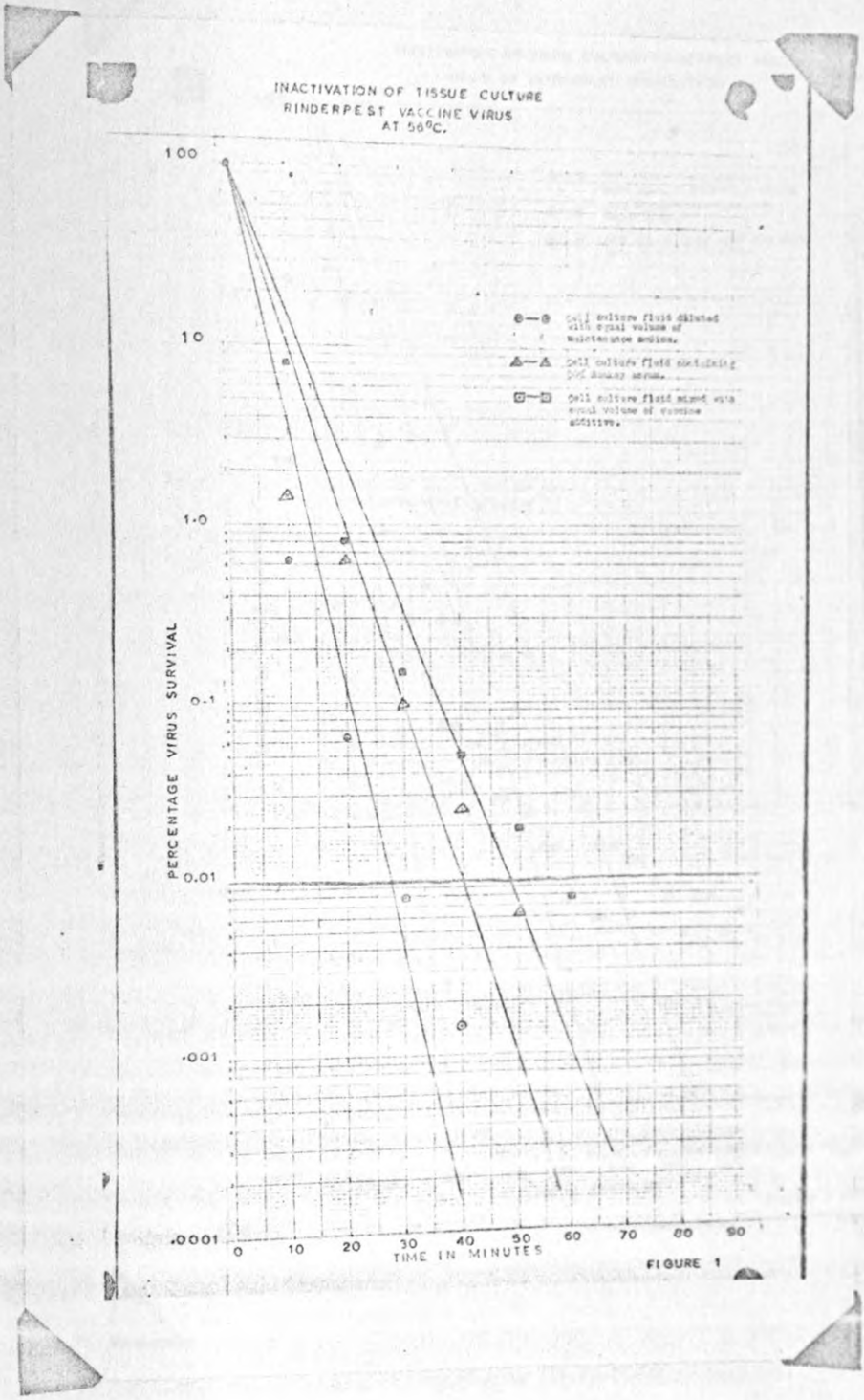

INACTIVATION OF TISSUE CULTURE
RINDERPEST VACCINE VIRUS
AT 56°C.
100
10
1.0
0.1
0.01
.001
.0001
PERCENTAGE VIRUS SURVIVAL
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES
FIGURE 1

FIG. 1.

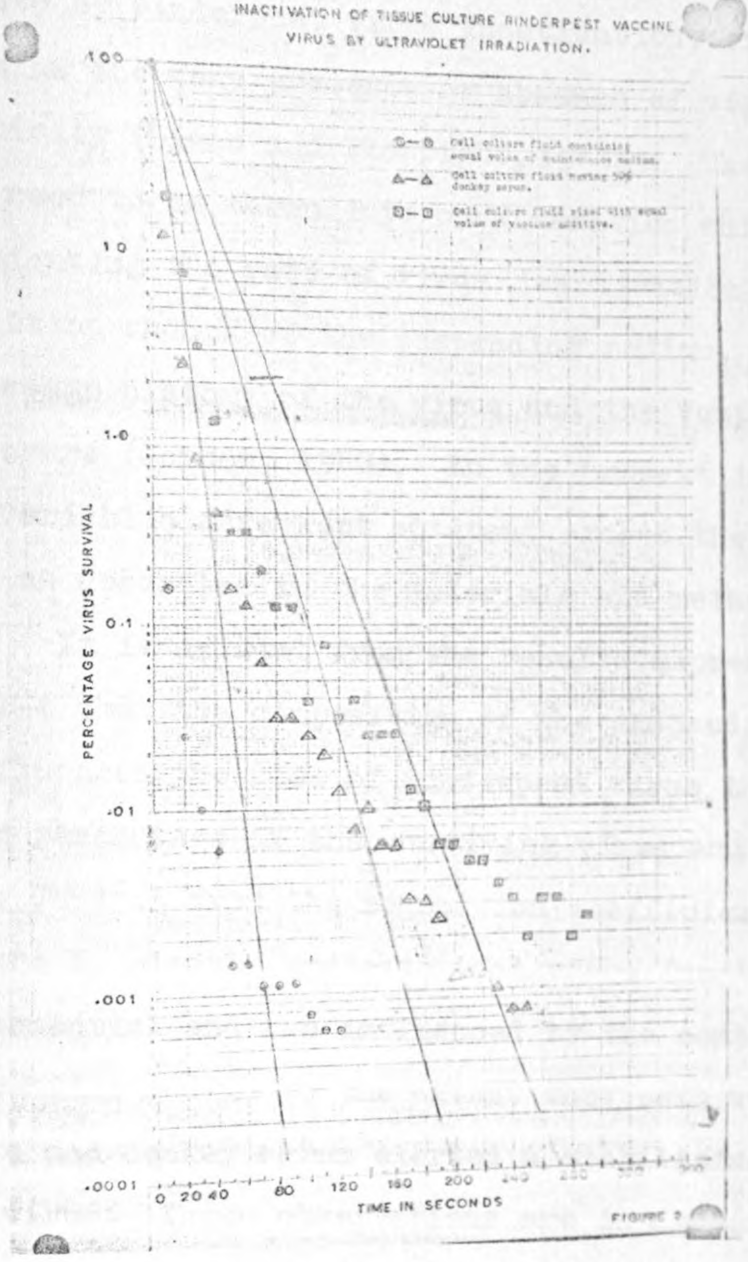

INACTIVATION OF TISSUE CULTURE RINDERPEST VACCINE VIRUS BY ULTRAVIOLET IRRADIATION.
100
10
1.0
0.1
.01
.001
.0001
PERCENTAGE VIRUS SURVIVAL
0 20 40 60 120 160 200
TIME IN SECONDS
Cell culture fluid containing equal volume of maintenance medium.
Cell culture fluid treated with equal volume of vaccine additive.

FIG. 2.

3. 4. DISCUSSION:

There appears to be no criteria in assessing the rate of rinderpest virus inactivation, except to determine the mere presence or absence of virus infectivity (Liess and Plowright, 1963a). The variables which need to be taken into consideration while investigating the rate of virus inactivation are the composition and pH of the suspending medium, the origin and passage history of the virus and the temperature of exposure (Gorham, 1960). In the present investigations these variables were kept constant except the suspending medium as described in the materials and methods.

It is evident from the results expressed in table 1-4 that the composition of the suspending medium had influenced the rate of rinderpest virus inactivation. Further, percentage of the surviving virus and half life periods derived from the regression coefficient presented in figure 1, clearly indicate that virus inactivation was exponential and was influenced by the composition of suspending medium to the extent that both vaccine additive and donkey serum exerted a stabilizing effect on the virus. These observations are in agreement with the findings of Gorham, (1960) in respect to the effect of the composition of the medium; and of Scott, (1959a); and Plowright and Ferris, (1961), regarding the shape of the inactivation curve. These findings differ in the half life period from that given by Plowright and Ferris, (1961), who observed a half life period of

3.5 minutes for the Kabete "O" strain of rinderpest virus suspended in Earle's medium containing 5% bovine serum and subjected to heat treatment at 56°C. The corresponding period in these studies was 2.5 minutes. The discrepancy might be due to the use of donkey serum as against steer serum used by Plowright and Ferris, (1961) or the use of very young cells in determining the residual virus infectivity because Plowright and Ferris, (1957, 1959a) observed that the CPE of rinderpest virus was extensive and striking in young actively dividing cells than in old cell cultures. As shown in Tables 1-3, there was no significant difference in the results obtained from replicated tests.

It is evident from the work reported in this chapter, that reported by Plowright, Rampton, Taylor and Herniman, (1970); and Johnson, (1962a) that vaccine additive and serum stabilize rinderpest virus to inactivation by heat. Musser and Underwood, (1960), also observed an increase in the storage life of measles virus by the addition of 5% calf serum to the storage medium.

Little work has been published on the inactivation of cultured rinderpest virus by ultraviolet irradiation. During the present investigation, all possible variables except the composition of the suspending medium were kept constant as described in the materials and methods. The results summarized in Table 5-8 and Figure 2 demonstrate inactivation of cultured rinderpest virus by U-V irradiation to be

exponential. It is also evident that vaccine additive and serum, again exerted a stabilizing influence on the virus to inactivation by U-V irradiation.

It is evident from the work reported in this chapter, also that by MacOwan, (1956); Johnson, (1962b); and Plowright, Hernimann and Rampton, (1971), that rinderpest virus inactivation proceeded as a first order reaction on exposure to U-V light. Addition of vaccine additive and serum stabilize rinderpest virus to inactivation by ultraviolet irradiation.

3. 5. SUMMARY:

The inactivation of cell culture rinderpest vaccine virus by heat (56°C) or U-V irradiation was studied after diluting in equal volume of maintenance medium, donkey serum or vaccine additive.

In both systems the pattern of inactivation was exponential. Virus was rapidly inactivated in maintenance medium as compared with donkey serum or vaccine additive. Addition of vaccine additive provided maximum protection to rinderpest virus against exposure to 56°C or ultraviolet irradiation.

CHAPTER 4

INTERFERENCE AND INTERFERON INDUCTION BY TISSUE CULTURE RINDERPST VACCINE VIRUS IN VITRO

4. 1. INTRODUCTION:

Plowright and Ferris, (1957, 1959a) observed that the CPE of rinderpest virus was more extensive and striking in young actively dividing cells than in old cultures. It was also observed that complete medium change accelerated the extension of CPE (Plowright, 1964) and the release of the virus in culture fluid (Plowright, Herniman and Rampton, 1969). These observations suggest that autointerference might be a limiting factor to the development and progression of rinderpest virus CPE. Plowright and Ferris, (1961) have also suggested autointerference to account for irregularities observed in the titrations of heat inactivated samples. The work reported in this chapter was carried out to investigate the ability of rinderpest virus to induce interference *in vitro* and also to characterise any such inhibitor as interferon.

4. 2. MATERIALS AND METHODS:

Media, Animals and tissue culture:

Description of animals, media and solutions used and procedures adopted in the preparation of cell culture have been described in chapter 3 of this thesis.

Interfering virus.

Kabete "O" strain of rinderpest virus (Plowright and Ferris, 1957), described in earlier experiments was used in these studies.

Challenge viruses.

Bovine tissue culture adapted, bovine virus diarrhoea (BVD), infectious bovine rhinotracheitis (IBR), para influenza-3 (PI-3), maintained in the EAVRO were used, while foot and mouth disease (FMD) virus, Asia type-1 collected from a field outbreak in Pakistan and adapted to grow in calf and goat kidney cells in the Veterinary Research Institute, Pakistan (VRI) was included in these studies.

Preparation of stock virus.

All the viruses were propagated on primary cultures. Cell culture fluid was harvested when CPE was evident in more than 80% of the cells. This was centrifuged at 3,000 r.p.m. at $4^{\circ}C$ for one hour and stored at $-70^{\circ}C$.

Immune serum.

Rinderpest reference immune rabbit serum maintained in EAVRO was used.

Virus inactivation and assay system.

TCRV diluted in maintenance medium was inactivated for 3 minutes by ultraviolet irradiation. Virus titrations and calculation of the $TCID_{50}$ were made as described in chapter 3 of this thesis.

Interference experiments.

Interference experiments were carried out in the following way: Active or inactivated TCRV at a titre of $10^{5.1}$ $TCID_{50}$ per ml was used. It was inoculated into 5 day old bovine kidney primary monolayer cultures in tubes. After 6 hours incubation at 37°C, maintenance medium was changed. Then after 48 hours of incubation, BVD and TCRV viruses were titrated in these and control cell cultures. The control cell cultures were similarly pretreated with ultraviolet treated maintenance medium. These were incubated at 37°C and observed daily for the development of CPE. CPE and virus yield were recorded. These experiments were carried out in triplicate.

Preparation of interferon from bovine kidney (BK) cells treated with inactivated TCRV.

Fluids from cell cultures inoculated with U-V inactivated TCRV and control cultures, collected at the time of challenge virus titration, were used as the source of interferon induced by inactivated TCRV and control fluids respectively.

Preparation of interferon from BK cells inoculated with live virus (TCRV).

One litre Blake bottles containing about $5x10^6$ freshly trypsinised BK cells per ml of growth medium were infected in suspension with $10^{5.1}$ $TCID_{50}$ of infective TCRV. These were incubated as described in chapter 3 and observed daily microscopically.

CPE appeared on the 3rd day p.i. and was allowed to progress without medium change for the next 48 hours by which time it was observed that no new foci of CPE were developing and some cell growth in the areas of CPE was observed. At this time, fluids from the infected and uninfected control bottles were harvested, centrifuged at 3,000 r.p.m. for 60 minutes at 4°C. The supernates were stored at 4°C for a maximum of 4 days as interferon and control fluids respectively.

Assay of interferon.

Principally, procedures described by Dinter and Philipson, (1962), were strictly adopted to assay interferon. 1 ml amounts of the serial two-fold dilutions in Hank's solution of interferon preparation were added to 5 tubes per dilution. Twenty-four hours later 100 $TCID_{50}$ of challenge virus was added. The interferon titre was expressed as the highest dilution which diminished about 50% of the CPE as compared to control cell culture fluid.

Characterization of interferon.

Fluid sample was aliquoted and subjected to the following studies:-

a. Acid stability.

The procedures adopted by Mirchamsay and Rapp, (1969) were used. The interferon fluid samples were acidified to pH 2 with 1:20 hydrochloric acid and stored at 4°C for 48 hours. Then the pH was brought to neutrality with 1-NaOH. The interferon c ntent c

the test culture fluids were assayed before and after acid treatment.

b. Non-sedimentability.

Interferon fluid was centrifuged at 30,000 r.p.m. for 1 hour at 4°C in a Spinco ultra centrifuge using the SW 30 rotor. The supernate was then assayed for interferon activity.

c. Non-dialyzability.

Dialyzability of TCRV interferon was assessed as described by Rosenquist and Loan, (1967). Fluid samples were dialysed in cellulose tubing at 4°C against 100 volumes of 0.1 M KCl-HCl buffer pH 2 (Bower and Bates, 1955; cited by Long, 1961), for 24 hours and then against 100 volumes of Hank's balanced salt solution pH 6.8 (Hank's and Wallace, 1949), for 24 hours and then tested for interferon activity.

d. Non-neutralizability.

Interferon samples obtained after acidification were mixed with equal volumes of 1:10 diluted rabbit anti-rinderpest serum and the mixtures incubated over night at 4°C. Interferon content of serum treated and untreated samples was assayed.

e. Virus-specificity.

Test interferon fluid was serially diluted two-fold in Hank's balanced salt solution. For each dilution 1 ml amounts were inoculated into each BK monolayer cultures. Twenty-four hours later, the cultures were superinfected with 100 $TCID_{50}$ of BVD virus or PI-3

or IBR virus or FMD virus Asia type-1. The cultures were examined daily for the development of CPE caused by the superinfecting virus.

f. Species-specificity.

1 ml amounts of the serial two-fold dilutions in Hank's balanced salt solution of interferon preparations were inoculated into bovine and goat kidney primary cell culture tubes. After 24 hours incubation at 37°C, these cultures were challenged with 100 $TCID_{50}$ of BVD or FMD Asia type-1 viruses. They were examined daily for five days for evidence of CPE due to challenging virus. Interferon titre was expressed as the reciprocal of the highest dilution that caused a 50% reduction in the challenging virus.

g. Trypsin sensitivity.

Interferon, after acid treatment was treated with trypsin at a final concentration of 0.05% (0.5 mg/ml) and incubated for two hours at 37°C, after which soybean inhibitor was added at a concentration of 0.1mg/ml of the original interferon preparation. Then rabbit immune rinderpest serum was added (1:10) and the mixture was incubated for two hours at 37°C after which it was assayed for interferon content.

h. Heat stability.

5 ml quantities of interferon were sealed in glass ampoules and heated in a water bath either at 56°C for 30 minutes or at 70°C for 1 hour. After

removal from the water bath the ampoules were immersed in ice cold water immediately for 20 minutes. The contents were then assayed for interferon activity.

4. 3. RESULTS:

Ultraviolet irradiated TCRV inoculated into BK cells induced a significant depression to BVD virus titre (table 9 and 10), which means that TCRV preparation interfered with BVD virus replication. Further, inactivated TCRV interfered with both homologous as well as heterologous viruses, but more depression in virus titre was observed with the heterologous than the homologous superinfecting viruses (table 11 and 12). Depression in BVD virus titre was more marked on the 3rd day p.i. but with active TCRV a constant depression of log $10^{1.0}$ $TCID_{50}$/ml was noticed from the 3rd day post challenge onwards. Likewise ultraviolet inactivated TCRV treated BK cells afforded a significant depression in virus yield of the superinfecting virus on the 3rd day post challenge (table 13 and 14).

TABLE 9

THE EFFECT OF TREATMENT OF BK MONOLAYER CULTURE WITH U-V INACTIVATED TCRV ON THEIR SUSCEPTIBILITY TO BVD VIRUS

Experiment Number	Day post BVD Virus Inoculation	BVD Virus Titre in log_{10} $TCID_{50}$/ml.		Depression in Titre
		A	B	B-A
1	3	4.5	5.3	0.8
	4	5.1	5.9	0.8
	5	5.5	6.5	1.0
2	3	4.3	5.5	1.2
	4	5.0	5.9	0.9
	5	5.5	6.7	1.2
3	3	4.5	5.7	1.2
	4	5.0	6.0	1.0
	5	5.5	6.1	0.6

A = BVD virus titre in BK cultures pretreated with U-V inactivated TCRV.

B = BVD virus titre in BK cultures pretreated with normal cell culture fluids (control).

TABLE 10

MEAN DEPRESSION OF THREE EXPERIMENTS IN BVD TITRE INDUCED BY INACTIVATED TCRV.

Experiment No.	Depression on P.I. Day		
	3	4	5
1	0.8	0.8	1.0
2	1.2	0.9	1.2
3	1.2	1.0	0.6
MEAN	1.1	0.9	0.9

TABLE 11

THE EFFECT OF TREATMENT OF BK MONOLAYER CULTURE WITH INACTIVATED TCRV ON THEIR SUSCEPTIBILITY TO BVD AND INFECTIVE TCRV

Experiment Number	Day post virus inoculation	Virus titre in $\log_{10}$ $TCID_{50}$/ml.				Depression in titre A - B	Depression in titre C - D
		A	B	C	D		
1	3	-	-	2.7	4.3	-	1.6
	4	-	-	4.1	6.5	-	2.4
	5	2.7	3.7	4.7	6.9	1.0	2.2
	6	2.9	3.9	4.7	6.9	1.0	2.2
	7	2.9	3.9	5.1	6.9	1.0	1.8
2	3	-	-	2.0	3.9	-	1.9
	4	-	-	2.5	4.3	-	1.8
	5	2.5	3.7	4.3	6.3	1.2	2.0
	6	2.9	3.9	4.7	6.7	1.0	2.0
	7	3.1	4.1	5.3	6.7	1.0	1.4
3	3	-	-	2.7	4.7	-	2.0
	4	-	-	4.1	5.7	-	1.6
	5	2.5	3.5	4.5	6.3	1.0	1.8
	6	2.9	3.9	5.0	6.3	1.0	1.3
	7	2.9	4.3	5.3	6.3	1.4	1.4

A = TCRV titre in BK cultures pretreated with inactivated TCRV.
B = TCRV titre in BK cultures pretreated with normal cell culture fluids (control)
C = BVD virus titre in BK cultures pretreated with inactivated TCRV
D = BVD virus titre in BK cultures pretreated with normal cell culture fluids (control)

TABLE 12

MEAN DEPRESSION IN HOMOLOGOUS AND HETEROLOGOUS CHALLENGE VIRUSES INDUCED BY INACTIVATED TCRV

Experiment Number	Depression in virus titre $\log_{10}$ $TCID_{50}$/ml. p.i. day									
	HOMOLOGOUS					HETEROLOGOUS				
	3	4	5	6	7	3	4	5	6	7
1	-	-	1.0	1.0	1.0	1.6	2.4	2.2	2.2	1.8
2	-	-	1.2	1.0	1.0	1.9	1.9	1.8	2.0	2.0
3	-	-	1.0	1.0	1.4	2.0	1.6	1.8	1.3	1.4
MEAN	-	-	1.1	1.0	1.1	1.8	2.0	1.9	1.8	1.7

TABLE 13

EFFECT OF PRETREATMENT OF BK CULTURES WITH U-V INACTIVATED TCRV FOR 24 HOURS ON THEIR SUSCEPTIBILITY TO BVD VIRUS

Experiment Number	Day post BVD virus inoculation	BVD virus yield in $\log_{10}$ $TCID_{50}$/ml.		Depression in titre A - B
		A	B	
1	3	5.1	5.7	0.6
	4	5.3	5.7	0.4
	5	5.7	6.1	0.4
	6	5.9	6.1	0.2
	7	5.9	6.1	0.2
2	3	4.9	5.7	0.8
	4	5.3	5.9	0.6
	5	5.7	6.1	0.4
	6	5.9	6.1	0.2
	7	6.1	6.1	-
3	3	5.1	5.7	0.6
	4	5.3	5.7	0.4
	5	5.7	5.9	0.2
	6	5.7	5.9	0.2
	7	5.9	5.9	-

A = BVD virus yield in BK cultures pretreated with U-V inactivated TCRV.

B = BVD virus yield in BK cultures pretreated with normal tissue culture fluids (control).

TABLE 14

MEAN DEPRESSION IN BVD VIRUS YIELD FROM THREE EXPERIMENTS AS ESTIMATED BY 24 HOURS PRETREATMENT OF BK CELLS WITH INACTIVATED TCRV.

Experiment Number	Depression in virus titre $\log_{10}$ $TCID_{50}$/ml. p.i. day				
	3	4	5	6	7
1	0.6	0.4	0.4	0.2	0.2
2	0.8	0.6	0.4	0.2	-
3	0.6	0.4	0.2	0.2	-
MEAN	0.7	0.5	0.3	0.2	0.1

Characterisation of interferon from rinderpest virus infected BK culture fluids.

a. Acid stability.

Titre (log $10^{2.4}$/ml) of TCRV induced viral inhibitor in BK cultures was not affected by acid treatment.

b. Non-sedimentability.

High speed centrifugation did not affect the titre of the viral inhibitor substance.

c. Non-dialysability.

Inhibitory action of the TCRV induced inhibitor was unaffected by dialysis as its titre before and after dialysis remained the same (log $10^{2.4}$/ml).

d. Influence of anti-rinderpest serum.

In this case, the activity to inhibit virus growth did not decrease. It is assumed therefore that this activity was not derived from the presence of rinderpest virus.

e. Virus-specificity.

Virus inhibitor at a dilution 1:16 inhibited CPE of EVD, IBR, PI-3 and FMD (Asia type-1 strain) viruses in BK cell cultures. Therefore it was assumed that the substance did not posses the so-called virus specificity.

f. Species-specificity.

The inhibitory activity of the interferon-like substance was demonstrated in the assay of interferon with the system of FMD and BK cultures, but not with the system of FMD virus in goat kidney cultures. Accordingly it was proved that the substance had species specificity.

g. Trypsin-sensitivity.

This enzyme destroyed the virus inhibitory property.

h. Heat-stability.

Heating at 56°C for 30 minutes decreased the virus inhibiting activity to log $10^{0.3}$, where as heat treatment at 70°C for 1 hour totally destroyed the virus inhibiting activity.

4. 4. DISCUSSION:

This study indicates that infective TCRV when rendered non-infectious by U-V irradiation interfered with the replication of superinfecting homologous as well as heterologous virus (Tables 9-12). These findings are in agreement with those of many workers (Isaacs and Lindenmann, 1957; Lindenmann, Burke and Isaacs, 1957; and Burke and Isaacs, 1958b), who observed similar behaviour with other viruses.

The present study also provides evidence that interferon is produced in BK cultures inoculated with infective or U-V inactivated TCRV. The identification of the inhibitor as interferon is supported by the evidence that virus was not present in the inhibiting fluid following acid treatment, and that its activity was not influenced by treatment with anti-chicken serum. These as live or inactived viruses are known to protect cell cultures against challenge viruses (Sheelock and Sibley, 1964), further treatment of culture fluid by acidification (Liess and Plowright, 1963a; DeFoor and Barber, 1964), and high centrifugation (Plowright, Cruickshank and Waterson, 1962) procedures, which would tend to either inactivate or eliminate virus particles did not affect the virus inhibitory substance.

The antiviral substance produced in BK cells was found to fulfill the biological criteria to be classified as an interferon (Levine and Nichol; 1970). It was a macromolecule of relatively small size as indicated by its lack of sedimentation at 30,000 r.p.m. for one hour and the fact that it was non-dialyzable. It possessed stability at pH 2 and was sensitive to trypsin. The latter indicated that the active antiviral substance was protein in nature. The antiviral property was partially inactivated by heating at 56°C for 30 minutes. Broad-spectrum antiviral activity was demonstrated by inhibition of BVD, PI-3, IBR and FMD viruses.

A narrow host-species specificity was shown by treating heterologous cell cultures with the antiviral substance produced on BK cells.

These properties justify the conclusion that the antiviral substance produced by the cells in response to TCRV is in fact an interferon.

The results obtained in this chapter clearly indicate that interferon mediated autointerference provides an explanation to the observation by Plowright, (1964); and Plowright, Hernimann and Rampton, (1969), that complete medium change of TCRV infected cultures accelerates the extension of CPE and the release of virus in culture fluid. This is because interferon

produced endogenously inhibits further replication of the same virus (Isaacs, 1962; and [illegible], whereas frequent change of medium reduce the interferon influence and thereby permit normal virus growth resulting in cell destruction (Isaacs, [illegible]). The induction of interferon system in BK cell cultures infected with TCRV coupled with the observations of Plowright and Finter (cited by Plowright, 1968), that an interferon preparation, produced in calf kidney cells infected with Sindbis virus suppressed the growth of virulent RGK/1 strain of rinderpest virus, suggest that the interferon system could play an important role in the pathogenesis of rinderpest virus infection *in vivo*.

4. 5. SUMMARY:

1) TCRV virus was found to interfer with infection by [illegible], parainfluenza, bovine virus diarrhoea and foot and mouth disease viruses.

2) Stimulation of bovine kidney cells with TCRV produced interferon.

3) Viral interference induced by TCRV was mediated by interferon.

4) The interfering factor was demonstrated to be interferon on account of the following characteristics:-

a. Stable at pH 2.

b. Non-dialyzable.

c. Not sedimentable at 30,000 r.p.m. for 1 hour.

d. Not affected by rinderpest immune serum.

e. Active against homologous as well as heterologous viruses.

f. Effective in calf kidney cells but not in goat kidney cells.

g. Partially stable at 56°C for 30 minutes.

h. Activity destroyed by 70°C for 1 hour heat treatment.

i. Activity destroyed by trypsin treatment.

CHAPTER 5

INTERFERON INDUCTION BY ATTENUATED AND VIRULENT STRAINS OF RINDERPEST VIRUS

5. 1. INTRODUCTION:

Enders in 1960 described that vaccine strain of measles virus induced cells to produce more interferon than virulent strains. Subsequently, De Meyer and Enders, (1961) reported that 5 attenuated strains of polio virus induced interferon. The titre was log $10^{0.3-0.6}$ in man. The virulent strains (Brunhilde, MEFI, Leon and Chat) did not produce any detectable amounts of interferon. Sellers in 1963 demonstrated that attenuated strains of FMD virus were better interferon inducers than virulent strains.

There is no published information available about the induction of interferon by different strains of rinderpest virus. Although it was described in chapter 4 that RPV induced interference against homologous as well as heterologous viruses, and that viral interference was mediated by interferon, it remained to find out the relationship between virus virulence and ability to induce cells to produce interferon.

The aim of this investigation was to study interferon inducing capability of the fully attenuated and virulent strains of rinderpest virus.

5. 2. MATERIALS AND METHODS:

Animals, media and solutions.

These have been described in chapter 3.

Procedure of cell cultures.

This has also been given in chapter 3.

Viruses.

1. Rinderpest virus strains.

a. Fully attenuated tissue culture vaccine strain "Kabete O" described in chapter 3.

b. Virulent non-contagious strain "REOK", passaged by subcutaneous inoculation over 60 years in cattle at EAVRO was obtained from Dr. Rweyemamu, Head Division of Virus Diseases EAVRO.

c. Fully virulent contagious strain "PAK", passaged by subcutaneous inoculation over 23 years in buffalo in VRI, where it is used in all research projects.

2. Foot and mouth disease virus Asia type-1.

This has been described in chapter 4.

Adaptation of the virulent rinderpest virus strains in BK cultures.

Virulent strains were once passaged in susceptible animals. Blood was collected during the acute febrile stage of the disease and virus was isolated following methods described by Plowright and

Ferris, (1962a). The isolates were further passaged for five times in BK cultures, using limiting virus dilution procedure.

Preparation of stock virus.

This was done by methods described in chapter 4.

Assay of virus infectivity.

Procedures described in chapter 3 were adopted.

Immune serum.

This has been described in chapter 4.

Preparation of interferon from BK cultures infected with rinderpest virus strains, characterization and assay of interferon.

These procedure have been detailed in chapter 4.

5. 3. RESULTS:

All the three strains of rinderpest virus included in these studies induced interferon production in BK cultures. Table 15 shows that fully attenuated vaccine strain "Kabete O" produced interferon of log $10^{2.4}$/ml when assayed against 100 $TCID_{50}$/0.1 ml of FMD virus. The virulent,non-contagious strain "RBOK" effected interferon production to a level of log $10^{1.8}$/ml. Where as stimulation by fully virulent contagious strain "PAK" produced interferon titre of log $10^{1.2}$/ml in BK cultures.

TABLE 15

INDUCTION OF INTERFERON BY ATTENUATED AND VIRULENT STRAINS OF RINDERPEST VIRUS

Experiment Number	Interferon titre in log 10		
	A	B	C
1	2.4	1.8	1.2
2	2.7	2.1	1.5
3	2.1	1.5	0.9
Mean	2.4	1.8	1.2

Key: A = Fully attenuated vaccine strain "Kabete O".

B = Virulent non-contagious strain "RBOK".

C = Fully virulent contagious strain "[illegible]".

FMD virus yield in BK cultures pretreated with interferon induced by fully attenuated vaccine strain "Kabete O" was log $10^{3.4}$ $TCID_{50}$/ml and showed a log $10^{2.3}$ depression ~~[illegible]~~ the test virus titre (Table 16-17). Similarly challenge virus yield in cultures pretreated with interferon of virulent

non-contagious strain "RBOK" was log $10^{4.2}$ and interferon treatment caused a log $10^{1.5}$ reduction in virus titre. Where as challenge yield in cultures pretreated with interferon produced following stimulation of BK cultures by fully virulent contagious strain "PAK" was log $10^{5.0}$/ml, only the decrease in virus titre was log $10^{0.7}$.

TABLE 16

EFFECT OF INTERFERON ATTAINED FROM BK CULTURES TREATED WITH DIFFERENT STRAINS OF RINDERPEST VIRUS ON FMD VIRUS REPLICATION IN VITRO

Experiment Number	Titre of FMD virus in log 10 $TCID_{50}$/ml on 3rd day p.i. of BK cultures 24 hours pretreated with interferon			
	A	B	C	D
1	3.7	4.1	5.1	5.7
2	3.1	4.7	5.3	6.1
3.	3.5	3.9	4.7	5.3
Mean	3.4	4.2	5.0	5.7

Key: A = Fully attenuated vaccine strain "Kabete O".

B = Virulent non-contagious strain "RBOK".

C = Fully virulent contagious strain "PAK".

D = Control virus titration.

TABLE 17

INFLUENCE OF RINDERPEST INTERFERON ON CHALLENGE VIRUS

Challenge Virus	Experiment Number	Depression caused by Interferon of Rinderpest Virus Strains on Challenge virus log 10 $TCID_{50}$/ml.		
		KABETE "O"	RBOK	PAT
ASIA-1 Foot and Mouth Disease Virus	1	2.0	1.6	0.6
	2	3.0	1.4	0.8
	3	1.8	1.4	0.6
	MEAN	2.3	1.5	0.7

~~For this~~ Statistical analysis of the effect of interferon attained from BK cultures treated with different strains of rinderpest virus on FMD virus replication *in vitro*, Table 18 shows that the highest level of interferon was induced by the fully attenuated strain, followed by the virulent non-contagious strain and the fully virulent contagious strain respectively.

At 5% probability level "Kabete O" and "RBOK" strains differ significantly from the control fluid, but the control fluid and "PAK" strain do not differ significantly. The "PAK" strain differs significantly from the "Kabete O" and "RBOK" strains of rinderpest virus with respect to their ability to induce interferon in BK cultures.

It is thus apparent that the attenuated strain produced more interferon than the virulent strains and that the enhanced interferon induce-ability of the attenuated strain may be a marker of rinderpest virus modification.

TABLE 16

STATISTICAL ANALYSIS SHOWING THE SIGNIFICANT DIFFERENCES BETWEEN THE RESULTS PRESENTED IN TABLE 16

ANOVA

S.O.V.	D.F.	S.S.	M.S.	F
Total	11	-	-	-
Tr.	3	8.68	2.89	22**
Error	8	1.04	0.13	-

$LSD = t.\ 05\ (8)\sqrt{\frac{2.13}{3}} = 0.68$

Difference table

Means of Virus Titre	[illegible]	[illegible]	[illegible]	[illegible]
	5.70	5.03	4.25	3.43
3.43	2.27	1.60	0.80	-
4.23	1.47	0.80	-	-
5.03	0.67	-	-	-
5.70	-	-	-	-

Significant difference at 5% probability level =

5.70 5.03 5.03 4.23 3.43

Results scored under one line differ nonsignificantly.

5. 4. DISCUSSION:

These investigations were undertaken in order to find out the possible difference in the ability of virulent and modified strains of rinderpest virus to induce interferon. The titres of interferon were log $10^{2.4}$, $10^{1.6}$ and $10^{1.}$ /ml for the fully attenuated vaccine strain "Kabete O", virulent non-contagious strain "KSOL" and the virulent contagious strain "PAK" respectively. These observations are in agreement with those of many workers (Enders, 1959b; Enders, 1960; DeHaeyer and Enders, Sellers, 1963, 1964), who observed a similar pattern with other viruses.

De Haeyer and Enders, (1965) suggested that the attenuation of measles virus strains was due to the activity of such strains to induce larger amounts of interferon than that induced by virulent strains of the same virus. This finding supports the observations presented in this report that the "Kabete O" vaccine strain of rinderpest virus was able to induce more interferon in the cells than the virulent strains. The virulence of a virus, can be defined only in terms of susceptibility of a specified host, is correlated with the rate and degree of viral multiplication in that host. Soon after the discovery of interferon by Isaacs and

Lindenmann, (1957), it was suspected of being one possible determinant of virus virulence. Although the fully virulent rinderpest virus strain "PAK" induced interferon in BK cultures, the less virulent non-contagious rinderpest virus strain "RBOK" and the fully attenuated vaccine strain induced much more interferon production. It is thus evident that interferon produced endogenously during the course of viral infection, may feed back into the system and inhibit further replication of the same virus that induced its formation and consequently, its contagiouness. This hypothesis is supported by the findings of Heller, (1963) and Wagner, (1964), who observed that the prevention of endogenous interferon production by actinomycin-D resulted in enhanced pathogenesis and virus yield. It is therefore evident that interferon induceability of a virus strain is correlated with its pathogenesis.

In the work described in this chapter it was possible to show virus virulence by the assessment of interferon induced and the depression in challenge virus yield. The finding that the avirulent strain produced more interferon, coupled with the observations made by Plowright and Ferris, (1959b); and Johnson, (1962b) that "Kabete O" vaccine strain interfered with virulent virus in cattle, suggest that the effect of virus virulence was masked by interferon.

Since a desirable attenuated strain for use as a vaccine is considered to be one that contains virus that replicate without production of lesions, an application of these findings would be the use of enhanced interferon production as a marker in the development of a modified strain of rinderpest virus.

5. 5. SUMMARY:

Fully virulent contagious, virulent non-contagious and fully attenuated vaccine strains of rinderpest virus induced interferon production in BK cultures. The ability of rinderpest virus strains to induce interferon however, varied with their virulence and that increased interferon inducing character of the virus may be used as a marker of virus attenuation.

CHAPTER 6

INDUCTION OF CIRCULATING INTERFERON AND ANTIBODIES IN BUFFALO FOLLOWING INOCULATION WITH TISSUE CULTURE RINDERPEST VACCINE VIRUS

6. 1. INTRODUCTION:

The demonstration of rinderpest virus induced interferon *in vitro* in chapters 4 and 5 raised the possibility that this virus may induce interferon *in vivo*. No work appears to have been carried out on the interferon induction by rinderpest virus in cattle or buffaloe, although such interferon has been detected from the serum of rabbits infected with the LV strain of rinderpest virus by Fujisaki, Ishii and Watanabe, (1968). The ability of cattle to produce interferon following inoculation with Newcastle disease (NDV), vaccinia, PI-3 and BHK viruses was described by Rosenquist and Doan in 1969.

The aim of this work was to study the development of interferon following inoculation of buffalo with TCRV in relation to viraemia and neutralizing antibody development.

6. 2 MATERIALS AND METHODS:

Most of the materials and methods have been described in chapters 3 and 4.

Detection of viraemia.

Viraemia was detected by methods as described by Plowright and Ferris, (1962a). Blood was collected in ethylenediamine-tetra-acetic acid (EDTA) disodium salt from the jugular vein. It was centrifuged and the buffy coat after aspiration was suspended in physiological saline. Blood cells were deposited again by way of centrifugation. The cell pellet was resuspended in maintenance medium volume, equivalent to that of blood and EDTA suspension. Two millilitres of this suspended buffy coat was inoculated into each of a group of five BK culture tubes. Next day, these tubes were washed twice with PBS and refilled with maintenance medium and treated like other infected cultures.

Neutralizing antibody assay.

Serum neutralization tests were conducted in BK cultures on the basis described by Plowright and Ferris, (1961). Two-fold serum dilutions were prepared in the maintenance medium and mixed with equal volumes of virus diluted to contain 100 $TCID_{50}$/ 0.1 ml. Serum-virus mixtures were kept overnight at $4^{o}C$ and 0.2 ml from each dilution was added to each of a group of five tubes.

Induction of interferon.

A group of five rinderpest susceptible buffalo calves were inoculated with 10 $10^{6.0}$ $TCID_{50}$ of TCRV and three buffaloes were similarly inoculated with tissue culture fluid from uninfected cultures. All the animals were bled from the jugular vein at 0, 24, 34, 48, 58, 72, 96, 120, 144, 168, 192, 216, 240, 288, 336, 384, 432 and 480 hours post inoculation. After keeping them for 30 minutes at 20°C, the blood clot was detached from the glass and were centrifuged at 2,000 r.p.m. for 30 minutes at 4°C. The clear serum samples were subjected to interferon and antibody assay.

Preparation, characterization and assay of interferon.

Procedures of acid treatment to serum samples for interferon studies described by Rosenquist and Loom, (1965) were followed with minor modifications. Sera were dialyzed in cellulose tubing at 4°C against 100 volumes of 0.1 M KCl-HCl buffer (pH 2) for 24 hours and then against 100 volumes of Hank's saline (pH 7.2) for an additional 24 hours. Precipitated materials developed in some specimens after dialysis and were removed by low speed centrifugation. Acidified serum samples were filtered through 300 mm Millipore filters and presumed as interferon preparation. Assay and further characterization of interferon was done as described in chapters 4 and 5.

6. 3. RESULTS:

Clinical response.

No rise in body temperature and visible abnormalities were detected in any of the experimental animals during the period of this investigation.

Viraemia.

The amount of virus, pathogenic to the BK cultures, was never sufficient to be expressed accurately as 50 percent end point. Consequently it was expressed as a proportion of tubes showing rinderpest virus CPE (Table 19). Viraemia was first detectable in 2 of the five animals on the third day post inoculation. Peak viraemia was attained on the 6th day, when also all the animals were viraemic. By the 8th day, only 2 of the 5 animals demonstrated low grade viraemia. From the 9th day p.i. onward none of the inoculated animals were viraemic. The mean viraemic response is plotted in figure 3.

Interferon response.

On the basis of procedures described in chapter 4, a viral inhibitor from the serum samples of vaccinated animals was characterized as interferon. Data of circulating interferon is presented in Table 20. The mean titre of circulating interferon is graphed in

figure 3. Out of vaccinated buffaloes interferon was detected only in the serum of animal No.3, following 48 hours of vaccination. At 58 hours post vaccination, buffalo No.5 showed interferon titre log $10^{0.7}$. Later on, interferon was detected in 4 out of 5 buffalo blood samples collected at 72 hours p.i. Where as peak interferon titre log $10^{1.8}$ was attained on the 6th day p.i., on the 12th day, circulating interferon reached a nondetectable level in all the animals. Interferon was, however, not detectable at any stage in the control animals during these studies.

Antibody response.

Results of the development of serum neutralizing antibodies in buffaloes following TCRV vaccination are presented in Table 21. The mean titre of the same data is plotted in figure 5. Antibodies were detected from the serum samples of two buffaloes only, on day 6 at the earliest. On day 8 p.i., antibodies were detectable in serum samples of all the animals vaccinated with log $10^{5.0}$ $TCID_{50}$ of TCRV and peak antibody mean titre log $10^{2.56}$ was attained on day 20 p.i.

TABLE 19

VIRAEMIA IN BUFFALO FOLLOWING TCRV INOCULATION

Animal Number	Virus titre; No. of tubes showing CPE out of 5 tubes inoculated.										
	Post inoculation day										
	1	2	3	4	5	6	7	8	9	10	11
2	0	0	0.2	0.4	0.4	1.0	0.4	0.2	0	0	0
3	0	0	0.2	0.2	0.2	0.2	0	0	0	0	0
5	0	0	0	0	0.4	0.2	0.2	0	0	0	0
7	0	0	0	0.4	0	1.0	0.2	0.2	0	0	0
8	0	0	0	0.2	0.2	0.8	0.2	0	0	0	0
Mean	0	0	0.08	0.24	0.24	0.64	0.20	0.08	0	0	0

Key: 0.2 = One test tube showing CPE

0 = No CPE.

TABLE 20

KINETICS OF CIRCULATING INTERFERON OF BUFFALOES INTRAV

LOG 10^6 $TCID_{50}$ OF TISSUE CULTURE RINDERPEST

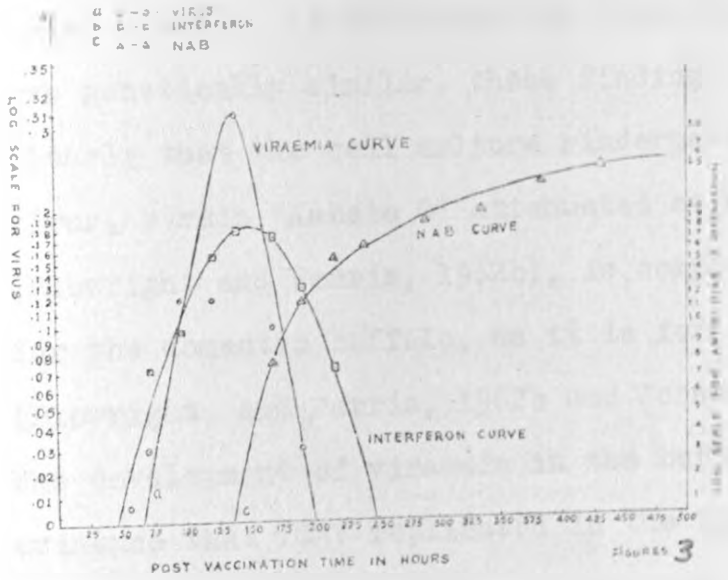
Animal Number	Inoculum	SAMPLING INTERVAL POST IN										
		0	24	34	48	58	72	96	120	144	168	19
2	Vaccine	-		-	-	-	1.0	1.3	1.6	1.9	1.8	1.
3	"	-		-	Heat	-	1.2	1.0	1.5	1.8	1.6	1.
5	"	-		-	-	0.7	-	1.3	1.6	1.8	1.6	1.
7	"	-		-	-	-	0.7	1.2	1.6	1.9	1.9	1.
8	"	-		-	-	-	0.7	-	1.5	1.6	1.8	1.
9	Control	-		-	-	-	-	-	-	-	-	-
10	Tissue	-		-	-	-	-	-	-	-	-	-
11	Culture Fluid	-		-	-	-	-	-	-	-	-	-
Mean						0.72	0.96	1.56	1.80	1.74	1.30	
S.D.						0.46	0.55	0.06	0.12	0.14	0.22	
95% Confidence Limits							0.05-1.39	0.16-1.76	1.48-1.64	1.66-1.94	1.57-1.91	1.02-1.59
S.E.							.21	.25	.03	.05	.06	.10

TABLE 21

KINETICS OF NEUTRALIZING ANTIBODY OF BUFFALOES WITH LOG 10^{6} CID_{50} OF TISSUE CULTURE RIND

Animal Number	Inoculum	SAMPLING INT										
		0	24	34	48	58	72	96	120	144	168	
2	Vaccine	-	-	-	-	-	-	-	-	-	0.2	
3	"	-	-	-	-	-	-	-	-	0.2	1.8	
5	"	-	-	-	-	-	-	-	-	-	-	
7	"	-	-	-	-	-	-	-	-	0.8	1.8	
8	"	-	-	-	-	-	-	-	-	-	-	
9	Control	-	-	-	-	-	-	-	-	-	-	
10	Tissue	-	-	-	-	-	-	-	-	-	-	
11	Culture Fluid	-	-	-	-	-	-	-	-	-	-	
Mean											0.76	1
S.D.											.95	
95% Confidence Limits											0-2.57	
S.E.											.42	

CIRCULATING ... INTERFERON AND NEUTRALIZING ANTIBODY (NAB) RESPONSE AFTER VACCINATION WITH TISSUE CULTURE RINDERPEST VACCINE IN BUFFALOES.



FIGURES 3

FIG. 3.

6. 4. DISCUSSION:

The lack of clinical response in buffalo to cell culture rinderpest vaccine virus was found to be in agreement with the findings of Singh, Omar, Raz, and El Cicy, (1967), who failed to record pyrexia or any visible abnormalities in Egyptian buffaloes inoculated with the strain of virus used in the work reported in this chapter (Kabete "O" 99th passage level virus). As Pakistani and Egyptian buffaloes are genetically similar, these findings demonstrate clearly that the cell culture rinderpest vaccine virus, strain "Kabete O" attenuated at EAVRO, MUGUGA (Plowright and Ferris, 1962b), is completely inocuous for the domestic buffalo, as it is for cattle (Plowright and Ferris, 1962b and Johnson, 1962b). The development of viraemia in the buffalo provided evidence that TCRV replicated in the tissues of this species. Although no attempt was made to simulate the work of Taylor and Plowright, (1965), it seems probable that such replication takes place in the lymphatic tissues as demonstrated for cattle by these authors.

The data on interferon and viraemia suggest a close correlation between viraemia and interferon in the blood. These findings are in agreement with those of others (Kono and Ho, 1965; Baron, 1966; and Baron, Buckler, McCloskey and Kirschstein, 1966), that

titres of interferon in serum often paralleled the degree of viraemia. Further evidence for the occurence of circulating interferon during viraemia comes from the reports on serum interferon during viraemia in mice (Baron, Du Buy, Buckler and Johnson, 1964; and Steinebring, and Youngner, 1964), chicken (Youngner and Stinebring, 1964) and man (Wheelock and Sibley, 1964; and Petralli, Merigan and Gregory, 1965).

It was found that the degree of viraemia with TCRV was not so high. The possible contributory factors of low or terminating viraemia seems to be strict lymphotropic characteristics of the virus strain (Taylor and Plowright, 1965) coupled with a close correlation of viraemia and interferon in blood. Further ability of the attenuated strain to induce more interferon (Chapter 5), may offer an answer to the quest, why TCRV did not cause high grade viraemia.

It is evident from the results of neutralising antibodies presented in Table 21 and Figure 3 that antibodies were detectable at the earliest on day 6 after inoculating log $10^{6.0}$ $TCID_{50}$ TCRV. This observation is similar to the findings of many workers (Plowright and Ferris, 1962b; Johnson and Smith, 1962; Plowright and Taylor, 1967; and

Okuna and Rweyemamu, 1974), who observed that following vaccination with TCRV antibodies become detectable between 6th-10th day. A point of speciel interest in this study was that both virus and interferon started declining with the appearance of antibodies which is possibly due to the blockage of the extracellular virus by antibodies and the suppression of the formation of viral components at the intracellular level by interferon.

6. 5. SUMMARY:

A study was made to determine *in vivo* the possibility of interferon induction by TCRV. Buffaloes responded to intravenous virus inoculation without showing any clinical reaction. Low level circulating interferon was identified as early as 48 hours p.i. Peak interferon titre was, however, attainable on day 6 p.i., which had a positive correlation with viraemia. Circulating specific antibodies were also detected at the earliest on the 6th day p.i. With the elevation of antibodies, the titre of viraemia and interferon started to decline. It is concluded that TCRV virus infection *in vivo*, first stimulated interferon production followed by antibody formation.

CHAPTER 7

AN INVESTIGATION OF THE ROLE OF INTERFERON IN EARLY PROTECTION AFFORDED BY TISSUE CULTURE RINDERPEST VACCINE

7. 1. INTRODUCTION:

Many workers have reported that animals inoculated with live rinderpest vaccines resist challenge as early as 2-3 days after vaccination (Pfaff, 1938; Hale, Walker, Maurer, Baker and Jenkins, 1946; Brotherston, 1951a; Simpson, 1954; Plowright and Ferris, 1959b; Wild and Scott, 1961; and Johnson, 1962b), although antibodies become first detectable between 6-10 days (Hale, Walker, Maurer, Baker and Jenkins, 1946; Plowright and Ferris, 1959b; Johnson, 1962b; Johnson and Smith, 1962; Taylor and Plowright, 1965; Plowright and Taylor, 1967; Okuna and Rweyemamu, 1974). Some workers have suggested that early protection afforded by TCRV was due to viral interference (Plowright and Ferris, 1959b; and Johnson, 1962b). Such protection mediated through interferon has been demonstrated in the case of many viruses, for instance, influenza (Isaacs and Hitchcock, 1960), vaccinia (Baron and Buckler, 1963; Glasgow and Habel, 1963), yellow fever (Wheelock and Sibley, 1965), NDV (Baron and Buckler, 1963; Baron, Buckler, Friedman and McCloskey, 1966), measles (Petralli, Merigan and Wilber, 1965a,b), and

LT strain of rinderpest virus (Fujisaki, Ishii and Watanabe, 1968). The demonstration of circulating interferon in buffalo inoculated with TCRV in chapter 6 has given some credence to the hypothesis first advanced by Plowright and Ferris, (1959b) and Johnson, (1962b) that this vaccine induces early protection through interference.

The aim of the present investigation was to clarify the mechanism of early protection following live rinderpest virus vaccine inoculation which develops prior to the appearance of neutralizing antibodies. Hence experiments were designed to study the development of interferon, neutralizing antibodies and the appearance of resistance to virulent virus infection in buffaloes inoculated with TCRV.

7. 2. MATERIALS AND METHODS:

Most of the materials and methods used in the present studies have been described in chapters 3, 4 and 6.

Twelve rinderpest susceptible young buffaloes were each inoculated with ~~log~~ $10^{6.0}$ $TCID_{50}$ TCRV intravenously. They were bled before vaccination and at 24, 34, 48, 58, 72 hours and then on 4, 6, 8, 10 and 14 days p.i. for serum collection. On day 2, 3, 4, 6, 10 and 14 p.i. two vaccinated animals were challenged by subcutaneous inoculation with ~~log~~ $10^{4.0}$ $TCID_{50}$ virulent contagious rinderpest virus strain "IAK"

and on day 2, 3, 4, 10 and 14 p.i. a susceptible control animal was also similarly inoculated with virulent virus. All the animals were examined clinically daily. The serum samples were assayed for interferon and neutralizing antibodies using methods described in chapters 4 and 6.

7. 3. RESULTS:

Clinical response.

None of the animals showed any clinical response to TCRV inoculation. Animals challenged at 48 hours post vaccination succumbed to infection in the same manner as the control, but those challenged 72-96 hours post vaccination were protected. They did show a transient mild reaction characterized by a rise of $3^{o}C$ in body temperature above normal. Detailed data of the development of resistance to rinderpest infection in buffaloes vaccinated with ~~log~~ $10^{6.0}$ $TCID_{50}$ TCRV is presented in Table 22. Temperature charts of the individual animals are attached as appendix 1-17.

Animals challenged on day 6, 10 and 14 were solidly immune and did not show any clinical response whatsoever. In all cases, control susceptible buffaloes reacted severely to challenge inoculation. They demonstrated typical symptoms and post mortem picture as described by Scott, (1967b).

Interferon response.

Detailed data of interferon production by buffaloes following TCRV inoculation is presented in Table 23. Low level interferon was detected at the earliest, 48 hours following TCRV inoculation in animal No.36. At 58 hours p.i. low level circulating interferon was detected from buffaloes No.17, 31, 32 and 36. On day 3 p.i. interferon was detectable from the blood samples of all the 12 buffaloes except No.29. Peak titre of interferon was observed on day 6 p.i. On the 10th day p.i.,low level interferon was detectable from 2 out of 12 animals only (No.15 and 20).

Challenge virus inoculation did not influence interferon production in any of the vaccinated animals. It however, stimulated the control animals to produce interferon which was detectable from the 3rd to the 10th day following inoculation with virulent rinderpest virus.

Antibody response.

All the animals responded to tissue culture rinderpest vaccination and developed neutralizing antibodies, which are shown in Table 24. Five out of the 12 vaccinated animals, showed low level circulating antibodies on day 6 at the earliest, which subsequently increased and attained a mean titre log $10^{2.35}$ on day 14 p.i.

Challenge virus inoculation to b
also stimulated antibody production in conv
animals. The earliest circulating neutrali
antibodies from the blood samples or reacti
were observed on day 8 p.i. Virulent virus
apparently did not influence the antibody
in vaccinated buffaloes.

TABLE 22

DEVELOPMENT OF RESISTANCE TO RINDERPEST INFECTION IN BUFFALOES

INOCULATED WITH ~~10G~~ $10^{6.0}$ $TCID_{50}$ TISSUE CULTURE RINDERPEST VACCINE

Animal Number	Interval Between Vaccination and Challenge	Interferon Titre *	Antibody Titre **	CLINICAL RESPONSE				Sequelae
				Pyrexis	Lacrimal discharge	Nasal Discharge	Diarrhoea	
12	48 Hours	-	-	H	+	+	+	Recovered
13	" "	-	-	H	+	+	+	"
14	Control	-	-	H	+	+	+	"
15	72 Hours	0.7	-	L	+	-	+	Recovered
17	" "	1.0	-	L	+	-	+	"
18	Control	-	-	H	+	+	+	"
21	96 Hours	1.5	-	-	-	-	-	Recovered
22	" "	1.2	-	L	-	-	-	"
26	Control	-	-	H	+	+	+	Died
28	6 Days	1.9	0.2	-	-	-	-	Recovered
29	" "	1.8	0.2	-	-	-	-	"
31	10 Days	-	1.8	-	-	-	-	Recovered
32	" "	-	2.0	-	-	-	-	"
33	Control	-	-	H	+	+	+	"
35	14 Days	-	2.2	-	-	-	-	Recovered
36	" "	-	1.8	-	-	-	-	"
37	Control	-	-	H	+	+	+	Died

TABLE 22

Key:

*	=	Log 10 IF_{50}
**	=	Log 10 SN_{50}
H	=	Rectal temperatu
L	=	Rectal temperatu
-	=	Below detectable
+	=	Positive
±	=	Moderate diarrhoe

TABLE 23

TITRE OF CIRCULATING INTERFERON IN BUFFALOES INOCULATED WITH CELL CULTURE RINDERPEST VACCINE AND SUBSEQUENTLY CHALLENGED WITH VIRULENT RINDERPEST VIRUS

Animal Number	Interval Between Vaccination and Challenge	POST VACCINATION SAMPLING INTERVAL											
		HOURS						DAYS					
		0	24	34	48	58	72	4	6	8	10	12	14
12	48 Hours	-	-	-	-	-	1.0	1.3	1.9	0.7	-	-	-
13	" "	-	-	-	-	-	0.7	1.0	1.8	1.0	-	-	-
14	Control	-	-	-	-	-	-	-	0.7	1.0	1.6	0.7	-
15	72 Hours	-	-	-	-	-	0.7	1.5	1.3	1.6	1.0	-	-
17	" "	-	-	-	-	0.7	1.0	1.3	1.9	1.3	-	-	-
18	Control	-	-	-	-	-	-	-	0.7	0.7	1.2	1.0	-
21	4 Days	-	-	-	-	-	1.0	1.5	1.9	1.5	0.7	-	-
22	" "	-	-	-	-	-	1.0	1.2	1.6	1.0	-	-	-
26	Control	-	-	-	-	-	-	-	-	Tr*	0.7	0.7	1.0
28	6 Days	-	-	-	-	-	0.7	1.2	1.9	1.5	-	-	-
29	" "	-	-	-	-	-	-	1.0	1.8	1.3	-	-	-
31	10 Days	-	-	-	-	-	0.7	1.3	1.3	1.8	1.2	-	-

TABLE 23

		HOURS					DAYS						
32	10 Days	-	-	-	-	0.7	1.3	1.6	1.5	1.0	-	-	-
33	Control	-	-	-	-	-	-	-	-	-	-	-	-
35	14 Days	-	-	-	-	-	1.0	0.7	1.8	1.0	-	-	-
36	" "	-	-	-	0.7	0.7	0.7	1.5	1.3	0.7	-	-	-
37	Control	-	-	-	-	-	-	-	-	-	-	-	-

Tr* = Traces

TABLE 24

TITRE OF CIRCULATING NEUTRALIZING ANTIBODY IN BUFFALOES INOCULATED WITH CELL CULTURE RINDERPEST VACCINE AND SUBSEQUENTLY CHALLENGED WITH VIRULENT RINDERPEST VIRUS

Animal Number	Interval Between Vaccination and Challenge	POST VACCINATION SAMPLING INTERVAL											
		HOURS						DAYS					
		0	24	34	48	58	72	4	6	8	10	12	14
12	48 Hours	-	-	-	-	-	-	-	0.2	1.2	1.5	1.8	2.2
13	" "	-	-	-	-	-	-	-	1.8	1.8	2.0	2.5	2.8
14	Control	-	-	-	-	-	-	-	-	-	0.8	1.8	2.3
15	72 Hours	-	-	-	-	-	-	-	-	1.8	2.2	2.2	2.8
17	" "	-	-	-	-	-	-	-	0.2	1.1	2.0	2.3	2.3
18	Control	-	-	-	-	-	-	-	-	-	-	0.9	1.1
21	4 Days	-	-	-	-	-	-	-	0.2	0.8	1.8	2.0	2.8
22	" "	-	-	-	-	-	-	-	-	0.4	0.9	1.8	2.2
26	Control	-	-	-	-	-	-	-	-	-	-	Tr.*	0.2
28	6 Days	-	-	-	-	-	-	-	-	0.2	1.1	1.8	2.3
29	" "	-	-	-	-	-	-	-	-	0.2	0.8	1.8	1.8
31	10 Days	-	-	-	-	-	-	-	-	0.2	0.9	1.8	1.8
32	" "	-	-	-	-	-	-	-	-	0.8	1.8	2.0	2.2
33	Control	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 24

		HOURS						DAYS					
		0	24	34	48	58	72	4	6	8	10	12	14
35	14 Days	-	-	-	-	-	-	-	0.2	1.1	1.8	2.3	2.2
36	" "	-	-	-	-	-	-	-	-	1.8	2.0	2.3	2.8
37	Control	-	-	-	-	-	-	-	-	-	-	-	-

*Tr. = Traces

7. 4. DISCUSSION:

In this investigation, factors which might limit virus propagation and thus aid in the animal recovery have been considered. The very first factor which strikes the mind is the development of specific anti-rinderpest antibody. Results of the present investigation shown in Table 24, indicate that antibody was first detected on day 6 p.i. with TCRV. This observation is in complete agreement with the findings of other workers who have studied the development of rinderpest neutralizing antibody in cattle and demonstrated it to be first detectable between 6 and 10 days p.i. (Plowright and Ferris, 1959b; Johnson, 1962b; Johnson and Smith, 1962; Taylor and Plowright, 1965; Plowright and Taylor, 1967; and Okuna and Rweyemamu, 1974).

Results described in Table 23, show that interferon was detected in the blood of vaccinated buffaloes on the 3rd day post vaccination. This aspect of the observations coincides with the response before the development of specific antibody in the case of many viruses, in vivo, such as influenza, vaccinia, yellow fever, NDV, measles, and LT strain of rinderpest virus (Isaacs and Hitchcock, 1960; Baron and Buckler, 1963; Glasgow and Habel, 1963; Wheelock and Sibely, 1965; Petralli, Merigan and Wilber, 1965a,b; Baron, Buckler, Friedman and McCloskey, 1966;

Observations recorded in Table 22, indicate that there was a tendency for animals that had circulating interferon to show marked ability of protection against virulent infection even when circulating neutralizing antibody was not detectable.

In addition to this, it is natural that some factor other than interferon must be taken into consideration to interpret the results hitherto obtained. But it is hardly possible that before the appearance of demonstrable level of neutralizing antibodies, the mechanism of preventing infection will come into operation by the participation of an amount of neutralizing antibodies so little to be detected before day 6 p.i. Absence of neutralizing activity from the serum samples collected in the early stages of TCRV infection (Table 24), appearance of interferon following 48 hours of vaccination (Table 23), and resistance to virulent infection on day 3 (Table 22), suggest a possibility that interferon might have participated in protection afforded in the early stages of infection. The ability of interferon produced in vivo to affect the course of viral infection has been described by many workers (Isaacs and Hitchcock, 1960; Hitchcock and Porterfield, 1961; Friedman, Baron, Buckler and Steinmuller, 1962). Thus the experiments reported in this chapter indicate

that interferon plays a role in early protection afforded by TCRV. Circulating interferon in buffalo was demonstrated after 48 hours of vaccine injection. This interval is long when compared with the findings of Baron and Buckler,(1963), who detected circulating interferon in mice one hour after intravenous inoculation of NDV. The results of TCRV stimulated interferon in buffaloes are, however, comparable with the findings of Fujisaki,Ishii and Watanabe, (1968), who detected circulating interferon in rabbits on the 2nd and 3rd day p.i. with lapinized rinderpest virus strain (LT). This seems to be a general character of interferon response in animals (Baron and Levy, 1966). The failure of interferon to persist may be related to the observation that cells which have absorbed or produced large amounts of interferon do not produce interferon on subsequent infection with the same virus and that the absorbed interferon is not extractable in active form (Wagner, 1961). On the other hand, low level intracellular interferon persists for several days and participates in later stages of recovery despite the absence of continued interferon production (Isaacs and Westwood, 1959; Lockart and Horn, 1963; Paucker and Cantell, 1963; and Friedman, 1964). Thus the relationship between

the appearance of resistance to challenge virus and the presence of interferon in the blood of buffaloes indicate that early protection afforded by TCRV is mediated by interferon production.

7. 5. SUMMARY:

1. Low level interferon was detected in the sera of buffaloes after 48 hours of ~~log~~ $10^{6.0}$ $TCID_{50}$ TCRV intravenous inoculation.

2. Animals challenged at 48 hours p.i. succumbed to virulent infection in the same manner as the control. Those challenged 72-96 hours p.i. were protected, but showed a transient mild reaction. Animals challenged on day, 10 and 14 were solidly immune

3. Interferon was also detected from the blood of control animals, 3-10 days following challenge infection.

4. Circulating neutralizing antibodies from the vaccinated buffaloes were detected at the earliest on day 6 p.i. Antibodies were detected on day 8 from the animals subjected to virulent challenge alone.

5. Early protection afforded by TCRV in buffalo to virulent virus infection has conclusively been shown to be due to the development of endogenous interferon.

CHAPTER 8

GENERAL DISCUSSION

The research reported in this thesis was designed to study viral interference in rinderpest, which has been a matter of conjecture for a long time (Pfaff, 1938; Brotherston, 1951a; Simpson, 1954; Plowright and Ferris, 1959b; Johnson, 1962b). Aspects of critical study were: the production of interference *in vitro* and *in vivo* as a result of rinderpest virus infection; whether interference played a part in protection from rinderpest infection, and to investigate and evaluate viral interference and its role in early protection of buffalo afforded by tissue culture vaccine.

These objectives have been achieved. The results show that virulent and fully attenuated rinderpest virus induced interferon production in BK cultures and in buffalo and that viral interference was mediated by interferon. Furthermore, these results indicate that it is possible to monitor virus virulence by assessing the interferon induced by the virus and

the depression in the challenge virus yield. Consequently, the finding that avirulent virus strain produced more interferon and the fact that "Kabete O" vaccine strain interfered with virulent virus in cattle (Plowright and Ferris, 1959b; Johnson, 1962b), suggest that the effect of virus virulence was masked by interferon in both the BK cultures and in buffalo. Moreover, these results also show that TCRV virus infection in buffalo stimulated the production of interferon followed by antibody formation and clearly demonstrate that the cell culture "Kabete O" strain rinderpest virus vaccine is completely inocuous for the domestic buffalo, as it is for cattle (Plowright and Ferris, 1962b; Johnson, 1962b).

The most significant findings are, that the addition of vaccine additive to the vaccine fluid stabilized the virus against exposure to heat at 56°C and against UV-irradiation. UV-irradiated or active TCRV virus had the ability to interfere with homologous

as well as heterologous viruses. The viral inhibitor produced by TCRV virus in BK cultures fulfilled the biological criterion to be classified as interferon (Levine and Nichol, 1970), and that the fully attenuated "Kabete O" rinderpest vaccine virus had an enhanced interferon inducing property compared with the virulent non-contagious "RBOK" and the virulent contagious "PAK" strains respectively. The relationship among the appearance of circulating interferon, the absence of detectable levels of neutralizing antibody and resistance to virulent infection show that interferon was responsible for early protection afforded by TCRV virus. The detection of interferon in the *in vitro* studies affords an explanation to the observations made by Plowright (1964), that complete change of media accelerated CPE and that the changing of media from cultures infected with TCRV released more virus into the surrounding fresh fluid.

It should be noted therefore, from these results that vaccine additives should be carefully considered and probably evaluated before they are

added to the vaccine fluid. Due to the enhanced interferon inducing property by fully attenuated "Kabete O" strain compared with virulent "RBOK" and "PAK" strains, increased interferon inducing character of the virus may be used as a marker of virus attenuation. These observations are also in favour of the use of TCRV virus in the event of rinderpest outbreaks because of its interferon inducing ability, instead of gamma globulin which is given for the same purpose.

Inspite of the homogeneous nature of rinderpest virus strains (Plowright, 1968), avirulent strains produce CPE of a different morphology when compared with the CPE produced by virulent strains (Plowright, 1962c; 1963b; Plowright and Ferris, 1962a: Liess, 1963). The explanation is that this difference may be due to differences amongst these strains with respect to their ability to induce interferon. There is therefore a need to pursue further studies to elucidate this point.

Some rinderpest virus strains such as "Kabete O" (Plowright and Ferris, 1962b) and "RBOK" (Brotherston, 1951b; Plowright, 1952), do not spread by contact. These strains do replicate in the body of inoculated animals (Taylor and Plowright, 1965; MacOwan, 1956) and during the present work, they have been shown to possess a more enhanced ability to induce interferon than the virulent contagious "PAK" strain. Secretion of interferon from nasal washings has been demonstrated in human subjects following virus infection (Jao, Wheelock and Jackson, 1970). It could therefore be postulated that due to the high interferon inducing potential by these strains, there is a higher level of interferon at the surface of the mucous membranes and parenchymatous tissues and this may account for the inability of these strains to spread by contact amongst susceptible animals. Since there is no relevant information to support this view, detailed investigation is therefore indicated in this direction.

Enhanced interferon inducing ability by viruses proved to be responsible for virus persistance in cell cultures (Ho, and Enders. 1959; Wagner, 1960; 1963c). The virus persistance theory, which may likely be responsible for the life long immunity in rinderpest, is still not ascertained. On the other hand, the influence of enhanced interferon inducing ability of viruses on antibody production in animals is not completely accepted (Hitchcock and Isaacs, 1960; Anderson, 1965; De Somer, Billiau and Clercq, 1967). The influence of endogenous interferon on antibody production therefore, needs a careful study in order to answer this question.

It is suggested that further research on the three above mentioned areas, is both desirable and necessary for the understanding of viral interference in rinderpest.

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SHED

ANIMAL No. 12

COLOUR Black AGE 2 years

DETAILS OF INOCULATION

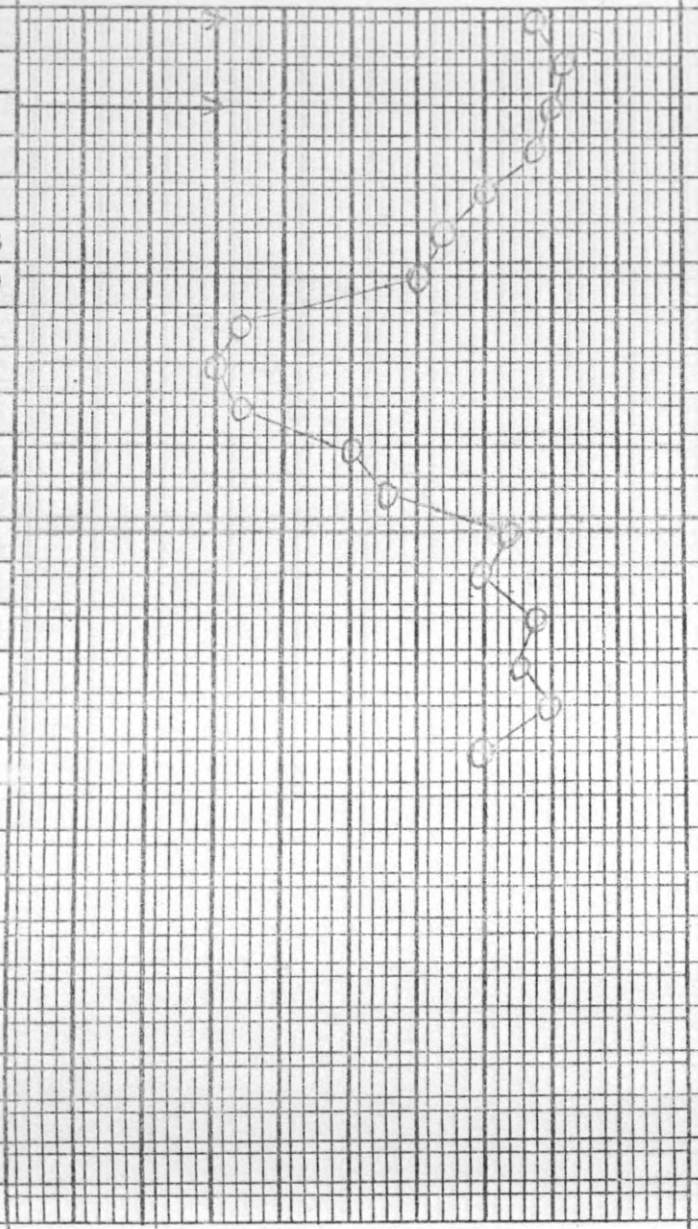
On 23.10.1974

log 10^6 TCID$_{50}$ TCRV batch No. 131 inoculated intravenously

On 25.10.1974

log $10^{4.5}$ TCID$_{50}$ rinderpest virus PAK strain inoculated subcutaneously

DISEASE :- VIRAL INTERFERENCE



DATE	DAY		SYMPTOMS AND	SPECIFIC LESIONS
23	0			
24	1			
25	2			
26	3			
27	4			
28	5			
29	6	mucoid lacrimal & nasal discharge		
30	7	muconurulent	lacrimal & nasal discharge	
31	8	" "	" "	" "
1	9	purulent	" "	" "
2	10	" "	" "	" "
3	11	" "	with moderate	diarrhoea
4	12	"	" "	" "
5	13	" "	with profuse	diarrhoea
6	14	" "	" "	" "
7	15	condition started improving	" "	" "
8	16			
9	17	condition better		
10	18			
11	19			
12	20			
13	21			
14	22			
15	23			
16	24			
17				
18				
19				

VETERINARY RESEARCH INSTITUTE, LAHORE CANTT

SHED

ANIMAL No. 13

COLOUR black AGE 2 years

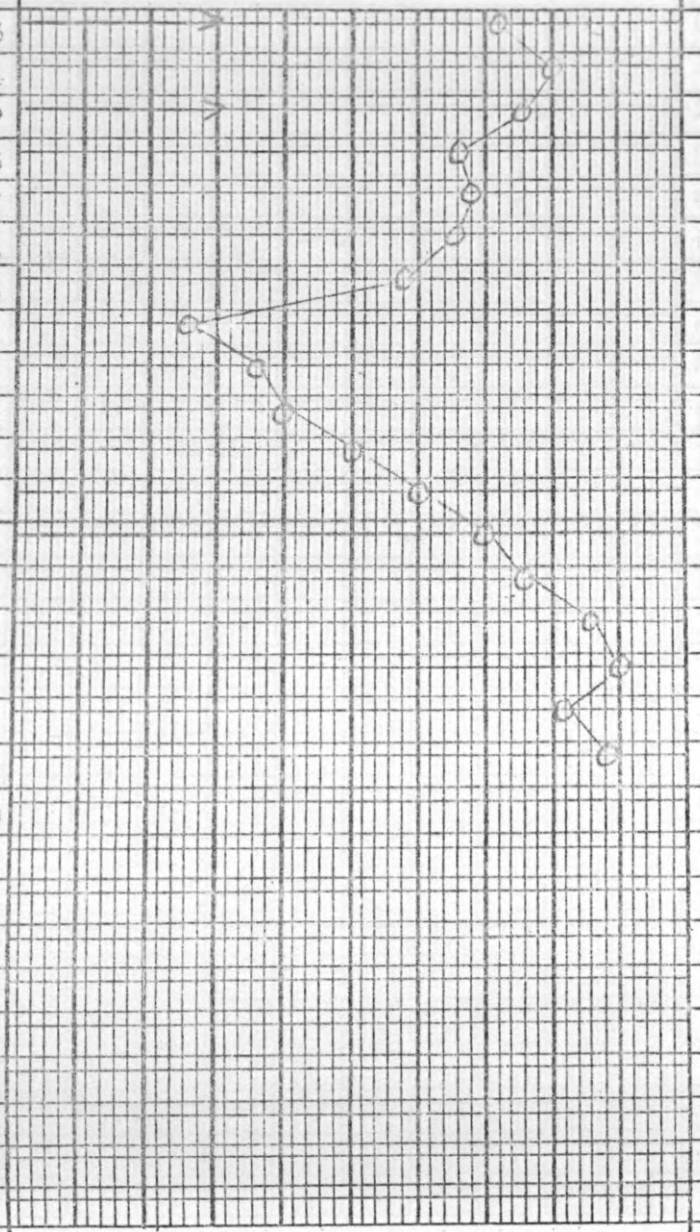
DETAILS OF INOCULATION

On 25.10.1974 log 10^{6} $TCID_{50}$ TCRV batch No. 431 inoculated intravenously

On 25.10.1974 log $10^{4.5}$ $TCID_{50}$ rinderpest virus PAK strain inoculated subcutaneously

(II)

DISEASE :- VIRAL INTERFERENCE



DATE	DAY	CONDITION AND DIET	SYMPTOMS AND	SPECIFIC LESIONS
23	0			
24	1			
25	2			
26	3			
27	4			
28	5	mucoid lacrimal discharge		
29	6	mucopurulent	lacrimal & nasal discharge	
30	7	" "	" "	" "
31	8	purulent	" "	" "
1	9	" "	" "	" "
2	10	" "	with moderate	diarrhoea
3	11	" "	with profuse	diarrhoea
4	12	" "	" "	" "
5	13	" "	" "	" "
6	14	condition started improving		
7	15			
8	16			
9	17	condition better		
10	18			
11	19			
12	20			
13				
14				
15				
16				
17				
18				
19				

SHED

ANIMAL No. 14

COLOUR: black AGE: 2 year

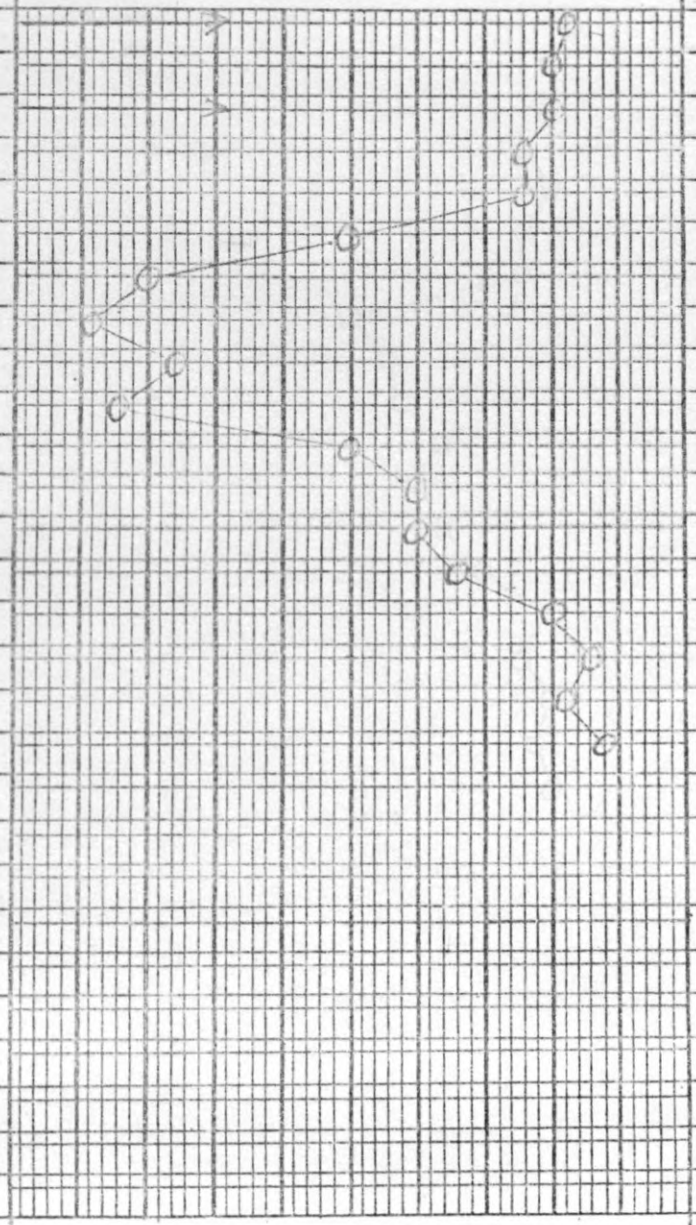
DETAILS OF INOCULATION

On 23.10.1974 10 ml cell culture fluid inoculated intravenously.

On 25.10.1974 log 10^6 $TCID_{50}$ TCRV batch NO. 431 inoculated subcutaneously.

(III)

DISEASE :- VIRUS CONTROL



DATE	DAY	CONDITION OF SHED	SYMPTOMS AND	SPECIFIC LESIONS
23	0			
24	1			
25	2			
26	3			
27	4			
28	5	mucoid lacrimal discharge		
29	6	muconurulent	lacrimal and	nasal discharge
30	7	purulent	" "	" "
31	8	" "	" "	" "
1	9	" "	" "	" "
2	10	" "	with moderate	diarrhoea
3	11	" "	" "	" "
4	12	" "	with profuse	diarrhoea
5	13	" "	" "	" "
6	14	condition started improving		
7	15			
8	16			
9	17	condition improved.		
10	18			
11	19			
12	20			
13				
14				
15				

SHED

ANIMAL No. 15

COLOUR: black AGE: 2 years

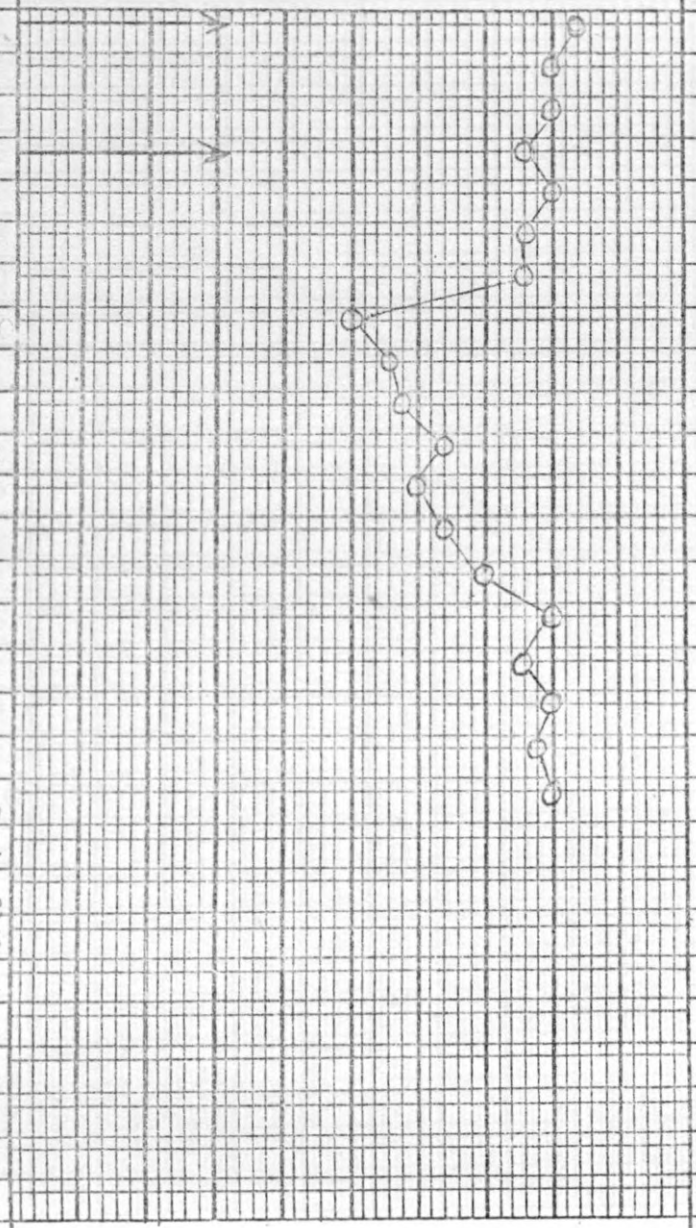
DETAILS OF INOCULATION

On 23.10.1974 log 10^{6} $TCID_{50}$ TCRV batch No. 431 inoculated intravenously.

On 26.10.1974 log $10^{4.5}$ $TCID_{50}$ rindermest virus PAK strain inoculated subcutaneously.

(IV)

DISEASE :- VIRAL INTERFERENCE



DATE	DAY	CONDITION AND DIET	SYMPTOMS AND	SPECIFIC LESIONS
23	0			
24	1			
25	2			
26	3			
27	4			
28	5			
29	6			
30	7	moderate lacrimal & nasal		discharge
31	8	" "	"	"
1	9	" "	with moderate	diarrhoea
2	10	" "	"	"
3	11	" "	"	"
4	12	condition improved		
5	13			
6	14	condition normal.		
7	15			
8	16			
9	17			
10	18			
11	19			
12	20			
13	21			
14				
15				
16				
17				
18				
19				

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SHED

ANIMAL No. 17

COLOUR black AGE 2 years

DETAILS OF INOCULATION

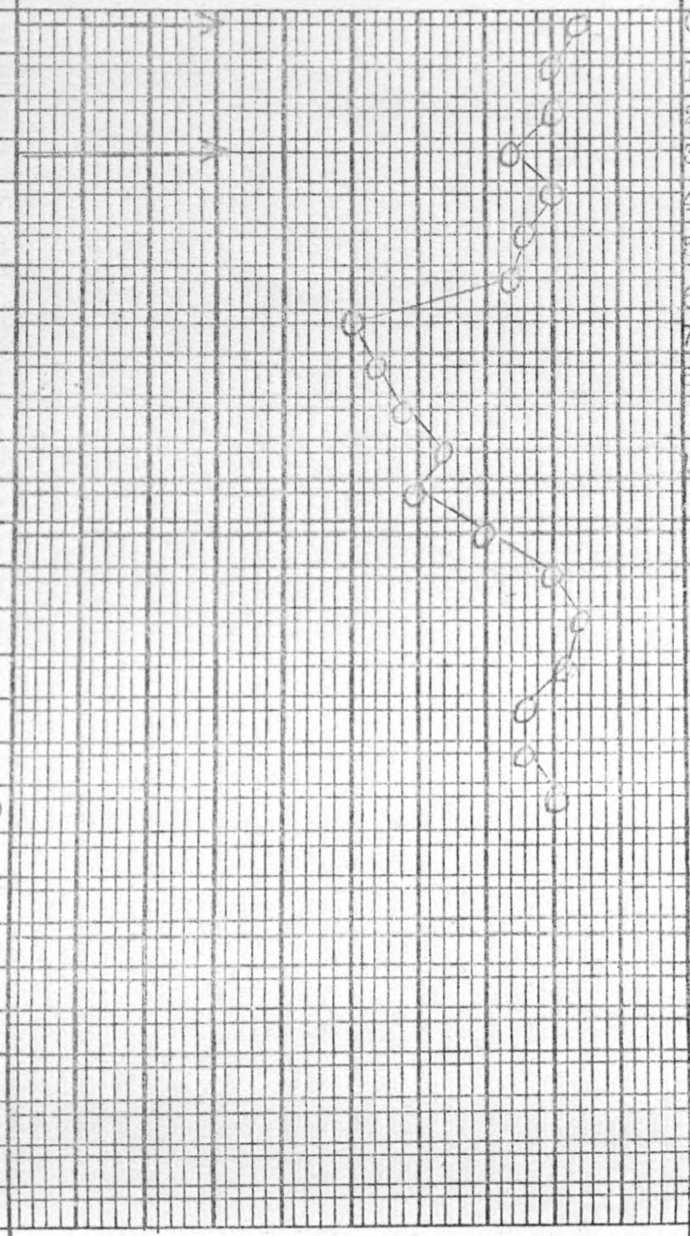
On 23.10.1974

log 10^{6} $TCID_{50}$ TCRV batch No. 431 inoculated intravenously.

On 26.10.1974

log $10^{4.5}$ $TCID_{50}$ rinderpest virus PAK strain inoculated subcutaneously.

DISEASE :- VIRAL INTERFERENCE



DATE	DAY	SYMPTOMS AND SPECIFIC LESIONS
23	0	
24	1	
25	2	
26	3	
27	4	
28	5	
29	6	
30	7	mucoid lacrimal & nasal discharge
31	8	" " " " "
1	9	" " with moderate diarrhoea
2	10	" " " " "
3	11	" " " " "
4	12	condition improving
5	13	
6	14	condition normal
7	15	
8	16	
9	17	
10	18	
11	19	
12	20	
13	21	
14	22	
15	23	
16	24	
17	25	
18	26	
19	27	

(V)

SHED

ANIMAL No. 18

COLOUR: black AGE: 2 years

DETAILS OF INOCULATION

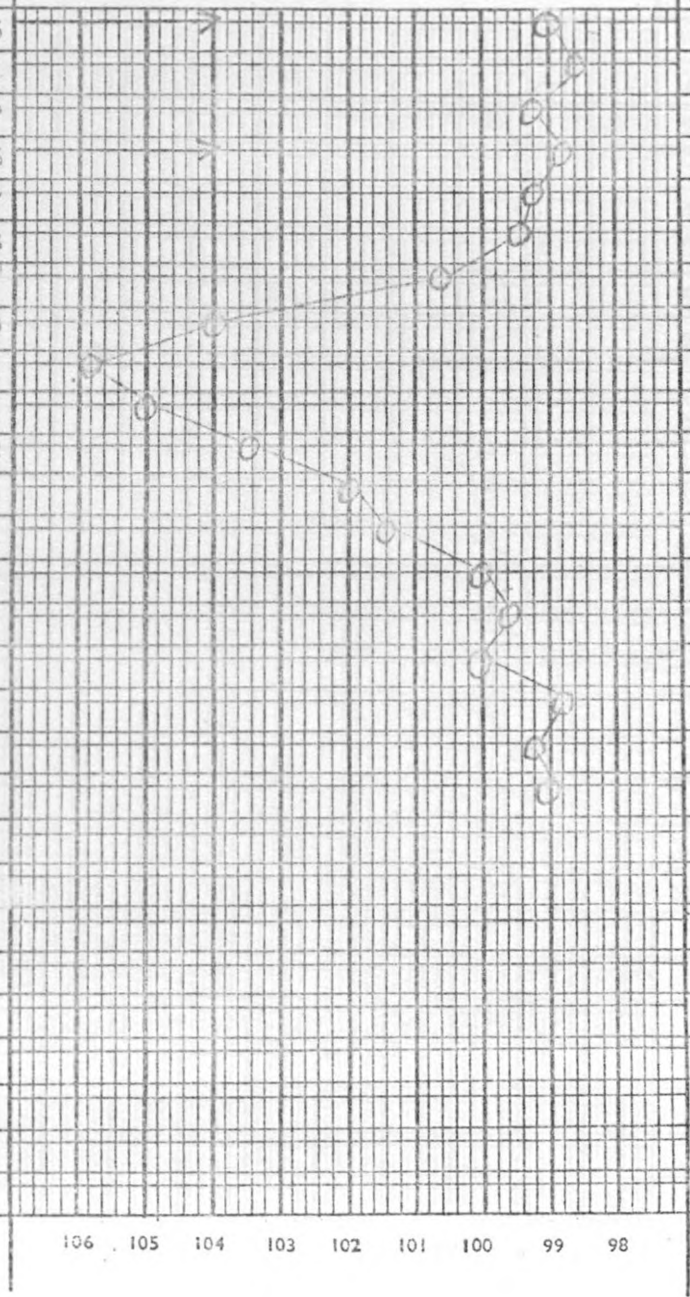
on 23.10.1974

10 ml cell culture fluid inoculated intravenously.

On 26.10.1974

10$^{4.5}$ TCID$_{50}$ rinderpest virus PAK strain inoculated subcutaneously.

DISEASE :- VIRUS CONTROL



DATE	DAY		SYMPTOMS AND	SPECIFIC LESIONS
23	0			
24	1			
25	2			
26	3			
27	4			
28	5			
29	6			
30	7	mucoid lacrimal & nasal discharge		
31	8	" "	" "	" "
1	9	mucopurulent	lacrimal & nasal discharge	
2	10	"	with moderate	diarrhoea
3	11	"	"	"
4	12	"	with profuse	diarrhoea
5	13	"	"	"
6	14	"	"	"
7	15	condition started improving		
8	16			
9	17			
10	18	condition better.		
11	19			
12	20			
13	21			
14	22			
15				
16				
17				
18				
19				

(IA)

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SHED

ANIMAL No. 21

COLOUR: black AGE: 2 years

DETAILS OF INOCULATION

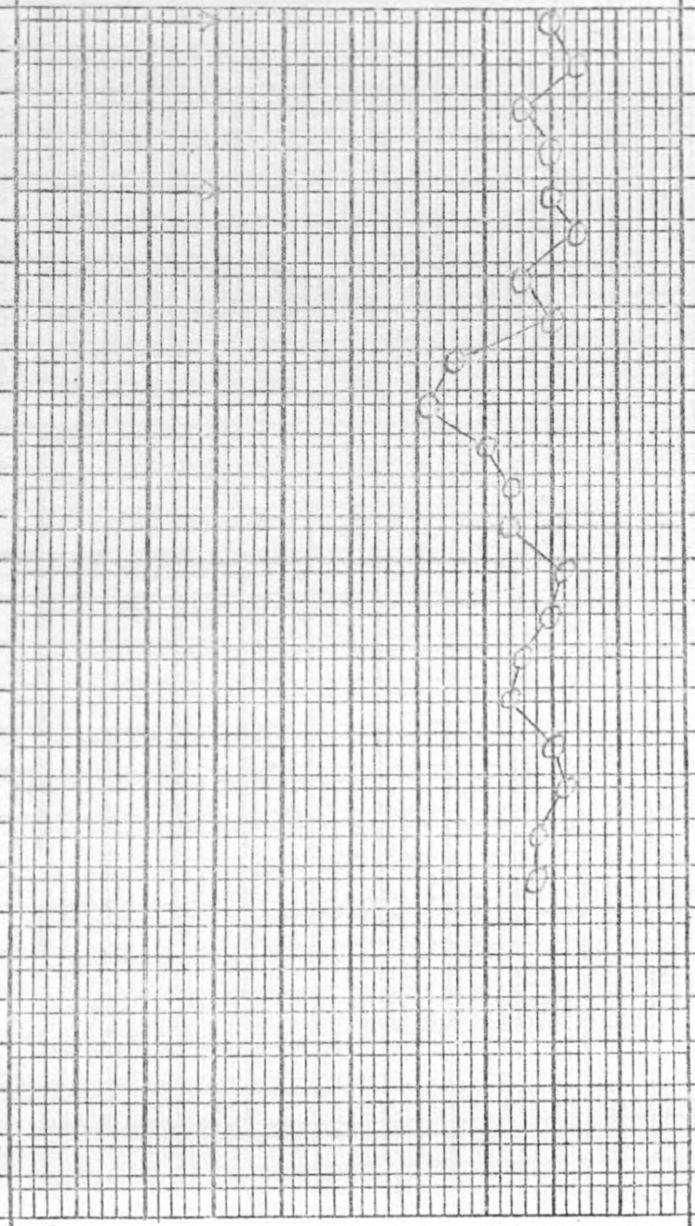
On 25.10.1974 log 10^{6} $TCID_{50}$ TCRV batch No. 431 inoculated intravenously.

On 27.10.1974 log $10^{4.5}$ $TCID_{5.0}$ rinderpest virus PAK strain inoculated subcutaneously.

(VII)

DISEASE :- VIRAL INTERFERENCE

DATE	DAY		SYMPTOMS AND	SPECIFIC LESIONS
23	0			
24	1			
25	2			
26	3			
27	4			
28	5			
29	6			
30	7			
31	8			
1	9			
2	10			
3	11			
4	12			
5	13			
6	14			
7	15			
8	16			
9	17			
10	18			
11	19			
12	20			



(VIIIA)

SHED

ANIMAL No. 22

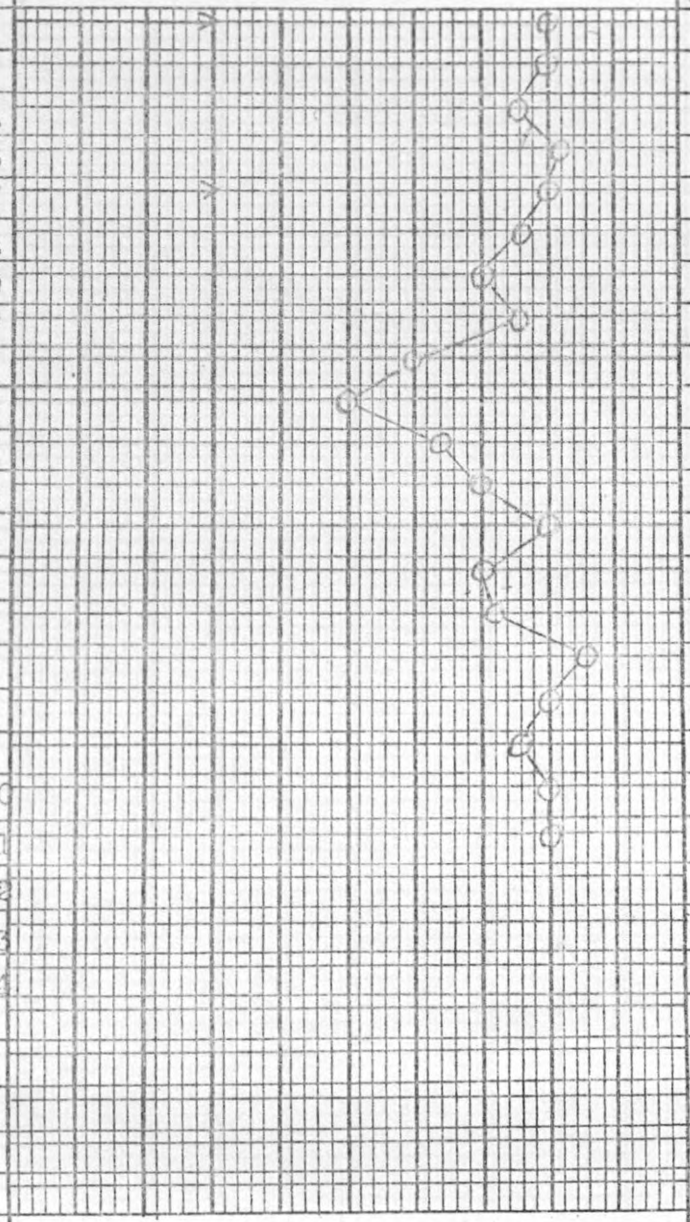
COLOUR black AGE 2 years

DETAILS OF INOCULATION

On 23.10.1974
log 10^6 $TCID_{50}$ TCRV
batch No. 431 inoculated intravenously.

~~On 27.10.1974~~
log $10^{4.5}$ $TCID_{50}$
~~rinderpest virus~~
~~PAK strain inoculated subcutaneously~~

DISEASE :- VIRAL INTERFERENCE



DATE	DAY		SYMPTOMS AND	SPECIFIC LESIONS
23	0			
24	1			
25	2			
26	3			
27	4			
28	5			
29	6			
30	7			
31	8			
1	9			
2	10			
3	11			
4	12			
5	13			
6	14			
7	15			
8	16			
9	17			
10	18			
11	19			
12	20			
13				
14				
15				
16				
17				
18				
19				
20				

ANIMAL No. 23

COLOUR: black

AGE: 2 years

DETAILS OF INOCULATION

On 23.10.1974

log 10^{6} $TCID_{50}$ TCRV

batch No. 431 inocu-

lated intravenously.

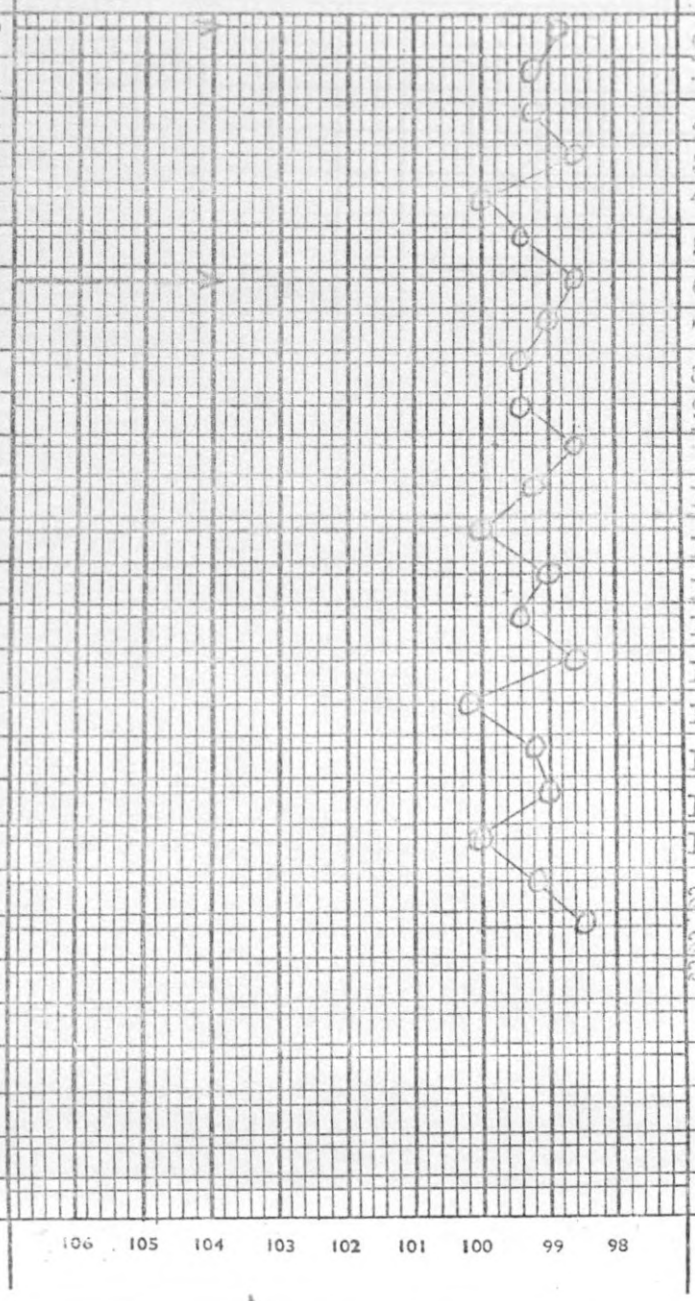
On 29.10.1974

log $10^{4.5}$ $TCID_{50}$

rinderpest virus

PAK strain inocul-

ated subcutaneously.



(XI)

SHED

ANIMAL No. 26

COLOUR AGE

black 2 years

DETAILS OF INOCULATION

On 23.10.1974

10 ml cell culture

fluid inoculated

intravenously.

On 27.10.1974

log $10^{4.5}$ $TCID_{50}$

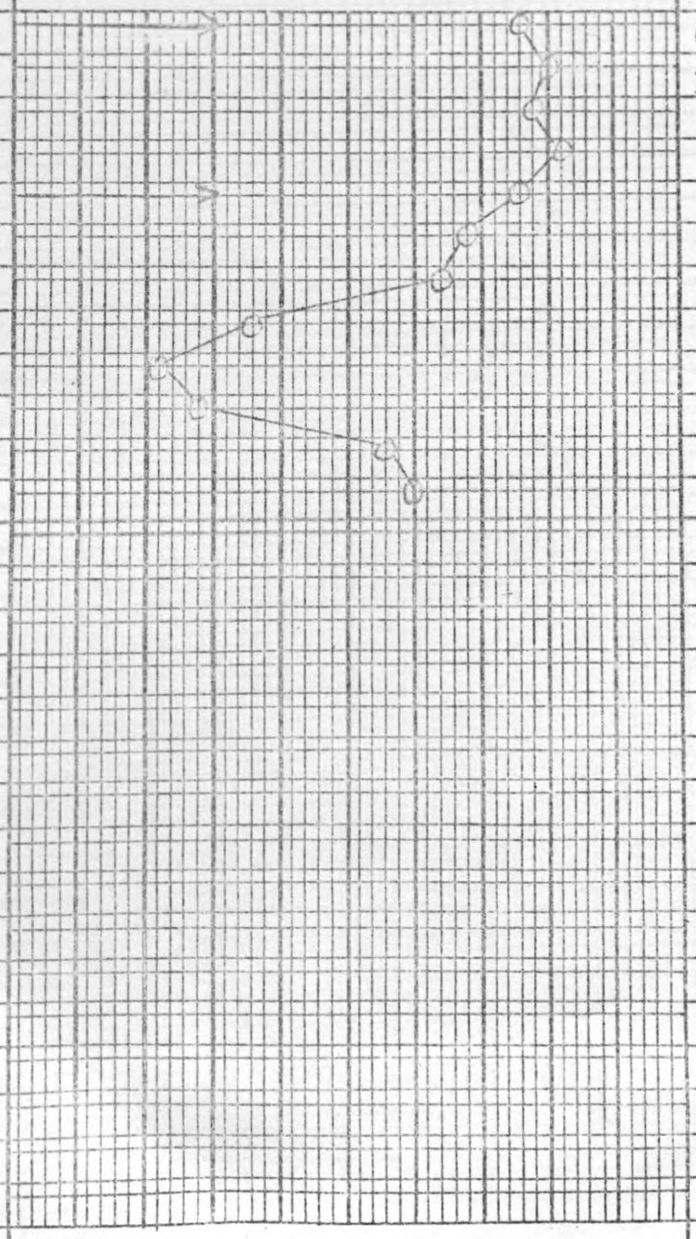
rinderpest virus

PAK strain inocul-

ated subcutaneous-

DISEASE :- VIRUS CONTROL

DATE	DAY	SYMPTOMS AND SPECIFIC LESIONS
23	0	
24	1	
25	2	
26	3	
27	4	
28	5	
29	6	
30	7	mucoid lacrimal discharge
31	8	" " & nasal discharge
1	9	" " " "
2	10	" " " "
3	11	" " " moderate " with diarrhoea.
4	12	found dead on the morning of 4.11.1974.
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		



SHED

ANIMAL No. 29

COLOUR black AGE 2 year

DETAILS OF INOCULATION

On 23.10.1974

log 10^{6} $TCID_{50}$ TCRV

batch No. 431 inoculated intravenously.

On 29.10.1974

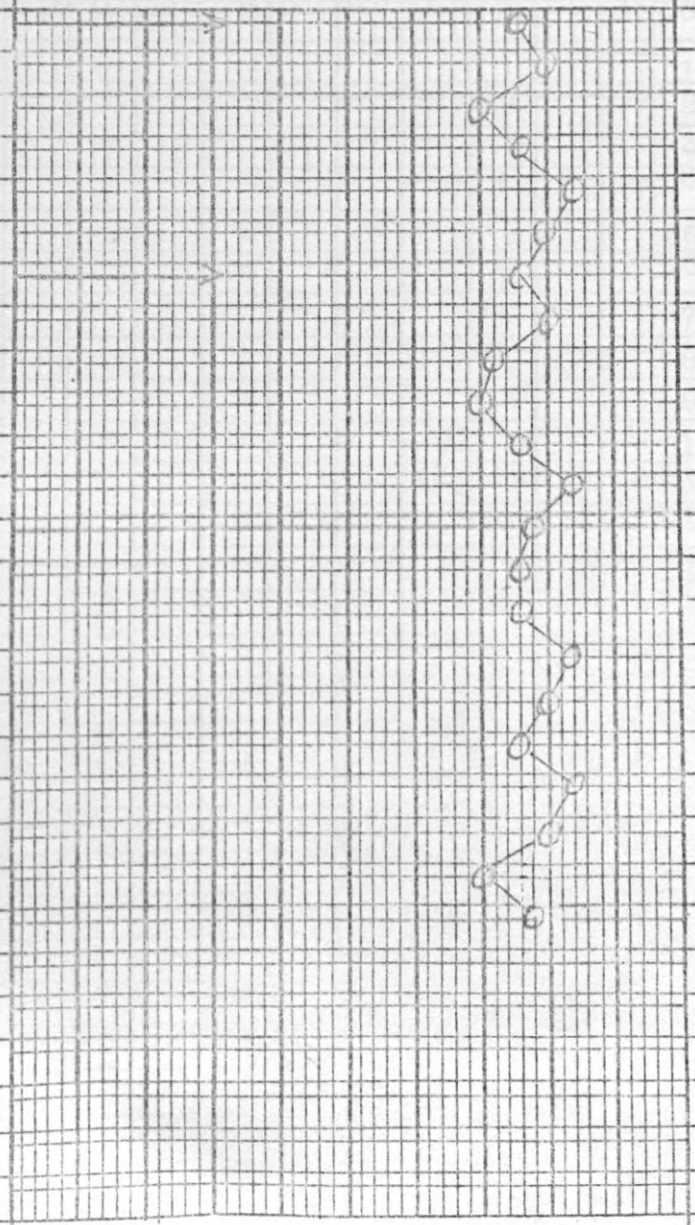
log $10^{4.5}$ $TCID_{50}$

rinderpest virus

PAK strain inoculated subcutaneously.

DISEASE :- VIRAL INTERFERENCE

DATE	DAY		SYMPTOMS AND	SPECIFIC LESIONS	
23					
24					
25					
26					
27					
28					
29					
30					
31					
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					



(XI)

SHED

ANIMAL No. 31

COLOUR black AGE 2 years

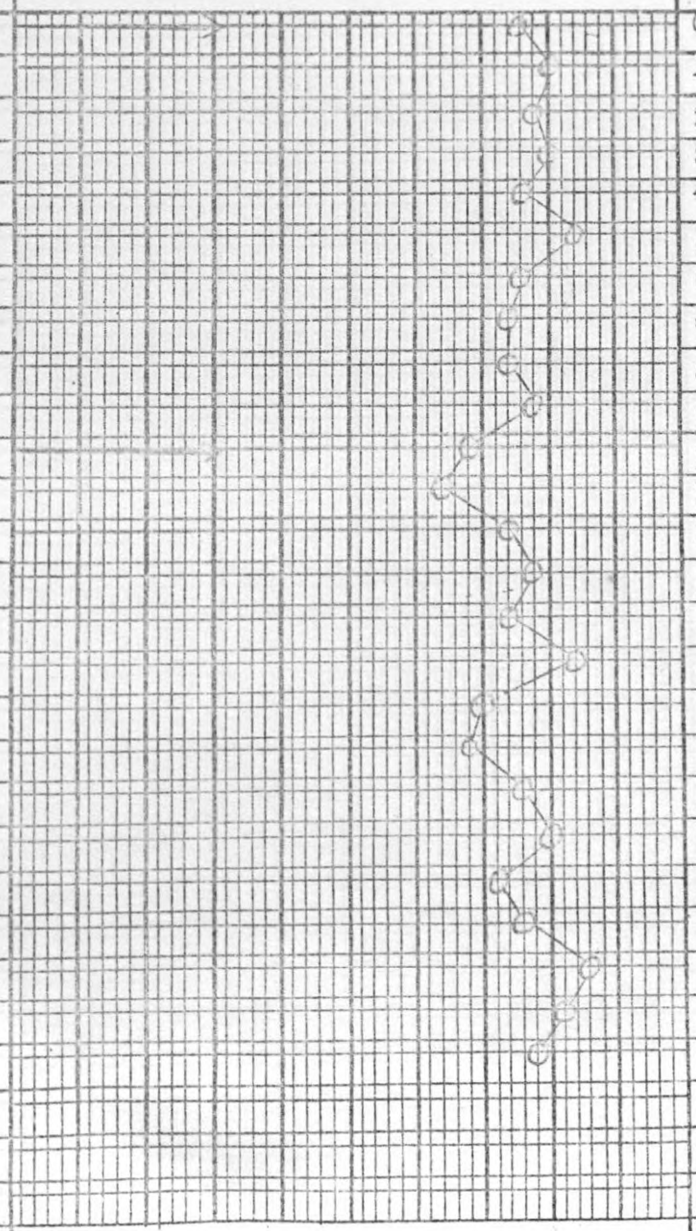
DETAILS OF INOCULATION

On 23.10.1974 log 10^{6} $TCID_{50}$ TCRV batch No. 431 inoculated intravenously.

On 2.11.1974 log $10^{4.5}$ $TCID_{50}$ rinderpest virus PAK strain inoculated suboutaneously.

DISEASE :- VIRAL INTERFERENCE

DATE	DAY
23	0
24	1
25	2
26	3
27	4
28	5
29	6
30	7
31	8
1	9
2	10
3	11
4	12
5	13
6	14
7	15
8	16
9	17
10	18
11	19
12	20
13	21
14	22
15	23
16	24
17	
18	
19	



SHED

ANIMAL No. 32

COLOUR black AGE 2 year

DETAILS OF INOCULATION

On 23.10.1974

log 10^6 $TCID_{50}$ TCRV

batch No. 431 inoculated intravenously.

On 2.11.1974

log $10^{4.5}$ $TCID_{50}$

rinderpest virus

PAK strain inoculated subcutaneously.

DISEASE :- VIRAL INTERFERENCE

DATE	DAY
23	0
24	1
25	2
26	3
27	4
28	5
29	6
30	7
31	8
1	9
2	10
3	11
4	12
5	13
6	14
7	15
8	16
9	17
10	18
11	19
12	20
13	21
14	22
15	23
16	24
17	25
18	
19	

106 105 104 103 102 101 100 99 98

(XIII)

SHED

ANIMAL No. 33

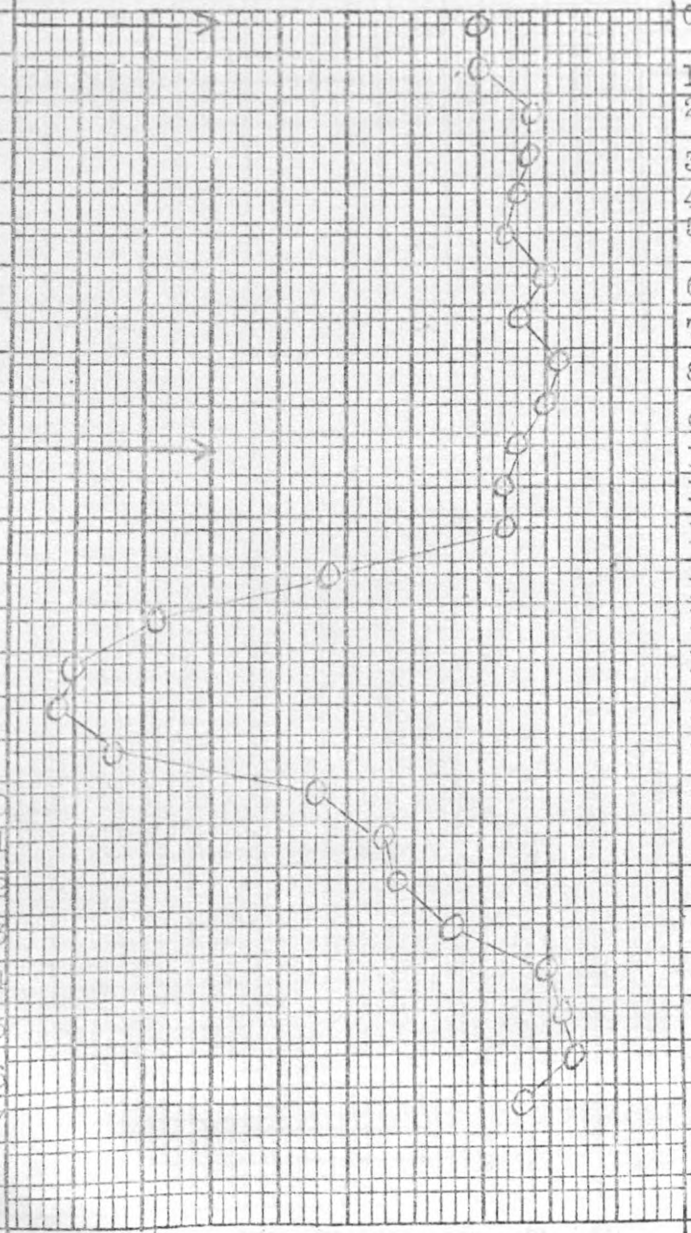
COLOUR: black AGE: 2 years

DETAILS OF INOCULATION

On 23.10.1974 10 ml cell culture fluid inoculated intravenously

On 2.11.1974 log $10^{4.5}$ $TCID_{50}$ rinderpest virus PAK strain inoculated subcutaneously.

DISEASE :- VIRUS CONTROL



DATE	DAY	SYMPTOMS AND	SPECIFIC LESIONS
23	0		
24	1		
25	2		
26	3		
27	4		
28	5		
29	6		
30	7		
31	8		
1	9		
2	10		
3	11		
4	12		
5	13	mucoid lacrimal discharge	
6	14	mucoid lacrimal & nasal discharge	
7	15	muconpurulent lacrimal & nasal discharge	
8	16	" " " " with moderate diarrhoea	" "
9	17	" " " " " "	" "
10	18	" " with profuse diarrhoea	" "
11	19	" " " " " "	" "
12	20	" " " " " "	" "
13	21	" " " " " "	" "
14	22	condition started improving	
15	23		
16	24		
17	25	condition improved.	

SHED

ANIMAL No. 35

COLOUR: black AGE: 2 years

DETAILS OF INOCULATION

On 23.10.1974 $\log 10^{6}$ $TCID_{50}$ TCRV batch No. 431 inoculated intravenously

On 6.11.1974 $\log 10^{4.5}$ $TCID_{50}$ rinderpest virus PAK strain inoculated subcutaneously.

DISEASE :- VIRAL INTERFERENCE

DATE	DAY		SYMPTOMS AND	SPECIFIC LESIONS	
23	0				
24	1				
25	2				
26	3				
27	4				
28	5				
29	6				
30	7				
31	8				
1	9				
2	10				
3	11				
4	12				
5	13				
6	14				
7	15				
8	16				
9	17				
10	18				
11	19				
12	20				
13	21				
14	22				
15	23				
16	24				
17	25				
18	26				
19	27				
20	28				

VETERINARY RESEARCH INSTITUTE, LAHORE CANTT

STUDIES OF VIRAL INTERFERENCE INDUCED BY RINDERPEST VIRUS

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B.Sc.(A.H.), M.Sc.(A.H.)

A Thesis submitted in fullfilment for the Degree of Doctor of Philosophy in the University of Nairobi.

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and Microbiology
Faculty of Veterinary Medicine
University of Nairobi
KENYA.

November, 1976.

DECLARATION

This thesis entitled STUDIES ON VIRAL INTERFERENCE INDUCED BY RINDERPEST VIRUS is my original work and has not been presented for a degree in any other University.

SYED FIDA HUSSAIN
B.Sc.(A.H.); M.Sc.(A.H.)

This thesis has been submitted for examination with our approval as University Supervisors.

.....

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.....

Dr. J.S. Kaminjolo,Jr.,
Senior Lecturer, Department
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CERTIFICATE

This is to certify that the thesis entitled STUDIES ON VIRAL INTERFERENCE INDUCED BY RINDERPEST VIRUS is bonafide work of Mr. Fida Hussain, Assistant Research Officer, Veterinary Research Institute, Lahore, Pakistan; and that the part of work completed in Lahore was carried out under my supervision, and that this thesis has been submitted for examination with my approval as External Supervisor.

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Dated: 1st October, 1976.

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SHED

ANIMAL No. 36

COLOUR black AGE 2 years

DETAILS OF INOCULATION

On 23.10.1974

log 10^{6} $TCID_{50}$ TCRV

batch No. 431 inoculated intravenously

On 6.11.1974

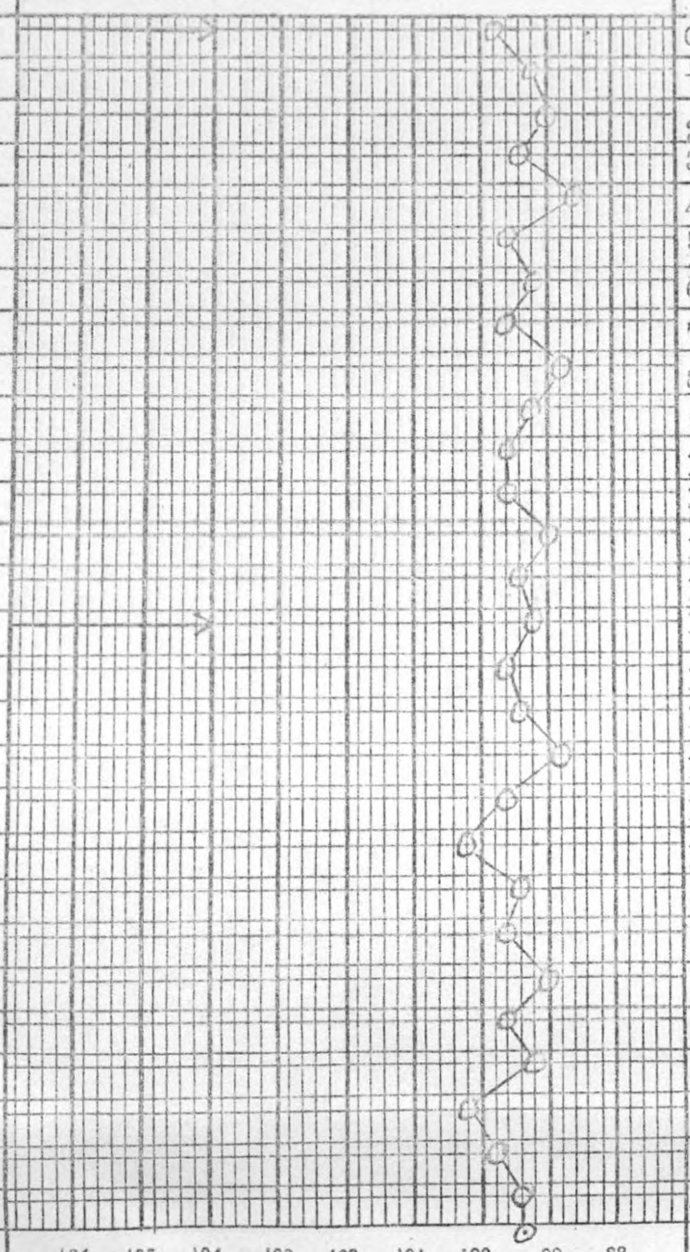
log $10^{4.5}$ $TCID_{50}$

rinderpest virus

PAK strain inoculated subcutaneously.

DISEASE :- VIRAL INTERFERENCE

DATE	DAY
23	0
24	1
25	2
26	3
27	4
28	5
29	6
30	7
31	8
1	9
2	10
3	11
4	12
5	13
6	14
7	15
8	16
9	17
10	18
11	19
12	20
13	21
14	22
15	23
16	24
17	25
18	26
19	27
20	28



SHED

ANIMAL No. 37

COLOUR black AGE 2 years

DETAILS OF INOCULATION

On 23.10.1974

10 ml cell culture fluid inoculated intravenously.

On 6.11.1974

log $10^{4.5}$ $TCID_{50}$ rinderpest virus PAK strain inoculated subcutaneously.

DISEASE :- VIRUS CONTROL

DATE	DAY
23	0
24	1
25	2
26	3
27	4
28	5
29	6
30	7
31	8
1	9
2	10
3	11
4	12
5	13
6	14
7	15
8	16
9	17
10	18
11	19
12	20
13	21
14	22
15	23
16	24
17	25
18	26
19	27
20	28