Study on Cell Mediated Immune Response in Mice to Foot and Mouth Disease Virus Serotype O

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

(FACULTY OF VETERINARY SCIENCE)

PUSA (SAMASTIPUR)

By

Sanjay Kumar Sinha

In partial fulfilment of the requirements

FOR THE DEGREE OF

Master of Veterinary Science

(MICROBIOLOGY)

POST GRADUATE DEPARTMENT OF VETERINARY MICROBIOLOGY

BIHAR VETERINARY COLLEGE

PATNA-800014

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P A T N A - 8 0 0 0 1 4

August 1987

Affectionately

Dedicated

To

My Reverend

Mother

Department of Veterinary Microbiology,
Bihar Veterinary College, Patna
RAJENDRA AGRICULTURAL UNIVERSITY, BIHAR

CERTIFICATE - I

This is to certify that the thesis entitled " Studies on Cell Mediated Immune Response in Mice to Foot-and-Mouth Disease Virus Serotype 'O' " submitted in partial fulfilment of the requirement for the Degree of Master of Veterinary Science (Microbiology) of the Faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar is the record of bonafide research carried out by Dr. Sanjay Kumar Sinha under my supervision and guidence. No part of the thesis has been submitted for any other Degree and Diploma.

It is further certified that such help or informations received during the course of this investigation and preparation of the thesis have been duly acknowledged.

C.B. Prasad
18.8.87

(C.B. Prasad)
Associate Professor & Head,
Department of Veterinary
Microbiology.

CERTIFICATE - II

We, the undersigned member of the Advisory Committee of Dr. Sanjay Kumar Sinha, a candidate for the Degree of Master of Veterinary Science with major in Microbiology have gone through the manuscript of the thesis and agree that the thesis entitled " Studies on Cell Mediated Immune Response in Mice to Foot-and-Mouth Disease Virus Serotype 'O' " may be submitted by Dr. Sanjay Kumar Sinha in partial fulfilment of the requirements for the Degree.

Prasad
18.8.87

(C.B.Prasad)
Chairman, Advisory Committee

Members of the Advisory Committee

1 B. K. Sinha 18/8/87

2 L. N. Prasad 18.8.87

3 Md. Murtuza

CERTIFICATE - III

This is to certify that the thesis entitled " Studies on Cell Mediated Immune Response in Mice to Foot-and-Mouth Disease Virus Serotype 'O' " submitted in partial fulfilment of the requirements for the Degree of Master of Veterinary Science (Microbiology) of the Faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar was examined and approved on 4.1.1988

(C.B.Prasad)
Chairman, Advisory Committee

(R.C.PATHAK)
External Examiner

Members of Advisory Committee

1 B.K. Sinha 4/1/88

2 L.N.Prasad 4.1.88

3 Md. Murtuza

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Dean, Post-Graduate Studies

I GRATEFULLY ACKNOWLEDGE THE
FINANCIAL ASSISTANCE PROVIDED BY THE
RAJENDRA AGRICULTURAL UNIVERSITY, BIHAR,

IN THE FORM OF
RESEARCH FELLOWSHIP DURING
THE PERIOD OF THE PRESENT STUDY

ACKNOWLEDGEMENT

ACKNOWLEDGEMENT

In presenting an interpretive account of "Studies on Cell Mediated Immune Response in Mice to Foot-and-Mouth Disease Virus Serotype 'O' " which by no means was easy task for the present scholar to complete without the help of many people with whom the author came in contact in course of FMD samples collection, enquiring and investigation. I very gratefully acknowledge their gracious outlook and endeavour in lending factual informations which immensely helped me to build up initial edifice of the present investigation.

I am very happy to express my deep sense of gratitude and sincere indebtedness to my esteemed teacher and advisor, Dr.C.B.Prasad, M.V.Sc., F.R.V.C.S., Ph.D., Associate Professor & Head, Department of Veterinary Microbiology, Bihar Veterinary College, Patna for his critical supervision, constant inspiration and encouragement during the present investigation. I recollect his critical trends which helped immensely to improve my knowledge over the years.

I owe a great deal of thanks to my minor advisor, Dr.M.Murtuza, Associate Professor & Head, Department of Veterinary Biochemistry, Bihar Veterinary College, Patna for his valuable suggestions and assistance rendered to me during this investigation.

Sincere gratitude is expressed to Dr.B.K. Sinha, Associate Professor, Department of Veterinary Microbiology, Bihar Veterinary College, Patna for his excellent suggestions, unlimited help and constant encouragement during the tenure of the present work.

Deep indebtedness is expressed to Dr.Lala Naresh Prasad, Associate Professor of Veterinary Pathology, Bihar Veterinary College, Patna for his valuable suggestions and assistance which facilitated my research work immensely.

It is my pleasure to express my sincere thanks to Dr.T.S.Sharma, University Professor and Chairman, Department of Veterinary Microbiology, Bihar Veterinary College, Patna for his valuable suggestions and assistance which facilitated my research work to a great deal.

It is my pleasure to express my sincere thanks to Dr.M.N.Sinha and Dr.C.R.Prasad, Associate Professors of Veterinary Microbiology and Dr.K.C.P. Singh, Assistant Professor of Veterinary Microbiology for their valuable suggestions and assistance.

Grateful obligations are expressed to Dr.Mani Mohan, Associate Professor and to Dr.D.Sharma, Assistant Professor, Department of Animal Breeding and Genetics, Bihar Veterinary College, Patna for providing Swiss Albino mice for the present study.

I am thankful to Dr.R.N.Singh, Dean and Principal, Bihar Veterinary College, Patna for providing the necessary facilities during the course of this study.

I am glad to extend my thanks to my collegue scholars Dr.(Mrs.) Neelam Kumari, Dr. Anup Kumar Roy, Dr.Yogendra Prasad Singh, Dr. Ajeet Kumar, Dr.Surendra Kumar Gupta, Dr. Bhubneshwar Singh, Dr. Ashok Kumar, Dr. Umesh Kumar, Dr. Bhawesh Gopal Deo and Dr. Mahendra Kumar for their cooperation and help in many ways during the present study.

I wish to express my thanks to Shri Subedar Prasad and other staffs of the department of Veterinary Microbiology, Bihar Veterinary College, Patna for their time to time help during the present study.

I am glad to extend my thanks to Mr. Raju Kumar, Typist, Legal Section, Rajendra Agricultural University, Bihar Veterinary College, Campus, Patna for his day and night labour to complete the thesis in time.

I express my deep sense of infinite gratitude to my reverend maternal grandmother and parent for their constant encouragement, sacrifice and devotion without which it would not have been possible to carry out the present piece of work and also I can not forget the help rendered to me by younger brother "Shrawan" and elder sisters Mrs. Sangita and Mrs.Sunita.

Last but not the least, I shall be faulty if I do not deliver my whole hearted appreciation and thanks to my dearest wife, Mrs. Manorama Sinha "RANI", M.Sc. for her endurance, enthusiasm and unfathomable love which in turn proved to be a deep rooted source of encouragement during the tenure of the study.

S.K.S.

C O N T E N T S

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INTRODUCTION

Foot-and-Mouth Disease (FMD) is an acute, febrile viral disease affecting almost exclusively cloven footed animals and is endemic in India occurring practically all the year round. Ellis and James (1978) provided a glimpse of its devastating impact on national economy as the disease leaves the survivors in extremely debilitated condition, decreasing milk production, causing spark of abortion and sterility and laming the draught animals. The impact is exaggerated in cross bred and exotic cattle with high calf mortality. The annual economic losses by way of reduction in milk production alone has been estimated to rupees four hundred crores (Adlakha and Sharma, 1982). Thus, this disease has been recognised as number one burning problem and stumbling block in the way of intensive livestock development programme.

Of the seven serotypes of FMD virus viz. 'O', 'A', 'Asia-1', 'C', 'SAT-1', 'SAT-2' and 'SAT-3', four serotypes, 'O', 'A', 'C' and 'Asia-1' have been recorded from outbreaks of foot and mouth disease in India. The problem of disease control becomes complicated because each of these serotypes possesses several subtypes. The condition becomes complex as recovery from infection or vaccination with one serotype does not render the animals immune to other types. In absence of antiviral agent, the only dependable method of dealing with FMD is its

prevention. The usual control and eradication measures adopted in advanced countries is slaughtering of all affected and in-contact animals which is impracticable in India due to economic and religious reasons. Thus, selective vaccination is the only available method for the control of this dreadful disease.

As regards the type of immune response induced in animals by different types of FMD virus following vaccination or infection, several workers have shown close relation between humoral immunity and protection of animals and have worked on the dynamics of antibody response at various intervals of time following vaccination (Van Bekkum et al.,1963; Lal et al.,1980; Sutmoller et al.,1980; Shorshnev et al.,1981). Studies conducted under "All India Coordinated Research Project for Epidemiological Studies on Foot and Mouth Disease" during the last 14 years have revealed that FMD virus type 'O' is most predominant and wide spread causing more than 70% of the FMD outbreaks. It has also been opined that probably FMD virus type 'O' is very weak immunogen.

However, only scanty reports are available proving relationship of cell mediated immune response with the protection of animals following vaccination or infection with different types of FMD virus (Lebedev

et al.,1972). Sharma et al. (1986) reported increased T-lymphocytes at 35 days post vaccination and demonstrated a positive delayed type hypersensitivity (DTH) following revaccination with FMD type 'O' suggesting cell mediated immune response. They found positive co-relation at 14 and 60 days post vaccination between T-cells and B-cells and it was suggested that T-cells mediated B-cells for humoral response.

Available literature show that a little concerted effort has been made to study the systematic immunological response to FMD virus types, specially in relation to cell mediated immune response and the role of macrophages as the effector limb of cell mediated immune response, which is considered essential in evolving a suitable immunoprophylaxis. In animals generally all the immunological apparatus remain intact and so it becomes virtually impossible to demonstrate the actual role of particular arm in immune system. However, the advent of immunodeficient models has made it possible to pin point the role of a particular arm by eliminating the other arm of the immune apparatus. Among the laboratory animals, mouse is being used increasingly for preparation of such immunodeficient models and simultaneously suckling mouse takes FMD virus infection easily by intramuscular or intraperitoneal route.

Keeping in view the above consideration, immunodeficient mouse model - FMD virus system has been used in the present investigation with the following objectives:

1. To study the role of cell mediated immune response to FMD virus type 'O' infection in T-cell deficient and normal mice.

2. To study the role of macrophages to FMD virus type 'O' infection in macrophage stimulated and macrophage depleted mice.

REVIEW OF LITERATURE

FMD Virus

FMD is considered as the most important economic disease of cloven footed animals and is endemic in India since long. Though it occurs in a mild form, but leaves the survivors in extremely deblitated condition affecting adversely the capacity to work and for production. Sterility and abortion are the usual after effects. The disease assumes a serious form in cross and exotic breeds. The problem of disease control is buffling and complicated due to plurality of virus serotypes and multiplicity of sub-types giving complexity to the immunization as recovery from infection with one serotype does not render the animal immune with other serotype.

Immune Response to FMD Virus

The immune system has been divided into (i) central lymphoid organs comprising thymus and bursa of Fabricius in birds or its equivalent in mammals and (ii) peripheral lymphoid organs represented by spleen, lymph nodes and gut and bronchus associated lymphoid tissues. Similarly, immune responses to any antigenic stimulus has been divided into (a) humoral immune response and (b) cell mediated immune response.

The stem cells for these responses are the

lymphocytes. One population of the lymphocytes matures in the bursa of fabricius in birds and in bone marrow of mammals and is differentiated into B lymphocytes. B lymphocytes become immunocompetent and after coming in contact with antigen subserve the function of humoral immunity by dividing into plasma cells which synthesize various mediators, the immunoglobulins. The second population of lymphocytes mature in the thumus and are differentiated into T-cells which become immunocompetent and responsible for provocation of cell mediated immune response (CMIR) . These T-cells when interact with an antigen become sensitised and proliferated and differentiated into effector cells which either destroy the target cells, or synthesize various effector substances known as lymphokines which cause local inflammation and delayed type hypersensitivity, promote maturation of B-cells and T-cells, or act as T-suppressors or T-helpers (Davis et. al.,1980). The macrophages are the most important ancillary cells which help humoral immune response processing antigen and help cell mediated immune response as well. After being activated by lymphokines, the macrophages play important role in removal of micro-organisms and foreign materials (Schwartz et al.,1970; Territo,1980).

As regards immune response to FMD virus, it has been well documented that FMD virus provokes both

humoral and cellular immune responses (Radisich et al., 1976; Sharma et al.,1985).

Humoral Immune Response

Several workers have studied various aspects of humoral immune response, specially the protective neutralizing antibody response to FMD virus serotypes 'O', 'A', 'C' and 'Asia-1' and sub-type 'A-22' both in cattle and in laboratory animals (Flachsel et al.,1982; Graves, 1969 and Lal et al.,1980) . The appearance of neutralizing antibody titre and its duration has also been subject of investigation by several workers (Fish et al.,1969; Bernath et al.,1979 and Sharma et al.,1983).

Van Bekkum et al. (1963) observed that neutralizing antibody titres in cattle vaccinated with FMD virus type 'O', 'A' and 'C' declined rapidly following primary vaccination. The antibody titres against 'O' was observed at 3 (three) weeks after primary vaccination followed by sharp decline by 12 weeks and then titres remained constant with marginal fluctuation.

More precise informations were furnished by Lucam et al. (1964) and Bernath et al. (1979) who considered a neutralizing antibody titre of 1:5 or greater to be protective and less than 1:5 to be unprotective against subsequent infection with FMD.

Wittmann et al. (1970) observed maximum neutralizing antibody titre ranging between 1:200 to 1:1300 at 6 weeks after immunization in cattle with inactivated 'O' FMD vaccine. The antibody titre declined to 1:10 from 1:260 at 8 weeks post vaccination and the titreswere not greater than 1:5 at 16 weeks post vaccination. After challenge with homologous virulent virus at 8 weeks post vaccination, all the four animals were found to be protected but two out of 4 developed generalized infection when challenged at 16 weeks post vaccination.

Negi and Kumar (1970) in India detected neutralizing antibody on the 7th days of vaccination in hill cattle vaccinated with 5,10 and 15 ml doses of 'Asia-1' vaccine. They reported full protection against homologous challenge at one month after vaccination and presence of protective neutralizing antibody upto 150 days.

Uppal et al. (1975) observed no significant difference in the antibody titres of calves either vaccinated with 20 ml or 40 ml dose of vaccine and they withstood virulent challenge after 6 weeks.

Radisich et al. (1976) observed neutralization index in respect of type 'O' virus following vaccination ('O' 'A' and 'C') to be 1.50, 2.72, 1.60, 1.42 and 1.13 at 10,21,90,120 and 180 days of vaccination respectively.

Lal et al. (1980) found that cattle inoculated with 2.5 ml dose of vaccine showed antibody titre ranging between 1.52 to 2.3 at 21 days post vaccination. The titre thereafter remained at a satisfactory level upto 150 days and later declined. The mean titre of 1.55 and 1.33 were recorded at 150 and 180 days post vaccination respectively.

Mathur et al. (1981) observed no significant difference in antibody titre in cattle inoculated with different doses of vaccine.

Tibor et al. (1985) studied duration of immunity in cattle immunized with polyvalent ('O','A' and 'C') FMD vaccine and challenged intralingually. Cattle immunized annually and 5 to 7 times during life time showed 70% protection in the 9th month following last vaccination, cattle immunized 3 to 13 times at interval of 6 months showed 75% protection and four months after the last innoculation,and cattle immunized only once at the age of one year was not satisfactory in the fourth month after immunization. Ninety percent of the cattle immunized twice at intervals of 4 months proved to be protected 3 and 6 months after 2nd innoculation, 70% after 9 months and 50% after 12 months of innoculation. It was pointed out that young animals should be revaccinated to maintain 80% protection of the stock.

Fernandez et al. (1986) studied susceptibility,

pathogenesis and immune response to FMDV experimental infection in 4 different strains (CF_1, C_3H, NIH-nude, BALB-C/J) of adult mice. Adult mice were found to be susceptible to FMDV under some experimental condition. Pathogenesis of infection, period of viremia and appearance of immune response were studied in these mice. Circulating neutralizing antibody appeared in CF_1 and C_3H mice at 72 and 96 hours post infection, respectively which persisted for 90 days period of study. Antibody against VIA was detectable for shorter period. Mice irradiated with one ID_{50} dose showed viremia for 40 days and low antibody response at the end of viremia.

Cell Mediated Immune Response

Scanty reports are available to ascertain the role of cell mediated immunity to FMD virus serotype in cattle and laboratory animals.

Lebedev et al. (1972) observed that when pig embryo kidney cells were infected with FMD in the presence of antiserum, most cells underwent cytocidal infection but a small proportion continued to multiply and some of them were transformed.

Kundsen et al. (1979) studied immunity to FMD serotype 'A-22' in guineapig with reference to cell mediated immunity. These workers showed the presence of

cell mediated immune response to FMD virus in guinea pigs by demonstrating macrophage inhibition factor and delayed skin response in guinea pigs.

Borca et al. (1984) studied the type of immune response in adult mice to FMDV infection following immunosuppression of animal with irradiation (750 R) and their reconstitution with mononuclear cells from allogenic mice. The donor allogenic mice were primed with 10,000 suckling mouse LD_{50} of FMDV type 'O', then 2 and 8 days post priming the mononuclear cells from blood, spleen, thymus and peritonial cavity were transferred to immuno suppressed mice. The parameters studies to determine the type of immune response were viremia, neutralizing antibody titre and sheep RBC haemaglutinating antibody titre in recepient mice. In irradiated mice viremia was found to be prolonged, antibody to FMDV or SRBC was found to be almost absent as compared to shorter viremia and greater neutralizing antibody response to FMD or SRBC in mice with reconstituted cells. It was concluded that humoral immune response play an important role in the recovery of mice from FMDV.

Mallick et al. (1985) studied non-specific immunostimulation of various laboratory animals viz., mice, chicks and Guinea pigs with chromogenic selected strain of Mycobacterium phlei against a variety of viruses, rabies,

Marek's disease and FMD. M. phlei produced strong CMIR against both specific and non specific viral antigens evidenced by lymphocyte migration inhibition test. A substantial quantity of inhibiting substances, which was not the classical antibody, was produced which neutralized FMD, IBR, rabies and NDV in cell culture, mice and embryonated eggs.

Sharma et al. (1985) reported increased T- lymphocytes at 35 days post vaccination and demonstrated a positive delayed type hypersensitivity (DTH) following revaccination in FMD virus type 'O' which suggested cell mediated immune response. A positive co-relation existed at 14 and 60 days post vaccination between T-cells and B-cells and it was suggested that T-cells mediated B-cells for humoral response.

The authors further assessed humoral immune response by micro SNI, ELISA and total Ig and cell mediated immune response by in-vitro LMI test, E-rosette test and in-vivo skin hypersensitive reactions in group I and II buffalo calves (8 to 12 months old) to type 'A-22' vaccine at 21 and 150 days post vaccination. Maximum Ig level (70 mg/ml) was observed on 28, 21 and 35 days post vaccination in group II. Average percent of LMI and E-rosette counts were 33.23 and 24.84 at 21 days post vaccination respectively, DTH in-vivo co-related LMI and E-rosette counts.

MATERIAL AND METHODS

Materials

Animals

Mice: Swiss Albino mice of either sex, weighing approximately 20-25 grammes and obtained from the Department of Animal Breeding and Genetics, Bihar Veterinary College, Patna were used throughout the study. Mice were kept in a group of three to five in a cage and were given sterilized feed and water ad libitum.

Rabbits: White rabbits of either sex, weighing 1.5 to 2.5 kilograms and obtained from the market were used throughout the study. They were fed on green vegetables, germinated grams and water ad libitum.

Guinea pigs: Male healthy guinea pigs, having body weight of about 600 to 700 grammes and procured from the market were used in the present study. They were maintained on green vegetables, germinated grams and water ad libitum.

Organisms

FMD Virus: FMD virus type 'O' used during the present study was obtained from the field samples as well as from the Department of Veterinary Microbiology (FMD Project), H.A.U., Hissar.

Methods

Serotyping of FMD virus from field samples by MCFT

A total of 77 samples were collected from FMD affected animals from the outbreaks under rural, urban and farm conditions. The samples included affected tissues of oral epithelium (tongue, gum and palate), feet epithelium, eye (inner and outer canthus) and vesicular fluid. Table-1 depicts the details of samples collected during the present investigation.

Method of Collection of Clinical Samples

Clinical materials for isolation and typing of FMD virus from suspected cases were collected at the height of reactions from a recently developed vesicles of affected animals. The collected materials comprised affected oral epithelium or feet epithelium but preferably from lesions of the tongue or elsewhere in the mouth. The affected tissues from feet epithelium were collected after washing with soap and water. The amount of epithelium varied from animal to animal, but a piece of 2 cms x 2 cms was preferred or maximum amount of covering epithelium was collected from the freshly developed vesicles.

The following methods were employed for collecting the epithelial tissues at different sites.

Table-1: Source and nature of clinical samples collected for typing of FMD virus

Sl. No.	Source of samples	Total	Nature of samples			
			Feet epith.	Tongue epith.	Gum lesions	Palate
1.	From FMD affected animals under village conditions:					
i)	Patna district	18	5	9	3	1
ii)	Nalanda district	9	5	4	-	-
iii)	Khagaria district	7	3	4	-	-
2.	under town condition					
i)	Patna	24	9	12	2	1
ii)	Nalanda	5	2	3	-	-
iii)	Khagaria	6	3	3	-	-
3.	Under farm condition					
i)	Exotic Cattle Farm, Patna	5	2	3	-	-
ii)	Gosala, Khagaria	3	1	2	-	-
	Total:	77	30	40	5	2

(i) Oral samples: The epithelial covering of the vesicles from tongues, gums and palates were collected by scrapping with the help of sterile forceps and scalpel. In case of ruptured vesicles, the epithelial covering was held with the help of sterile forceps and the tissues were removed by cutting.

(ii) Feet samples: The epithelial covering of recently developed vesicles formed in the interdigital spaces (the clefts of hoof) and on the bulbs of the heels were collected with the help of sterile forceps and scissors. Materials collected from one or more animals of the same species at one place comprised one sample, while materials from the other species constituted second sample. While collecting samples in the vial with glycerine saline, particulars of animals viz., species, age, sex and breed were noted.

Virus Preservation

About two grammes of epithelial tissues were collected from each infected animal and preserved in a glass vial containing about 30 ml of 50% phosphate buffered glycerine as preservative. Each sample was then stored in a refrigerator at $4^{\circ}C$.

50% Glycerine Phosphate Buffer

Solution - A

Disodium hydrogen phosphate (Na_2HPO_4)	...	21.390 gm
Distilled water	...	3000 ml

Solution - B

Dihydrogen potassium phosphate (KH_2PO_4)	...	2.725 gm
Distilled water	...	500 ml

pH - 7.4

Equal parts of phosphate buffer and analytical grade neutral glycerine were mixed and autoclaved at 15 lbs pressure for 30 minutes. The pH was checked and solution was distributed in small vials and stored at $4^{o}C$ till used.

Typing of Original Samples

Samples were typed by Micro Complement Fixation Test (MCFT). Each clinical sample was divided in two parts, the first part was subjected directly to typing by MCFT and the second unmanipulated part was used for mouse passage.

First part of the clinical samples was subjected directly to MCFT following the technique described by Rai and Rao (1977) along with modifications of Rai (1980).

Preparation of Antigen: The tongue or feet epithelia collected from infected animals were used as the source of viral antigen. The viral suspension was prepared as per the method of Rai (1980) from these tissues . One gramme of the collected epithelial tissues was taken in a mortar and washed thrice with phosphate buffer saline (PBS), pH-7.2 in order to remove the preservative. The epithelial tissues were triturated along with sterile sand particles with pestle and mortar in PBS sufficient to prepare suspension was centrifuged at 3000 rpm for 30 minutes in a refrigerated centrifuge. The supernatant was taken out in another centrifuge tube with the help of a pipette. An equal volume of chloroform was added to it and the suspension was again centrifuged at 3000 rpm for 30 minutes. Three separate layers were visualized after centrifugation. The uppermost aqueous layer contained the virus,the middle slimy thick layer consisted of debris and lipid and the chloroform remained at the bottom. The viral suspension of the uppermost layer was taken out with the help of a pipette and used as antigen in the test proper.

Phosphate Buffer Saline (PBS)

Solution - A

Sodium Chloride ... 64 gm

Potassium Chloride	...	1.6 gm
Disodium hydrogen phosphate (Na_2HPO_4)	...	17.36 gm
Potassium dihydrogen phosphate (KH_2PO_4)	...	1.6 gm
Distilled water	...	6400 ml.

Solution - B

Calcium Chloride ($Cacl_2.2H_2O$)	...	1.06 gm
Distilled water	...	800 ml

Solution - C

Magnessium Chloride ($Mgcl_2.6H_2O$)	...	1.70 gm
Distilled water	...	800 ml.

All the three solutions were autoclaved separately at 15 lbs pressure for 30 minutes.

After cooling, solution B was added to A and then solution C was mixed with B A. 1.00,000 I.U. of crystalline penicillin (1,00,000 I.U.) and streptomycin (100 mg) per litre of PBS were added with sterile precautions. The solution was stored at $4^{o}C$ till used.

Guinea pig anti FMD virus hyperimmune serum

The guinea pig hyperimmune sera (FMD antisera) against virus types 'O', 'A', 'C', 'Asia-1' and sub-type 'A-22' were obtained from Central Laboratory of All India Coordinated Research Project for Epidemiological Studies on Foot and Mouth Disease, Mukteshwar.

The sera were diluted as per the direction of the supplier. The diluent used was veronal buffer (Brooksby,1952). The diluted sera were inactivated at 56°C in water bath for 30 minutes before use.

Veronal Buffer (Brooksby,1952)

The stock solution (5x) was prepared as follows:

Sodium chloride	...	85.00 gm
5,5 diethyl barbituric acid	...	5.75 gm
Sodium 5,5 diethyl barbiturate	...	3.75 gm
Magnesium chloride ($Mgcl_2.6H_2O$)	...	1.68 gm
Calcium chloride ($Cacl_2.2H_2O$)	...	0.28 gm

The stock was prepared by dissolving diethyl barbituric acid in 500 ml hot distilled water, sodium chloride was then added and volume was made upto 1400 ml. Sodium barbiturate was dissolved separately in 500 ml

distilled water and added to sodium chloride barbituric acid solution. The volume was finally made upto 2000 ml after addition of the remaining ingredients. It was distributed in 200 ml amounts and autoclaved at 15 lbs pressure for 20 minutes. After autoclaving, the solution was stored at 4°C. In order to make it working solution, it was diluted 1:5 in sterile distilled water.

Complement

The fresh guinea pig serum constituted the source of complement in complement fixation test. Great care was ex ercised in storing it always in refrigerator or keeping it over ice while working, considering its thermolabile property.

(i) Collection of Sera: Healthy guinea pigs of musculine sex, having body weight of about 600 to 700 grammes were allowed to fast overnight. Next morning, the guinea pigs were bled by cardiac puncture with the help of sterile 20 gauze needle and syringe. The blood was collected in clean sterile tubes and left at room temperature for an hour for clotting. The clot was broken and tubes were left at 4°C for several hours till maximum contraction took place to yield maximum amount of serum. The serum was finally separated by refrigerated centrifuge at 4°C and transferred in amber coloured glass vials.

The complement was preserved using Richardson's solution (Richardson,1941) as preservative.

Richardson's solution

Two stock solutions were prepared:

Solution - A

Boric acid (Na_3BO_3)	...	0.93 gm
Borax ($Na_2B_4H_7.10\ H_2O$)	...	2.29 gm
Sorbitol	...	11.74 gm
Saturated Nacl soln. upto	...	100.00 ml

The boric acid,borax and sorbitol were dissolved in saturated sodium chloride solution.

Solution - B

Borax	...	0.57 gm
Sodium Azide* (NaN_3)	...	0.81 gm
Saturated Nacl soln. upto	...	100. 00 ml.

The borax and sodium azide were dissolved in saturated sodium chloride solution and the volume was made upto 100 ml with saturated sodium chloride solution.

The serum (C^1) and Richardson's solution were mixed in the following amounts and order:

---

*:- Toxicity comparable to KCN.

Serum (C')	...	8 parts
Richardson's solution - B	...	1 part
Richardson's solution - A	...	1 part

(ii) Titration of complement: The complement was titrated, using the following steps.

One ml of Richardson's preserved complement was mixed with 7 ml of distilled water thus making the complement dilution to 1:10 . From 1:10 stock, further dilutions of complement to 1:20, 1:25, 1:30, 1:40, 1:45 and 1:60 were prepared using veronal buffer as diluent. Complement in a volume of 25 microlitres of each dilution was transferred in each well of microtitre plate using three wells for each dilution.

Veronal buffer in a 50 microlitre volume was added in each well containing complement dilutions.

In three separate wells of microtitre plates without complement, 75 microlitres of veronal buffer was added to serve as erythrocyte control.

The contents of the wells were sealed with the adhesive tape and incubated in a water bath at 37°C for 30 minutes.

After incubation, the plate was taken out and adhesive tape was removed.

Then 50 microlitre of sensitized haemolytic system was added in each of the wells.

Again, the contents of the wells were sealed with the adhesive tape and incubated in the water bath at 37°C for 30 minutes.

Special care was taken to prevent the entrance of water in the wells incubating the plate in water bath.

The plate was removed and kept at 4°C in refrigerator.

The final reading was taken after keeping the plate overnight in refrigerator, thus allowing the erythrocytes to settle completely.

Observation: The degree of haemolysis was ascertained by the degree of settling of unlysed erythrocytes in the bottom of the wells. The scoring was scaled from 0 to 4 as mentioned in Table-2.

The diameter of the button formed by settling of erythrocyte control (without complement) was given a score of 4. The diameter of the button formed in these wells by settling of erythrocytes provided a scale of comparison for other wells. The highest dilution of complement giving a score of 2 was considered to contain one haemolytic dose (1 HD_{50}) or one unit of complement.

Table-2: Showing titration of complement and its scoring

Percentage of erythrocytes settled	percentage of haemolysis	Score
Nil	100	0
25	75	1
50	50	2
75	25	3
100	Nil	4

In the test proper three units of complement were used.

Haemolytic System

Haemolytic system containing 4 MHD (minimal haemolytic dose) of haemolysin in each ml of 1% sheep erythrocyte suspnsion was prepared and stored at 4°C in a refrigerator. It was sensitized by incubating at 37°C for 30 minutes in water bath before use.

(i) Erythrocyte suspension: Blood was collected from jugular vein of a healthy sheep in equal volume of Alsever's solution. It was mixed by gentle thorough shaking and kept at 4°C in a refrigerator. Sheep RBC suspension was m-de as per the following steps.

The Alsever's preserved sheep blood was centrifuged at 1500 rpm for about 15 minutes.

The supernatant was removed, normal saline solutions (0.85%) was added to packed erythrocytes in sufficient amount and the contents were mixed gently by pipetting. Again, it was centrifuged at 1500 rpm for 15 minutes. The procedure of washing was repeated once more, then final centrifugation was done at 3000 rpm for 15 minutes in order to pack the erythrocytes at the bottom.

The sedimented erythrocytes were taken out

in desired volume to prepare 1% erythrocyte suspension in veronal buffer which was stored at 4°C in a refrigerator.

Alsever's solution (Newman,1967)

Dextrose	...	20.50 gm
Sodium citrate	...	8.00 gm
Sodium chloride	...	4.20 gm
Citric acid	...	0.55 gm
Distilled water	...	1000 ml

pH - 6.1

The solution was sterilized by autoclaving at 10 lbs pressure for 15 minutes.

(ii) Haemolysin: The procedure for preparation of haemolysin has been described by Kolmer et al. (1952) and Kabat and Mayer (1961). The method followed at the Central Laboratory of "All India Coordinated Research Project for Epidemiological Studies on Foot and Mouth Disease" Banglore was followed during the present study for preparation of haemolysin.

Freshly collected sheep RBC cells were washed in normal saline solution and then diluted to make 20% suspension in normal saline. Four healthy albino rabbits of 600-800 gms weight were inoculated with 20% sheep erythrocyte suspension by intravenous

(I/V) route as per the schedule given in Table-3 for preparation of haemolysin.

On the ninth day of last inoculation i.e. 28th day of the first inoculation, last bleeding of individual rabbit was performed by cardiac puncture. The sera were separated and inactivated at 56°C for 30 minutes in a water bath. The sera were screened for the presence of haemolytic activity by complement fixation test adopting the technique of Brooksby (1952).

Preservation: If the titre was found to be satisfactory, all the rabbits were exanguinated. Their blood was collected and sera were separated.

The pooled haemolytic sera were inactivated at 56°C for 30 minutes in water bath in order to inactivate the complement present in the sera and then stored at 4°C in a refrigerator.

Titration: The haemolysin was titrated using 2 units of complement, using the following steps.

The glycerine preserved haemolysin was diluted to sub-dilutions of 1:1000, 1:2000, 1:3000, 1:4000, 1:5000, 1:6000, 1:7000 and 1:8000 using veronal buffer as diluent.

Table-3: Inoculation schedule of rabbits for the preparation of haemolysin.

Sl. No.	Days post primary inoculation	Size of the inoculum	Route	Remarks
1.	0	1.0 ml	I/V	20% of sheep erythrocyte.
2.	5	0.5 ml 1.5 ml	S/C I/V	After 15 minutes of subcutaneous inoculation.
3.	10	0.5 ml 2.0 ml	S/C	After 15 minutes of S/C inoculation
4.	15	0.5 ml 2.5 ml	S/C I/V	After 15 minutes of S/C inoculation.
5.	20	0.5 ml 3.0 ml	S/C I/V	After 15 minutes of S/C inoculation.
6.	28	-	-	Sera screened for haemolytic activity

A volume of 0.5 ml of each dilution of haemolysin was transferred into a clean wassermann tubes.

A volume of 0.5 ml of 2% sheep erythrocytes suspension was added to each tube.

For the purpose of erythrocyte control, a volume of 0.5 ml of erythrocyte suspension and 0.5 ml of veronal buffer w·s taken into one tube.

All tubes were incubated at 37°C for 30 minutes in a water bath.

After that 0.5 ml of appropriate complement dilution and 1.0 ml of veronal buffer was added to each tube.

All the tubes were incubated at 37°C for 45 minutes in a water bath again.

Thereafter percentage of haemolysis in each tube was recorded.

The highest dilution of haemolytic sera causing complete and sparkling haemolysis was considered to contain one unit or one MHD (minimal haemolytic dose) of haemolysin.

(iii) Haemolytic system preparation: The haemolytic system contained haemolysin and sheep erythrocyte in veronal buffer as follows:

Haemolysin	...	4 MHD/ml
Sheep erythrocytes	...	1%
Diluent	...	Veronal buffer

The haemolytic system thus prepared was incubated at 37°C for 30 minutes in a water bath for sensitization. The system was kept at 4°C for 5 days without further sensitization.

MCF Test Procedure

The MCFT was conducted as per the method of Rai (1980) using microlitre plates in the following stages.

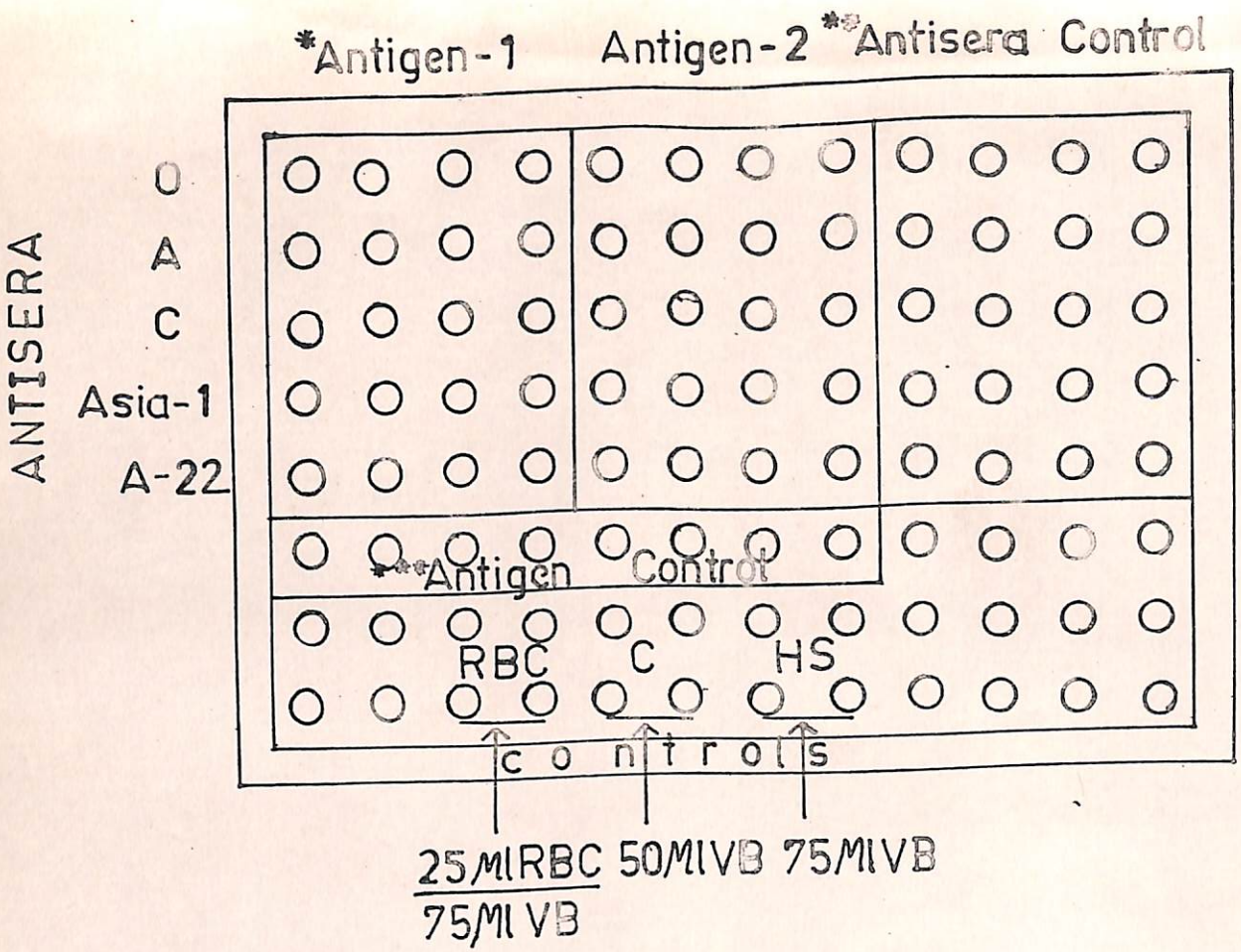
The antigen was added in 25 microlitre amount per well keeping four wells for each type of antisera (Figure-I).

The diluted and inactivated FMD antisera against virus types 'O','A', 'C' and sub-type 'A-22' were transferred in 25 microlitre amount per well of the microlitre plate keeping one row for each type of antisera.

The appropriate complement dilution containing 3 units was added to each well in 25 microlitre amount.

The amount of veronal buffer added to various controls was 25 microlitre for antisera and

Figure-I Schematic Representation Of Micro Complement Fixation Test



PHASE-1: Ag Ab C
25 Ml + 25 Ml + 25 Ml
Incubated at 37°C for 30 mts.

PHASE-2: HS (50Ml) Incubation at 37°C for 30 mts.

REFERENCES

Ag = Antigen

Ab = Antisera

C = Complement

HS = Haemolytic system

VB = Veronal buffer

*(a) Ag – 25Ml
Ab – 25Ml
C – 25Ml
(b) HS – 50Ml

**Antisera control
(a) Ab – 25Ml
VB – 25Ml
C – 25Ml
(b) HS – 50Ml

***Antigen control
(a) Ag – 25Ml
VB – 25Ml
C – 25Ml
(b) HS – 50Ml

antigen control, 50 microlitre for complement control, and 75 microlitre for erythrocyte control.

The plate was shaken gently and thoroughly to mix the reagents.

The plates were sealed by adhesive tapes and incubated at 37°C for 30 minutes in a water bath.

After incubation, adhesive tape was removed and 50 microlitres of freshly sensitized haemolytic system was added to each well, except one maintained as erythrocyte control in which 1% erythrocyte suspension was added in 50 microlitre amount.

Again, the plate was sealed after thoroughly mixing the contents and incubated at 37°C for 30 minutes in water bath.

After incubation, the plate was kept overnight at 4°C in refrigerator to allow settling of RBC and result was recorded in the following morning.

Interpretation of MCFT: In the RBC control wells the diameter of button of erythrocytes settled was scored as 4, that means 100% RBC settled and there is no haemolysis. The other wells were compared with the same RBC control. The row of wells which is giving settled RBC and no haemolysis indicated the presence of that type of virus against which the antisera was used

in that well. If the button of RBC was observed in the well of either antigen or antisera, that antigen or antisera was declared as anticomplementary and the test was repeated with best possible effort to eliminate the effect.

MCFT with mouse passaged samples

The samples which yielded FMD virus type 'O' by direct MCFT was passaged through the unweaned suckling mice for obtaining more potent of the FMD virus type 'O'. A total of 20 samples were positive for FMD virus type 'O'. All of which were passaged through the mice and then subjected to MCFT to ascertain the FMD virus types.

The virus inoculum for mouse passage was prepared as per the method mentioned above under preparation of antigen except that the chloroform was omitted. Streptomycin 1 /ug/ml, penicillin 1000 units/ml and mycostatin 250 units/ml were added to the antigen preparation and allowed to remain at room temperature for one hour. Four to seven day old unweaned suckling mice (Albino) were used as host for inoculation of suspension containing FMD virus type 'O'.

Inoculation: For virus passage one litter (3 to 5 mice) was used for each of the samples, keeping one mouse uninoculated as control. Tip of the tail of

control mouse was cut for immediate identification. The mice were inoculated with 0.1 ml of 10% of virus suspension intramuscularly (I/M) in thigh region. The mice were observed daily upto 7 days for the development of paralysis of hind quarters. Any death occuring within 24 hours post inoculation was taken as non specific. Usually by third day, mice died showing paralytic symptoms followed by death. The mice carcass was cleaned of the skin, appendages and viscera. Only skeletal muscle portion was collected and used as antigen after processing as per the method mentioned above under preparation of antigen. Materials were passaged further in mice, if needed.

MCFT: The mouse carcass antigen was then subjected to MCFT to ascertain the FMD virus type and also to ascertain the potency of the virus from the samples. The procedure of MCFT was the same as described above.

Preparation of T-cell deficient mouse model

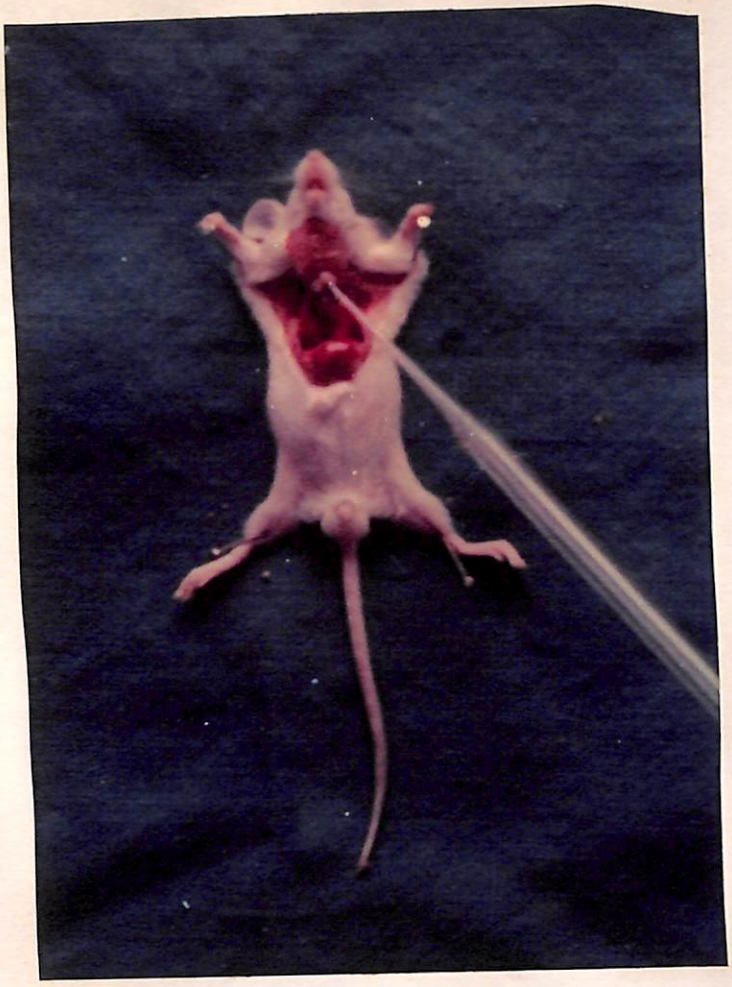
Preparation of antithymocyte serum (ATS)

Antithymocyte sera against mouse thymocyte was raised in rabbits by the method of Levey and Medawar (1966).

About four weeks old mice were taken, sacrified by stunning and breaking spinal cord of the

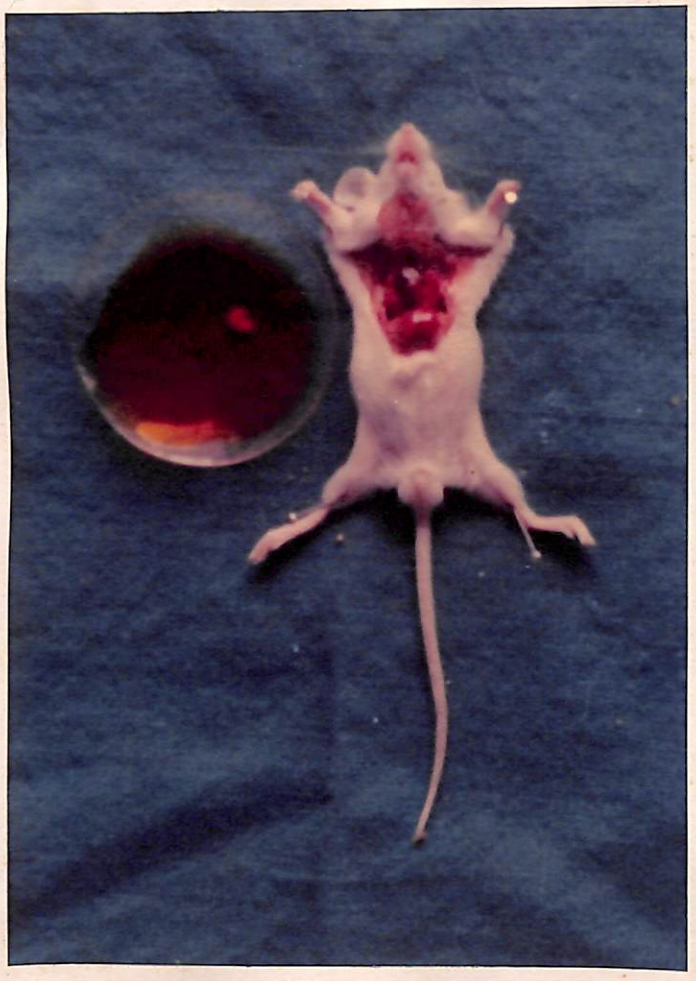
neck and then fixed on the board facing ventral surface upwards. Skin was removed along with fascia and pined on the board and then ribs of both sides were cut out. After cutting whitish bilobed thymus situated near the auricle of the heart was removed asceptically with the help of forceps. Due care was taken while taking out the thymus so that not even a trace of blood is attached to it. Thymuses were collected in cold Hank's solution and washed with Hank's balance salt solution (HBSS) atleast twice. The composition of HBSS has been depicted in Annexure-A.

Single cell thymocyte suspension was prepared by crushing thymus on stainless steel wire mesh with the help of a sterilized piston of glass syringe into a 50 ml glass beaker. The wire mesh was rinsed with cold HBSS atleast for two to three times. Large clumps of cells were broken out by pipetting the suspension several times. The thymocyte cells were centrifuged in a refrigerated centrifuge at $4^{o}C$ at 5000 rpm for 10 minutes and washed thrice with HBSS. The process was repeated three times. Last wash was done with normal saline solution. Thymocytes cells were suspended (1:10 dilution) in normal saline solution. The thymocytes were counted in haemocytometer as done in counting of WBC. Trypan blue dye exclusion test was applied for estimating thymocyte viability. For this purpose few drops of thymocyte



Photograph-1:

" Showing the position of thymus in mice "



Photograph-2:

" Showing removed thymus kept in watch glass containing Hank's solution "

suspension were taken in a test tube and was diluted by adding about 10 drops of normal saline to the suspension. Then one drop of 1% trypan blue solution was added. Thereafter one drop was placed on the glass slide and covered with cover slip and seen under compound microscope. The thymocytes which took trypan blue staining were considered as viable.

First injection of the thymocytes containing $2x10^8$ cells in 2 ml was given intravenously in each of 3 rabbits. Second injection was given after 15 days containing $2x10^9$ thymocyte cells in 2 ml amount in each rabbit intravenously. One week after the second injection, rabbits were bled by cutting ear vein and blood was collected in sterilized test tube adopting sterile precautions. The blood was kept at room temperature for about four to five hours. Sera were pooled out in sterilized vials. Antithymocyte sera was collected over a period of four weeks. Antithymocyte sera were decomplemented at 56^oC for 30 minutes and stored at -20^oC till used. The cytotoxic assay was used for assessing the activity of antithymocyte sera.

Cytotoxic Assay

(i) Thymocytes: For obtaining viable thymocytes five mice were taken and their thymuses were removed aseptically as described above, and collected in

Hank's solution. After crushing, pipetting, washing and centrifugation, thymocytes suspension was adjusted in cold Hank's solution to contain $1x10^7$ viable thymocytes per ml and kept in small test tubes.

(ii) Complement: Complement was collected from healthy guinea pigs as per description given earlier under MCFT.

The cytotoxic assay was done by the method of James et al. (1970). Cytotoxic assay is based on the principle of destruction of the thymocytes in the presence of complement by antithymocyte sera. For the test 0.1 ml of diluted antithymocyte serum, 0.1 ml of thymocyte suspension and 0.1 ml of guinea pig serum as source of complement were taken in a test tube.

The controls were:

(a) 0.1 ml antithymocyte sera plus thymocyte cells but no complement.

(b) 0.1 ml thymocyte cells plus 0.1 ml complement

(c) 0.1 ml thymocyte cell plus 0.1 ml cold Hank's solution.

All tubes including controls were mixed properly and incubated in a water bath for 90 minutes

at 37°C. After incubation 1.6 ml of diluted trypan blue solution (one volume of 1% trypan blue plus 14 volume of Hank's solution) was added in all tubes and left for 10 minutes. The counting of dead thymocyte cells were done with the help of haemocytometer. The dilution of antithymocyte serum causing fifty percent viability of thymocytes was considered as the end point.

Thymectomy of Mice

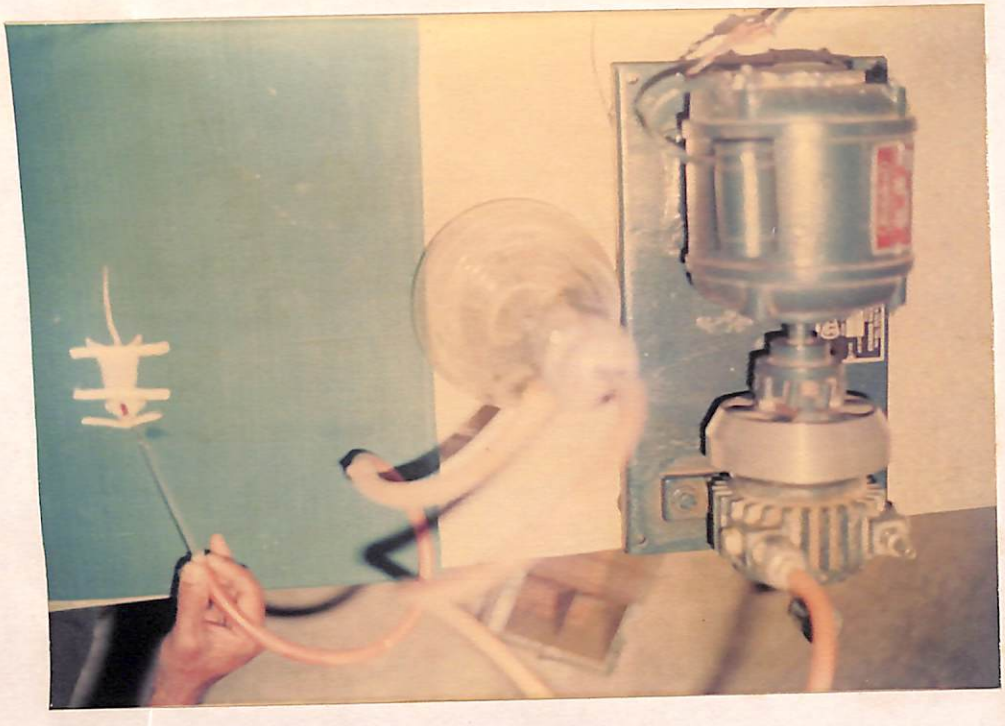
The experimental mice, about two weeks old were taken and their thymuses were removed by suction according to the technique of Miller (1961). The mice were anaesthetised with reconstituted "Avertin" at the rate of 0.02 ml/gm body weight administered intraperitoneally. Half ml of Avertin was mixed with 25 ml normal saline solution and 2 ml of methanol.

The anaesthesia was achieved within two minutes and lasted for about 60 to 90 minutes.

The anaesthetised mice were taken on a dissection board, ventral surface facing upwards. The mice were fixed with strips of adhesive tapes at three positions i.e. at the hind leg, in the middle and through the mouth. The skins of the mice at neck region and chest were sterilised with a few drops of 70% alcohol. Thereafter, with the help of a sharp, sterilised scissors and

sterilised forceps a median incision was made extending from the chin to the level of second rib, skin was lifted out with the help of pointed forcep and the bilobed thyroid gland was separated gently. The peritracheal fascia and the upper part of the sternum was incised gently upto the level of second rib. The incision was made cautiously in mid line taking care that not a single major blood vessel was cut or damaged and special precaution was taken for heart. After cutting of sternum, the upper end of the sternum was lifted out with the help of sterilised pointed forcep. The thymus lobes were gently sucked into a pasteur pipette which was connected to a suction pump through a reservoir. After removing the thymus lobes, the fascia, thyroid gland and other tissues were put back into its original position and four to five interrupted sutures were given to close the skin perfectly. For making interrupted suture, the black braided silk thread no. 3-0 was used (Johnson & Johnson), Neosporin powder was sprinkled over the suture uniformly to avoid infection and early healing. All experimental mice were subjected to identical surgery.

Thymectomised mice were given tetracycline at the rate of 250 mg of tetracycline per litre of drinking water to prevent recurrent infection. Administration of antibiotics was stopped a week before infecting mice with foot and mouth disease virus



Photograph-3: Thymectomy done by suction pump

serotype 'O'. The control group of mice were subjected to identical surgery but their thymuses were not removed.

Administration of ATS to thymectomised mice: Nine days after thymectomy, each mouse was given 0.25 ml of ATS by intraperitoneal inoculation. The dose of ATS was repeated in each mouse daily for 10 days.

Control mice received normal rabbit serum 0.25 ml per day for 10 days.

Standardization of models

T-cell deficient mice were standardized by using the following methods:

(a) detection of antibody against sheep RBC,
(b) delayed type hypersensitivity (DTH) to sheep RBC,
(c) differential leucocyte count.

(a) Detection of antibody against sheep RBC: T-cell deficient animals do not synthesize antibody against T-dependent antigen like sheep RBC. In this experiment T-cell deficient mice along with control were inoculated intraperitoneally with 0.5 ml of 5% sheep RBC. The mice were bled on fifth day post inoculation. The sera were tested for the presence of antibodies to sheep RBC by direct haemagglutination test.

(b) Delayed type hypersensitivity to Sheep RBC:

DTH was determined in sheep RBC sensitized mice by inoculating sheep RBC (0.03 ml, 20%) intradermally in the left hind foot pad while the right foot pad was kept as control (0.03 ml of NSS I/d). The thickness of foot pad was measured with the help of calipers before and 24 hours after inoculation. The degree of DTH reaction was expressed as the difference in foot pad thickness (mm) of left hind foot pad over the control right hind foot pad.

(c) Differential leucocyte count: The blood smear of T-cell decicient and control mice were prepared and stained with Leishman stain. Differential leucocyte count was done after counting hundred leucocytes and then percentage of different leucocytes were determined.

Preparation of macrophage stimulated model

Treatment of mice with Bacillus calmette Guerine (BCG)

BCG is a knownmacrophage stimulator and hence it was used to stimulate the macrophages population of the mice. BCG was obtained from T.B.Centre, Bankipore,Patna-4.

The mice were placed in the V-box, holding

its tail through the bottom of the slot. A pad of cotton wool soaked in hot water was wrapped around the base of the tail and left for one to two minutes. The cotton wool was removed and the prominent lateral coccygeal vein was selected for injecting 10^5 viable BCG with the help of tuberculin syringe using needle no. 30. The tail was held with the left hand, droping it over the forefinger and securing it with thumb. The syringe with needle level uppermost was brought upto the tail in line with the part of the tail lying over the forefinger but slightly below its surface. The needle was inserted along the line of the vein and within its lumen. The plunger of the syringe was gently pressed and 0.25 ml of the saline containing 10^5 viable BCG was inoculated. Mice were left for two weeks and then a second dose of 10^5 viable BCG organisms was administered.

Standardization of macrophage stimulated mice model: The level of macrophage stimulation was ascertained by carbon clearance test as compared to that of control.

Carbon clearance test: Carbon clearance test was performed to ascertain the functioning of macrophages as per the method of Levy and Wheelock (1975). For this 0.1 ml of colloidal carbon was injected with tuberculin syringe into tail vein of each mouse. At 3 and 15 minutes

intervals 20 /ul of blood samples were taken from inner canthus of eye by rupturing the retro orbital plexuses of each mouse and haemolysed in 4 ml of 1% (vol/vol) acetic acid. The optical density of the blood was determined with the help of spectronic-20 at 650 mm using red filter and amount of carbon was ascertained by the phagocytic index K, by using the following formula:

$$K = \frac{\text{Log}_{10}\ \text{O.D. 3 min.} - \text{Log}_{10}\ \text{O.D. 15 min.}}{12\ \text{min.}}$$

Preparation of macrophage deficient model

Preparation of Silica: Silica particles (1-5 /u) (Sigma Chemicals, St. Louis, Missouri,U.S.A.) were purified as described by Allison (1976).

About 400 mg of silica particles taken in a large screw capped vial were boiled in 50 ml IN-HCl to remove contaminating ferric chloride ($FeCl_3$). The boiling followed by cooling was repeated till the disappearance of green colour. The green colour indicated presence of ferric chloride. After boiling, the silica was washed ten times with double glass distilled water to remove excess of HCl. Thereafter, the treated silica particles were dried in hot air oven. The dried silica particles was kept separately in 60 mg lot in separate

small screw capped vials and autoclaved at 15 lbs pressure for 20 minutes. The silica particles were suspended in HBSS a day prior to use.

Treatment of mice with silica: Mice were inoculated intravenously with 0.5 ml of 3 mg of silica particles suspension in HBSS. The control mice were similarly injected with 0.5 ml of HBSS only.

The state of macrophage deficiency was measured by carbon clearance test (Levy & Wheelock,1975) as described under macrophage stimulation test.

FMD Virus type 'O' infection in T-cell deficient, macrophage stimulated and macrophage depleted mouse models

Groups of T-cell deficient,macrophage stimulated and macrophage depleted models of mice along with their controls were inoculated with 0.1 ml of 10% suspension of type 'O' FMD virus intramuscularly. After infection, the experimental mice were observed for a week for ascertaining different parameters of susceptibility of these animals to the virus.

Various parameters used for detecting the susceptibility of mice to FMD virus infection were:

(a) Appearance of clinical symptoms,its onset and duration,

(b) Mean survival time (MST) of dying mice &

(c) Virus content of dead or sacrified mice.

(a) Appearance of clinical symptoms, its onset and duration: After virus challenge, the experimental mice were observed for the onset of clinical symptoms namely, appearance of illness, paresis, staggering gait and paralysis of hind quarters, coma and death, if any. Death within 24 hours after challenge was treated as non specific.

(b) Mean survival time: Mean survival time (MST) of mice dying of infection was calculated. For calculation of MST, days taken for each mouse to die were added and then the total was divided by the number of animals dead. Recovery if any, from infection was also recorded.

(c) Virus content of dead or sacrified mice: The virus content of dead or sacrificed mice belonging to groups of T-cell deficient, macrophage stimulated and macrophage depleted models as compared to their controls (normal infected mice) was considered as an important critarion for susceptibility, specially in case of mice which recovered after infection. In case the experimental mice died, its carcass was triturated and a 10% of stock suspension was made in phosphate buffer saline

and then processed for MCFT for assay of its virus content, using different dilutions of stock virus suspension, the highest virus dilution giving positive MCFT was determined. The mice which survived, were sacrified on the same days and processed for assaying its virus content as above.

RESULTS

Serotyping of FMD virus by MCFT from original materials

Out of 77 samples collected from FMD outbreaks, 34(45.45%) could be typed and 43 (54.55%) remained untyped. The untyped samples included 21 (48.84%) negative ones and 22 (51.16%) showing anticomplementary activity. FMD virus serotypes recovered from different epithelial lesions were 21 (52.5%) from tongue, 9(30%) from feet, 3(60%) from gum and 1(50%) from palate. Among 34 samples found positive by MCFT, the maximum of 20 (25.99%) were type 'O' 7 (9.09%) 'Asia-1', 5(6.49%) sub-type 'A-22' and 2(2.59%) type 'C' (Table-4).

Preparation of T-cell deficient mouse models

Antithymocyte serum (ATS): ATS was prepared in rabbits by I/V inoculation of thymocytic cells ($2x10^8$ cells). The titre of ATS was determined and was found to give cytotoxicity titre of 1:32.

Standardization of T-cell deficient mouse models

Detection of antibody against sheep RBC: The haemagglutinating antibody response in T-cell deficient mice to sheep RBC was assayed. The results of haemagglutinating antibody response have been shown in Table-5. The haemagglutinating titre in T-cell deficient mice varied from 0 to 1:16. The titre in case of control

Table-4: Showing the details of result of serotyping of FMD virus by MCFT from original samples

Sl. No.	Nature of samples	Total number of samples tested	Number of +ve samples yielding serotypes							No. of untyped samples		
			'O'	'A'	'C'	'Asia-1'	'A-22'	Cross reacting	Total	Anti-complementary	-ve typing	Total
1.	Affected tongue epithelium	40	11	-	2	5	3	-	21 (52.5)	9 (47.36)	10 (52.64)	19 (47.5)
2.	Affected feet epithelium	30	6	-	-	1	2	-	9 (30)	11 (52.38)	10 (47.62)	21 (70)
3.	Affected gum lesions	5	2	-	-	1	-	-	3 (60)	1 (50)	1 (50)	2 (40)
4.	Affected palate lesions	2	1	-	-	-	-	-	1 (50)	1 (100)	-	1 (50)
	Total	77	20	-	2	7	5	-	34 (45.45)	22 (51.16)	21 48.84)	43 (54.55)

Figures in parenthesis represents the percentage of data.

Table-5: Haemagglutinating antibody response of T-cell deficient and control mice following immunization with sheep RBC.

Tests	No. of animals	Dilution of antisera giving +ve Haemagglutin. 1:0	1:2	1:4	1:8	1:16	1:32	1:64	1:128	Mean Haemagglutinating titre
T-cell deficient mice	1	+	+	-	-	-	-	-	-	0 to 1:16
	2	+	+	+	+	+	-	-	-	
	3	+	+	+	+	-	-	-	-	
	4	+	-	-	-	-	-	-	-	
	5	+	+	+	+	+	-	-	-	
Control mice	1	+	+	+	+	+	+	+	+	1:64 to 1:128
	2	+	+	+	+	+	+	+	+	
	3	+	+	+	+	+	+	+	-	
	4	+	+	+	+	+	+	+	+	
	5	+	+	+	+	+	+	+	-	

mice were found to vary from 1:64 to 1:128.

Delayed type hypersensitivity to sheep RBC: The T-cell deficient and control mice were immunized with sheep RBC and the increase in the thickness of foot pad after 5 days of inoculation was measured. The data of the experiment have been presented in Table-6. The mean foot pad thickness in T-cell deficient mice was calculated to be 0.006mm± 0.0003162 while in control mice, the thickness was 0.326mm± 0.0116619.

Differential leucocyte count: Differential count of leucocytes of T-cell deficient and control mice were carried out. The mean lymphocytes and neutrophils count in T-cell deficient mice was found to be 16.4% and 79.9% respectively. In control mice the mean counts of lymphocyte and neutrophils was 81.9% and 12.2% respectively, (Table-7).

FMD Virus infection in T-cell deficient and control mice

Symptoms of infection and mean survival time: The clinical symptoms which included staggering gait and paralysis of hind quarters appeared on the third day of infection in T-cell deficient mice. Only two of the five T-cell deficient mice died on fourth day post infection and the rest survived. The mean survival time was found to be 4.0 days in T-cell deficient mice. The

Table-6: The foot pad thickness of T-cell deficient and control mice after inoculation with sheep RBC showing DTH.

Tests	No. of animals	Foot pad thickness in mm	Mean thickness in mm
T-cell deficient mice	1	0.006	0.006 ± 0.0003162
	2	0.005	
	3	0.006	
	4	0.007	
	5	0.007	
Control mice	1	0.330	0.326 ± 0.0116619
	2	0.290	
	3	0.310	
	4	0.350	
	5	0.350	

Table-7: Differential leucocyte counts of T-cell deficient and control mice.

Tests	No. of animals	Lympho-cytes %	Mean lympho cytes %	Neutro-phils %	Mean neutro-phils %
T-cell deficient mice	1	15	16.4	81	79.9
	2	17		80	
	3	17		79	
	4	16		80	
	5	17		79	
Control mice	1	79	81.9	12	12.2
	2	80		13	
	3	80		12	
	4	82		12	
	5	82		13	

symptoms of FMD infection in control were noticed on the fourth and the fifth days. The severity of symptoms was seen to be more in T-cell deficient mice as compared to control group. None of the control mice died. They recovered later on (Table-8).

Virus content in T-cell deficient and control mice:

The virus content of both T-cell deficient and control mice was determined by MCFT using standard FMD antisera. The highest dilution of the virus suspension made from both the groups of T-cell deficient and control mice carcass giving positive MCFT was determined and the data are presented in Table-9.

In case of T-cell deficient mice, the highest virus content of the carcass dying of FMD infection gave positive MCFT in 1 in 80 dilution of the virus. The virus content of the control groups of mice on sacrifice gave positive MCFT in 1 in 20 dilution of the virus.

Macrophage Stimulated Mice

The macrophages of mice were stimulated by inoculation of interavenous dose of 0.25 ml of the saline containing 10^5 viable BCG organisms. Thereafter carbon clearance test was done to ascertain the extent of stimulation of macrophage. In the macrophage stimulated mice the phagocytic index (K) was 0.47 while

Table-8: Appearance of symptoms and mean survival time of T-cell deficient and control mice dying of FMD virus infection.

Tests	No. of animals	Days of appearance of symptoms						Days on which mice died						Mean survival time
		1st	2nd	3rd	4th	5th	6th	1st	2nd	3rd	4th	5th	6th	
T-cell deficient mice	1	-	-	+	+			-	-	-	+			4.00 days
	2	-	-	-	+	+	+	-	-	-	-	-	-	
	3	-	-	+	+			-	-	-	+			
	4	-	-	-	-	+	+	-	-	-	-	-	-	
	5	-	-	+	+	+	+	-	-	-	-	-	-	
Control mice	1	-	-	-	+	+	+	-	-	-	-	-	-	-
	2	-	-	-	-	+	+	-	-	-	-	-	-	
	3	-	-	-	+	+	+	-	-	-	-	-	-	
	4	-	-	-	+	+	+	-	-	-	-	-	-	
	5	-	-	-	-	+	+	-	-	-	-	-	-	

Table-9: Highest dilution of virus suspension of T-cell deficient and control mice carcass giving positive MCFT.

Tests	No. of carcasses	+ve MCFT in highest virus dilution				
		1:10	1:20	1:40	1:80	1:160
T-cell deficient mice	2 (pooled)	+	+	+	+	-
Control mice	2 (Pooled)	+	+	-	-	-

in control mice it was 0.058 i.e. macrophage stimulated mice cleared coloidal carbon nine times more as compared to control.

FMD virus infection in macrophage stimulated and control mice

Mean Survival Time: The macrophage stimulated mice along with controls were infected with 0.1 ml of 10% suspension of type 'O' FMD virus. The mean survival time of both the groups of mice was observed and the virus content of the mice dying of infection was assayed. However, in this experiment some descripency was observed. Three of the five BCG stimulated mice after FMD infection died (mean survival time 5.3 days) and the rest of the BCG stimulated mice showed resistance and then recovered. In case of control mice (not stimulated with BCG but infected with FMD) showed slight symptoms but they all recovered. The experiment could not be repeated due to lack of mice and limitation of time. Data of the experiment have been presented in Table-10.

Virus content: Virus content of both the macrophage stimulated and control mice after FMD infection was ascertained. BCG group showed positive MCFT in 1:80 dilution of the virus while controls gave positive MCFT in 1:20 dilution of the virus (Table-11).

Table-10: Appearance of symptoms and mean survival time of macrophage stimulated (with BCG) and control mice infected with FMD virus.

Test	No. of animals	Days of appearance of symptom						Days on which the mice died						Mean survival time
		1st	2nd	3rd	4th	5th	6th	1st	2nd	3rd	4th	5th	6th	
Macrophage stimulated mice	1	-	-	-	+	+	+	-	-	-	-	-	-	5.3 days
	2	-	-	+	+	+		-	-	-	-	+		
	3	-	-	-	+	+	+	-	-	-	-	-	-	
	4	-	-	-	+	+		-	-	-	-	+		
	5	-	-	-	+	+	+	-	-	-	-	-	+	
Control mice	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	+	+	-	-	-	-	-	-	-	
	3	-	-	-	+	+	-	-	-	-	-	-	-	
	4	-	-	-	-	-	-	-	-	-	-	-	-	
	5	-	-	-	+	+	+	-	-	-	-	-	-	

Table-11: Highest dilution of virus suspension of macrophage stimulated and control mice carcass giving positive MCFT.

Test	No. of carcasses	+ve MCFT in highest virus dilution				
		1:10	1:20	1:40	1:80	1:160
Macrophage stimulated mice	2	+	+	+	+	-
Control mice	2	+	+	-	-	-

Macrophage depleted mice

For depletion of macrophages in mice, silica was administered by intravenous route. The extent of depletion of macrophages was ascertained by carbon clearance test on days 1,7 and 21 following silica treatment. The carbon clearance was found to be higher in controls as compared to the macrophage depleted mice . The datas of the tests have been shown in Table-12. In the macrophage depleted mice the phagocytic index (K) was 0.011 ± 0.001 while in the control mice it was 0.055 ± 0.022.

FMD virus infection in macrophage depleted mice

Mean Survival Time: Both the silica treated and control mice were infected with 0.1 ml of 10% FMD virus suspension. The appearance of symptoms of infection and mean survival time of mice dying of infection was ascertained. In both the groups only fewer mice showed symptoms of virus infection and died of infection while majority of mice survived. Only one of the macrophage depleted infected mouse died while all the remaining control infected mice survived. In macrophage depleted mice the mean survival time was 5.0 days (Table-13).

Virus content: The virus content of macrophage depleted and control mice dying or showing symptoms of FMD infection was ascertained and datas shown in Table-14.

Table-12: Effect of silica on the phagocytic capacity of the reticuloendothelial system.

Days post silica treatment	Silica treated Mice	Control Mice
	Phagocytic index (K)	Phagocytic index (K)
1	0.025 ± 0.013 (2)	0.055 ± 0.022 (2)
7	0.011 ± 0.001 (2)	0.065 ± 0.002 (2)
21	0.055 ± 0.022 (2)	0.069 ± 0.023 (2)

Table-13: Appearance of symptoms and mean survival time of macrophage depleted and control mice inoculated with FMD virus infection.

Tests	No. of animals	Day of appearance of symptoms						Days on which the mice died						Mean survival time
		1st	2nd	3rd	4th	5th	6th	1st	2nd	3rd	4th	5th	6th	
Macrophage depleted mice	1	-	-	-	-	-	-	-	-	-	-	-	-	5.0 days
	2	-	-	-	+	+		-	-	-	-	+		
	3	-	-	-	-	+	+	-	-	-	-	-	-	
	4	-	-	-	-	-	-	-	-	-	-	-	-	
	5	-	-	-	+	+	-	-	-	-	-	-	-	
Control mice	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	+	+	+	-	-	-	-	-	-	
	3	-	-	-	-	+	+	-	-	-	-	-	-	
	4	-	-	-	+	+		-	-	-	-	-	-	
	5	-	-	-	-	+	-	-	-	-	-	-	-	

Table-14: Highest dilution of virus suspension of macrophage depleted and control mice carcass giving positive MCFT

Test	No. of carcasses	+ve MCFT in highest virus dilution				
		1:10	1:20	1:40	1:80	1:160
Macrophage depleted mice	2 (pooled)	+	+	+	-	-
Control mice	2 (pooled)	+	+	+	-	-

In both the macrophage depleted and control mice, the virus dilution of 1:40 gave positive MCFT.

DISCUSSION

The recovery of animals from viral infection depends on various factors acting in concert or independently which include induction of humoral immunity, cellular immunity and various non specific host resistance factors like macrophages, ambient temperature etc. As regards recovery of animals from FMD infection, several workers have recognised the role of humoral immunity in protection of the host (Radisich et al., 1976; Van Bekkum et al., 1969), some workers have thrown light on the role of cellular immunity (Sharma et al., 1986), while a few reports are also available on the role of non specific factors (Mallick et al., 1985). However, there seems to be several gaps in our knowledge on the relative role of each of these host factors in protection against FMD infection. All such responses in an intact host usually act in concert and hence the role of each of them is identified by suppressing or eliminating one factor while keeping the other aspect intact and vice versa. The traditional techniques used for immunomodulations include whole body x-radiation (North, 1971), various immuno suppressive drugs, administration of antilymphocytic serum (Lance et al., 1973), thymectomy and bursectomy (Rogers and Balish, 1980). In the present study an attempt has been made to assess the role of cellular immune response and the role of macrophages in recovery of mice from FMD infection in T-cell deficient, macrophage

stimulated, macrophage depleted and normal mice models which may help in understanding the mechanisms operating during recovery of animals from FMDV infection and may lead to evolve a suitable preventive measure against the same.

Role of Cell Mediated Immune Response

T-cells deficient mouse models

It is well established fact that T-cells subserve the function of (i) protection against intracellular infections, namely viruses, fungi and intracellular bacteria, (ii) induction of DTH, (iii) transplantation reaction, (iv) tumour immunity and (v) host verses graft reaction (Davis & Blood, 1970). Therefore, it was pertinent to produce T-cells deficient model amongst laboratory animals susceptible to FMD virus, so that the role of cellular immunity could be assessed. The current methods which are in vogue nowadays for making T-cells deficient model include whole body x-radiation, use of immuno suppressive drugs, use of antithymocyte serum (North, 1971), neonatal thymectomy, and adult thymectomy followed by antilymphocytic serum. In the present study thymectomy was adopted soon after birth of mice and followed by administration of ATS as advocated by Lance *et al*. (1973). It was presumed that T-cells

deficient mouse would not respond to T-dependent antigens, would not produce DTH, and would show increased susceptibility to FMD virus infection. Such efforts are in conformity with the works already done by the above noted workers to assess the role of cellular immune responses to various infections in animals.

Thymectomy, administration of ATS and standardization of models: Before conducting experiments on the T-cells deficient mice, it was confirmed whether these animals have really been made T-cells deficient. Three important parameters of testing T-cells deficient animal models selected for the present study were (i) haemagglutinating antibody response to sheep RBC, (ii) induction of delay hypersensitivity and (iii) differential leucocyte counts. Therefore, various experiments were carried out to ascertain whether mechanisms for these parameters were operating or not.

Haemagglutinating antibody response: It is expected that T-cells deficient mice will not provoke immune response to T-dependent antigens like sheep RBC. It is evident from the result (Table-5) that T-cells deficient mice induced very meagre response to sheep RBC by producing haemagglutinating titre of only 0 to 1:16. In control normal mice the mean haemagglutinating titre was higher varying from 1:64 to 1:128. Thus the

present work collaborates the findings of Waldman et al. (1979) and Sinha et al. (1986) and showed that mice have been made T-cells deficient and suitable for further study.

Delayed type hypersensitivity: As stated earlier, T-cells,provoke development of DTH which is measured by increase in the thickness of skin at the site of antigen inoculation in sensitized mice. Thickening of skin is produced by infilteration of sensitized T-lymphocytes at the site of antigen inoculation (Reuben et al., 1979).In T-cells deficient models, there will not be any infilteration of T-lymphocytes and hence no increase in thickness will be observed. Similar results have been obtained during the present study when skin thickness in T-cell deficient mice was found to be only 0.006 ± 0.0003162, while in control mice skin thickness was 0.326 ± 0.0116619 or more (Table-6). This findings is in agreement with Reuben et al. (1979).

Differential leucocyte count: Leucocyte counts of the T-cells deficient mice showed only 16.4% lymphocytes, whereas in the control mice it was 81.9%. The neutrophil counts in T-cells deficient and control mice were 79.9% and 12.2% respectively. These observations are in total agreement with the works already done by Arnason et al. (1964) and Salvin et al. (1965) who

showed that administration of ATS to thymectomised mice selectively depleted circulating lymphocytes of the animals leaving other cells intact.

FMD virus type 'O' infection in T-cell deficient and control mice

After ascertaining that thymectomised mice have actually been made deficient in T-cells, FMD virus infection was given to T-cells deficient mice as well as normal mice (control). It was noted that signs of infection appeared earlier in T-cells deficient mice with staggering gait and paralysis of hind quarters. In contrast, the symptoms appeared two days later in control mice. The T-cells deficient mice also died of FMD infection earlier with mean survival time of 4.0 days. On the contrary, in control mice clinical symptoms were less severe. It was further noted that in T-cells deficient mice the virus multiplied to a greater extent giving positive MCFT in 1:80 dilution of the virus (Table-9). In control mice virus multiplied slowly and hence the virus concentration was lesser to give positive MCFT in 1:20 dilution.

However, there were certain genuine difficulties encountered during the present investigation which is worth mentioning here. It is well known fact that FMD

infection (Fernandez et al.,1986). After repeated passage of FMD virus in mice,the animals took infection upto 3 weeks of age but older ones still showed age resistance. It may be mentioned here that during the process of preparation of T-cells deficient mouse model, i.e. thymectomy and administration of ATS, mice attained the age of 4 weeks and more. Therefore, FMD infection had to be given at this stage where mouse developed age resistance to the virus to a large extent. This is evident by the fact that non of the control mice died of FMD infection. They showed some symptoms of infection and then recovered and hence the experimental and control mice had to be sacrificed to study the relative susceptibility and the virus content of experimental and control mice after giving FMD virus infection. Due to limitation of time alloted to Post graduate students and non avaibility of required number of mice,it was not possible to get these experiments repeated. Notwithstanding the above limitations,the present study has shown that T-cells deficient mice models have been produced which fulfilled all the parameters of T-cells deficiency and that these animals showed slightly increased susceptibility to FMD virus under limited circumstances.

Macrophage stimulated and depleted mice models

After assessing the role of T-cells,it was thought proper to ascertain the role of macrophages in protection against FM^D infection in mice.

In recent years the prominent role of macrophages in resistance to various types of infection has been well established (Dubuy,1975; Sher et al.,1975; Clark et al., 1976 and Wozencraft et al.,1984). Macrophages are the most important auxillary cells which act both non specifically in removing the foreign substances and infectious agents as well as remain involved in various phases of immune responses. But its role as secondary cells in cell mediated immunity is most important. After sensitization by T-cells,macrophages become the 'angry cells' when its killing power is increased several folds by increased phagocytosis (Benacerraf et al.,1959).

To observe the independent role of macrophages, it was essential to produce mouse models with its macrophages kept in the condition of stimulation, depletion and in normal states. BCG was used to activate macrophage (Mackaness,1968). It has been reported that activated macrophages,besides killing the specific organisms,also possess increased killing power against several unrelated microorganisms like bacteria,fungi,

protozoa, viruses and tumour (Dubuy,1975; Sher et al., 1975; Clark et al.,1976 and O' Brien et al.,1979).

It is evident from the result presented in Table-10 that the macrophages after treatment with BCG actually got stimulated and showed enhanced carbon clearance. However, some discrepency was observed during these experiments because three of the BCG stimulated and FMD challenged mice died while the control mice survived (Table-10). The presumption, whether this has been caused by the adverse effect of BCG or some other infection associated with BCG has to be ascertained before making any generalization on FMD infection to assess the role of macrophages. Moreover,for macrophage stimulation two I/V injections of BCG ,one given at an interval of 7 days each which would not be given in mice younger than 10 days. It means that FMD challenge infection would have to be given at least after 4 weeks of age and by this time mice developed age resistance to FMD virus to a great extent.

Lastly, an endeavour was made to see whether depletion of macrophages by silica treatment will affect ability of mice to FMD virus infection. Silica is said to be toxic and acts probably by causing lysis of its membrance (Allison et al.,1966) due to which macrophages are depleted from lymphoid population.

Efficacy of silica treatment has been established against Salmonella typhimurium (O'Brien et al.,1979) and tumours. In the present study silica particles were used to deplete the macrophages and depletion was ascertained by reduced carbon clearance test after silica treatment. These mice alongwith the controls were infected with FMD virus. The results (Table-13) indicated slightly enhanced mortality with only one out of 5 mice dying with mean survival time 5 in silica treated group than in untreated control mice.

SUMMARY

1. The present study was undertaken to ascertain the role of CMIR and the role of macrophages in recovery of mice following infection with FMD virus type 'O' using T-cells deficient, macrophage stimulated and macrophage depleted mice models alongwith their controls.

2. FMD virus type 'O' strains were procured by serotyping of the clinical samples obtained from field outbreaks of FMD. A total of 77 clinical samples were collected and were subjected to direct MCFT of which 34 (45.45%) proved viable and 43 (54.55%) proved non-viable. The maximum number of FMD outbreaks was caused by serotype 'O' numbering 20 (25.99%) followed by 'Asia-1' 7(9.09%), 'A-22' 5(6.49%) and 'C' 2 (2.59%).

3. T-cells deficient mouse models were prepared by thymectomy of two-fold mice followed by intraperitoneal inoculation of 0.25 ml of ATS daily for one week. ATS was prepared in rabbits by I/V administration of mouse thymocytes ($2x10^8$ cells in 2 ml NSS) twice at an interval of 15 days.

4. T-cells decicient mice models were standardized by using three parameters, namely (i) the haemagglutinating antibody response in T-cells deficient mice to sheep RBC, (ii) delayed type hypersensitivity to sheep RBC and (iii) differential leucocyte count.

5. The haemagglutinating antibody titre in T-cells deficient mice varied from 0 to 1:16, whereas in control mice the titre varied from 1:64 to 1:128.

6. Delayed type hypersensitivity to sheep RBC in T-cells deficient mice was ascertained by the foot pad thickness which was found to be 0.006 ± 0.0003162 mm, while in control mice the thickness was 0.326 ± 0.0116619 mm.

7. Differential leucocyte counts in T-cells deficient mice revealed neutrophils and lymphocytes to be 79.9% and 16.4%, respectively, whereas in control mice these counts were 12.2% and 81.9%, respectively.

8. The macrophages of the mice were stimulated by administration of 10^5 viable BCG organisms in 0.25 ml saline intravenously twice at two weeks interval. The extent of macrophage stimulation was ascertained by carbon clearance test with the help of spectrometer which was found to be 0.47.

9. The mice were depleted of their macrophages by administration of 3 mg of silica particles contained in 0.5 ml saline intravenously.

10. T-cell deficient, macrophage stimulated and macrophage depleted mice were infected with FMD virus type 'O' by intramuscular inoculation of 0.1 ml

of virus suspension. Various parameters used to ascertain the susceptibility of mice to FMD virus were (i) appearance, onset and duration of clinical symptoms of infection, (ii) the mean survival time of mice dying of infection and (iii) the virus content in the dead or sacrified mice.

11. The clinical symptoms in T-cells deficient mice after FMD virus infection was evidenced earlier, while the symptoms were seen a bit later in control mice. Two out of five T-cells deficient mice died with mean survival time of 4 days while non of the control mice died of infection. The virus content was found to be higher in T-cells deficient mice as compared to the controls.

12. In macrophage stimulated mice infected with FMD, the clinical symptoms appeared earlier as compared to the controls. Three of the five macrophage stimulated mice died, while non of the control mice died. The virus content was found to be higher (1:80) in macrophage stimulated mice as compared to the controls (1:20).

13. In the macrophage depleted mice the symptoms of infection appeared a bit earlier as compared to control. The virus content was similar in both the macrophage depleted and the control mice.

14. During the process of preparation of T-cells deficient, macrophage stimulated and macrophage depleted mice models, the mice become older and attained the age of about four weeks during which time age resistance to FMD virus increased.

BIBLIOGRAPHY

Adlakha and Sharma (1982). Problems of FMD in India. VIIth Workshop of All India Coordinated Research Project on Foot and Mouth Disease.

Allison, A.C., Harington, J.S. and Birback, M. (1966). An examination of the cytotoxic effect of silica on macrophages. J. exp. med. 124: 141-144

Allison, A.C. (1976). Fluorescence microscopy of lymphocytes and mononuclear phagocytes and the use of silica to eliminate the latter. In in-vitro methods in cell mediated and tumour immunity, pp. 395-404, Bloom B and David J. (eds), Academic Press, Inc., New York.

Arnason, B.G., De Vaux St. Cyr, C. and Shaffner, J.B. (1964). A comparison of immunoglobulins in the normal and thymectomized mouse. J. Immunol, 93: 915-925.

Benacerraf, B., Kivy, R.E., Sebstyen, M.N. and Zweifach, B.W. (1959). The effect of high doses of x-irradiation of the phagocytic, proliferative and metabolic properties of the reticuloendothelial system. J. exp. med. 110: 49-64.

Bernath, S., Kucsera, G. and Soos, T. (1979). Evaluation of the virus neutralization test (S-index) in the efficacy control of foot and mouth disease vaccines. Magyar Allatorvosok Lapja 34(i): 29-32 (vide vet. bull. 49: 4406)

Borca, M.V., F.M. Fernandez, A.M. Sadir and A.A. Schudel (1984). Reconstitution of immunosuppressed mice with mononuclear cells from donors sensitized to foot and mouth disease virus. (recd., 1985) Vet. Microbiol. 10(i): 1-12.

Brooksby, J.B. (1952). The technique of complement fixation in foot and mouth disease research. Agric. Res. Council Report series no. 12.

Clark, I.A., Allison, A.C. and Cox, F.E. (1976). Protection of mice against Babesia and Plasmodium with BCG, Nature 259: 309-311.

Davis, B.D., Dulbecco, R., Eisen, H.N. and Ginsberg, H.S. (1980). Microbiology including immunology and molecular genetics. 3rd ed. J.B. Eippincott. Co., Philadelphia, U.S.A., pp. 291-293.

Dubuy,H. (1975). Effect of silica on virus infections in mice and mouse tissue culture. Infect. Immun. 11: 996-1002.

Ellis,P.R. and James,A.D. (1978). Foot and mouth disease: India. In " New techniques in veterinary epidemiology and economics ", edited by Ellis, shaw and Stephens. Reading UK; Deptt. of Agri. Univ.,p. 118-122.

Fernandez,F.M.,M.V.Borca,A.M. Sadir,N. Fondevila,J. Mayo and A.A.Schudel (1986). Foot and mouth disease virus experimental infection: Susceptibility and immune response of adult mice. Vet. microbiol 12(i): 15-24.

Fish,R.C.,Van Bekkum,J.G.,Lehmann,R.P. and Richardson, G.V. (1969). Immunogenic response in Dutch cattle vaccinated with FMD vaccines under field conditions:Neutralizing antibody responses to 'O','A' and 'C' types. Am. J. Vet.Res.30: 2115-2123.

Flachsel,P., Hubik,R.,Polak,L. (1982). A study of cattle immunization against FMD types 'O','A' & 'C'. Evaluation of mass immunoprophylaxis of cattle against FMD. In XVIthe conference of FMD commission,Paris,France,office International des Epizooties. 275-290.

Graves,J.H. (1969). Immune response of cattle to different serotype antigens in monovalent and trivalent FMD vaccines. J. Immun. 102: 58-62.

James,K.,Pullar,D.M.,James,V.S.,Wood,A.,Epps,H.B.C. and Raha,L. (1970). The development and distribution of antilymphocyte and other antibodies in horses immunized with human lymphoid antigens. Transplantation 10: 208-213.

Kabat,E.A. and Mayer,M.M. (1961). Experimental immunochemistry.Chales,C.,Thomas.Publrs,Springfield Ilinoss,U.S.A.

Kolmer,J.A.,Spoulding,E.H. and Robinson,H.W. (1952). Approved laboratory technique,H.K. Lewis & Co.,Ltd.,London.

Lal,S.M.,Vasantha,S.,Mathur,B.B.,Kathuria,B.K.,Negi,B.S. and Pandey,M.C.(1980). Immune and antibody response of cattle to foot and mouth disease type 'A' vaccine. Ind.Vet.J. 57: 714-718.

Lance, E.M., Medwar, P.B. and Taub, R.N. (1973). Antilymphocyte serum. Adv. Immunol. 17: 2-92.

Lebedev, A.I., Mutuzkin, L.I., Gorbatov, V.A., Gogolev, M.M., Koromyslov, G.F. (1972). Mechanisms of cellular immunity in foot and mouth disease. Veterinariya (Moscow) No. 5, 38-40 (Ru) Viev, Moscow Zh, 472.

Levey, R.H. and Medwar, P.B. (1966). Nature and mode of action of antilymphocytic antiserum. Proc. Nath. Acad. Sci. U.S.A., 56: 1130-1137.

Levy, M.H. and Wheelock, E.F. (1975). Effects of intravenous silica on immune and non immune functions of the murine host. J. Immunol. 115: 41-48.

Lucam, F., Fedida, M. and Dannacher, G. (1964). Bull. Acad. vet. 37: 175 (cited by Lal et al., 1980)

Mackaness, G.B. (1968). The immunology of antituberculous immunity. Amer. Rev. resp. Dis. 94: 337-344.

Mallick, B.B., Subodh Kishore, S.K. Das and Archana Garg, (1985). Non specific immunostimulation against viruses. Comp. Immunol. Microbiol. Infect. Dis. 8(i): 53-64.

Mathur, B.B.L., Butchiah, G., Vasantha, S., Lal, S.M., Joshi, S.V. and Narayanaswamy, M. (1981). Note on antibody response of cattle to FMD monovalent type 'O' vaccine (reliability of SN-index). Ind. J. of Anim. Sc. 51(1): 109-110.

Miller, J.F.A.E. (1961). Immunological function of thymus. Lancet, II: 748-749.

Negi, B.S. and Kumar, S. (1970a). Immunological studies on FMD type 'O' and 'C' vaccines with cell cultured virus adsorbed on aluminium hydroxide gel and inactivated by formalin. Ind. J. Anim. Sci. 40: 418-424.

Negi, B.S. and Kumar, S. (1970b). Immunological studies with cell cultured aluminium hydroxide gel adsorbed formalin inactivated FMD type 'Asia-1' monovalent vaccine. Ind. Vet. J. 47: 555-561.

North, R.J. (1971). The action of cortisone acetate on cell mediated immune response to infection. J. exp. med. 134: 1485-1500.

O'Brien, A.D., Scher, I. and Formal, S.B. (1979). Effect of silica on the innate resistance of inbred mice to Salmonella typhimurium infection. Infect. Immun. 25: 513-520.

Radisich, T., Degiorgi de Burghi, E., Durini, L.E., Fernandez de Albarracin, R.G., Frick, E.C. and Marenco, C. (1976). A study of foot and mouth disease immunity following vaccination of calves. Gac.Vet.(B-Aires) 38 (314): 367-376.

Rai, A. and Rao, B.U. (1977). Use of BHK cell culture and MCFT for the isolation and typing of FMD virus. Ind. Sci. 47: 794-798.

Rai, A. (1980a). Note on the micro complement fixation test for the typing of FMD virus. Ind. J. Anim. Sci. 50: 280-282.

Rai, A. (1980b). A comparative study of the MCFT and MNT in subtyping of FMD virus. Ind.J.Anim.Sci. 50: 961-965.

Reuben, C., Sundaram, K. and Phondke, G.P. (1979). Suppression of secondary immune response by antilymphocyte serum: Time relationship between immunization and administration of antilymphocyte serum. Immunology 36: 159-164.

Richardson, G.M. (1941). The preservation of liquid complement serum. Lancet 2: 696.

Rogers, T.J. and Balish, E. (1980). Immunity of Candida albicans . Microbiol. Rev. 44: 660-682.

Salvin, S.B., Petersen, R.D.A. and Good, R.A. (1965). The role of the thymus in resistance to infection and endotoxin toxicity. J. Lab. Clin.Med. 65: 1004-1022.

Schwartz, R.S., Ryder, R.J.W. and Gottlieb, A.A. (1970). Macrophages and antibody synthesis. Prog. Allergy, 14: 81-85.

Sharma,G.,Vasantha,S. and Lal,S.M. (1983).Foot and mouth disease virus neutralizing antibodies in bovine serum collected from slaughter house. Ind.Vet.J. 60: 333-335.

Sharma,R.S.,Prasad,K.L.,Ahuja,M.M.Rahman and A. Kumar (1985). Cell mediated immune response following foot and mouth disease vaccination in buffalo calves. (recd.1986).Acta Virol (Prague) (Engl end) 29(6): 509-513.

Sher,N.A.,Chaparas,S.D.,Greenberg,L.E. and Bernard,S. (1975). Effects of BCG,Corynebacterium parvum and methanol extraction residue in the reduction of mortality from Staphylococcus aureus and Candida albicans infections in immunosuppressed mice.Infec. Immun.12:1325-1330.

Shorshnev,V.I.,Onufriev,V.P.,Brokhanov,M.M.,Yakovleva, N.M. and Pronin,I.A. (1981).Dynamics of antibody formation in experimental foot and mouth disease (in cattle). Veterinariya, Moscow,1: 38-39 (vide vet.bull.51: 4020).

Sinha,B.K.(1985). Protective immune response in candidiasis. Thesis submitted to the Haryana Agricultural University for the award of Ph.D. Degree.

Sutmoller,P. and Vieira,A.(1980).The relationship of neutralizing antibody titres for foot and mouth disease virus and the protection of cattle.Boln.Cent.Pan-am Fiebre Aflosa.39/40: 57-62 (vide vet.bull.52: 4584).

Territo,M.C. (1980). Clinical investigation of macrophage function. In:Recent advances in clinical immunology vol.2.Ed.Thompson,R.A.,Churchill Livingstone,New York,pp. 276-292.

Tibor,Soos and Fazekas Arpad (1985). Studies on the duration of immunity of Hungarian foot and mouth vaccines. Magy Allatorv Lapja 40(7): 397-402.

Uppal,P.K.,Kumar,S.Das,S.K. and Bhatt,P.N. (1975).Foot and mouth disease vaccination in newly born calves. Ind.J. 52: 282-288.

Van Bekkum,J.G.,Fish,R.C. and Dale,C.N.(1963). Immunogenic studies in Dutch cattle vaccinated with foot and mouth disease vaccines under field conditions.I.Nutralizing antibody responses to 'O' and 'A' types. Am.J.Vet. Res.24: 77-82.

Van Bekkum, J.G., Fish, R.C. and Nathans, I. (1969). Immunogenic response in Dutch cattle vaccinated with FMD vaccines under field conditions: Neutralizing antibody response and Immunity to 'O', 'A' and 'C' types. Am. J. Vet. Res. 30: 2125-2129.

Waldman, R.H., Cruz, J.M. and Rowe, D.S. (1979). Immunoglobulin levels and antibody to Candida albicans in human cervico vaginal secretions. Clin. exp. Immunol. 9: 427-434.

Wisniewski, J., Jankowska, J., Kobusiewicz, T., Szkilnik, S. and Baranowski, C. (1971). Determination of the degree of resistance in cattle on the basis of neutralizing antibody titres after immunization with a Frenkel type 'C' FMD vaccine. Medycyna Weterynaryjna. 27(9): 528-531.

Wisniewski, J., Kobusiewicz, T., Szkilnik, S., Baranowski, C. and Jankowska, J. (1972). Determination of the degree of immunity in cattle from the serum neutralizing antibody level after immunization with a type 'O' Frenkel vaccine. Medycyne Weterynaryjna. 28(10): 586-588.

Wittmann, G., Bauer, K. and Mussgay, M. (1970). Experimental immunization of cattle with an EEI/DEAE-D vaccine against infection with subtype 'O' virus. Zentbl. Vet.med. 17B: 1067-1068.

Wozencraft, A.O., Dockrell, H.M., Taverne, J., Targett, G.A.T. and Playfair, J.H.Z. (1984). Killing of Human malaria by macrophage secretory products. Infect. Immun. 43: 664-669.

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ANNEXURE-'A'

Hank's Balanced Salt Solution

Stock Hank's solution was made up with ten times the concentration of all the components except for the sodium bicarbonate solution as follows:

Stock Solution A:

1.	Sodium Chloride (Nacl)	160.00 gm
	Potassium Chloride (Kcl)	8.00 gm
	Magnesium sulphate ($MgSO_4 . 7H_2O$)	2.00 gm
	Magnesium chloride ($Mgcl_2 . 6H_2O$)	2.00 gm
	Distilled Water	800.00 ml
2.	Calcium Chloride ($Cacl_2$)	2.8 gm
	Distilled Water	100.00 ml.

These two solutions were mixed slowly and the volume was adjusted to 1000 ml with distilled water. After that 2.0 ml chloroform was added and stored in a flask at $4^{o}C$.

Stock Solution B:

Disodium hydrogen phosphate ($Na_2HPO_4 . 2H_2O$)	1.05 gm
Potassium dihydrogen phosphate (KH_2PO_4)	1.2 gm

Glucose	20.00 gm
Distilled water	800.00 ml

These ingredients were dissolved in 100 ml phenol red solution (0.4%) and made to 1000 ml by adding distilled water. After that 2.0 ml chloroform was added and stored at $4^{o}C$.

Sodium Bicarbonate Solution:

Sodium bicarbonate ($NaHCO_3$)	1.4 gm
Distilled Water	100.00 ml

This solution was sterilized by autoclaving in a flask at $115^{o}C$ (9 tlb pressure) for 10 minutes.

Hank's solution was made by adding one volume of stock solution 'A', one volume of stock solution 'B' and 18 volumes of distilled water. After that it was sterilized by steaming for one and half hours. A volume of 0.5 ml of sterile 1.4% sodium bicarbonate solution was added to each 20 ml of Hank's solution immediately before use.

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