DIAMOND JUBILEE

SOUVENIR



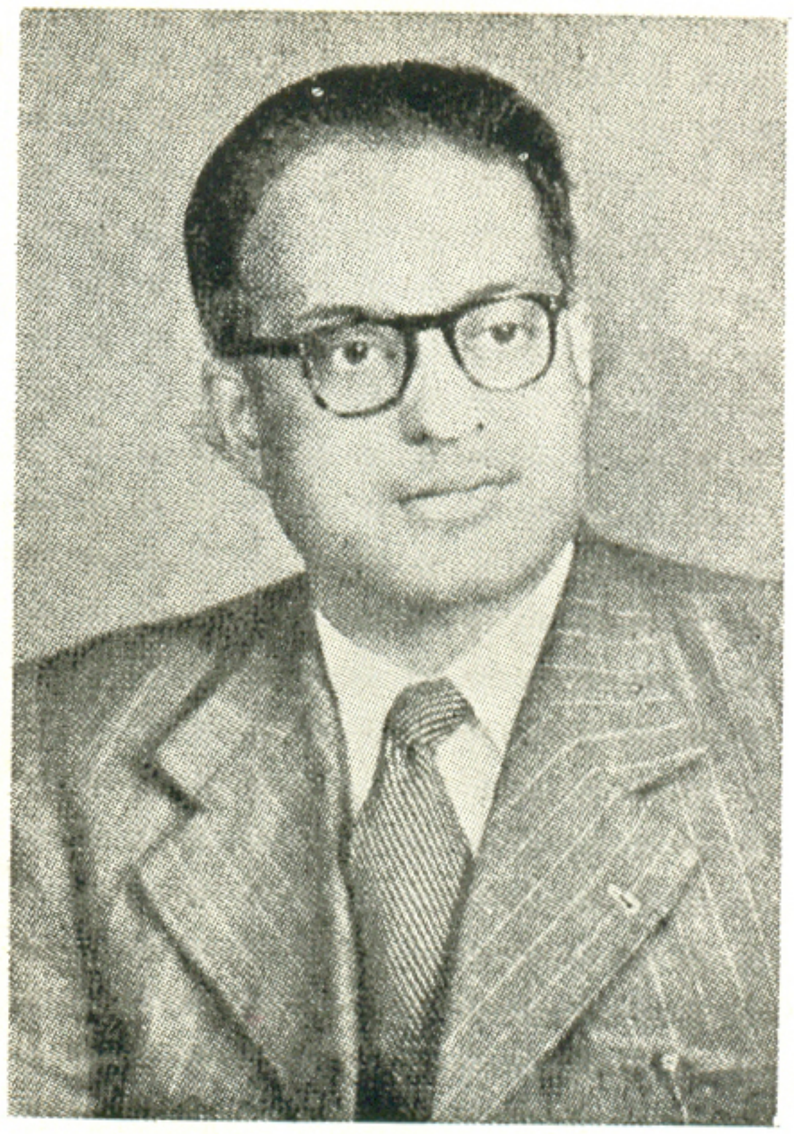
PASTEUR INSTITUTE OF SOUTHERN INDIA

COONOOR

1907 - 1967



MAJOR W.S. PATTON, M.B., F.E.S., I.M.S.
Acting Director (1919–'20)



DR. I.G.K. MENON, M.B.B.S., Ph.D.
Acting Director (1951)



DR. P.V. KURIAN, M.B. B.S., D.T.M., M.S.
Deputy Director (1962–)



DR. A. BALASUBRAMANIAN, M.B.B.S., M.SC.
Assistant Director (1962–)

Donated by
Thangaraman
27/11/68

PASTEUR INSTITUTE
OF
SOUTHERN INDIA
COONOOR

HAFFKINE INSTITUTE
LIBRARY
BOMBAY

DIAMOND JUBILEE
SOUVENIR

HAFFKINE INSTITUTE LIBRARY
BOMBAY.
Call No. Acc No.
Price Source Purchased.
Donated

1907–1967



WHO EXPERT COMMITTEE ON RABIES, GENEVA (1959)



L. PASTEUR
1822–1895

"...then, I implore you, take some interest in those sacred dwellings meaningly described as *laboratories*. Ask that they may be multiplied and completed. They are the temples of the future, of riches and of comfort. There humanity grows greater, better, stronger; there she can learn to read the works of Nature, works of progress and universal harmony, while humanity's own works are too often those of barbarism, of fanaticism and of destruction."

LOUIS PASTEUR,
January 1868.



WHO SPECIAL MEETING ON RABIES RESEARCH, PARIS (1961)



M. PASTEUR et Mme PASTEUR
en 1884



WHO EXPERT COMMITTEE ON RABIES, GENEVA (1965)

TABLE OF CONTENTS

Section			Page
	Foreword		2
I. 1.	President, Members of the Central Committee and Association and Honorary Secretary and Treasurer as on 1st September 1967		3
2.	Members of the Staff as on 1st September 1967		5
II. 1.	Progress During 1957—67		8
2.	Office-Bearers of the Association and Staff of the Institute since 1907		
	(*a*) Certificate of Incorporation		19
	(*b*) First Members of the Committee of Management of the Pasteur Institute of Southern India, Coonoor ...		19
	(*c*) Presidents of the Association		20
	(*d*) Directors of the Institute and Honorary Secretaries and Treasurers		21
	(*e*) Deputy Directors		21
	(*f*) Assistant Directors		21
	(*g*) Senior Research Officers		21
	(*h*) Research Officers		22
	(*i*) Veterinary Officers		22
	(*j*) Medical Officers		22
	(*k*) Veterinary Assistant Surgeons		23
	(*l*) Head Clerks		23
3.	Training		24
4.	Post-Graduate Qualifications		26
5.	Advisory Work and Meetings		26
III.	Scientific Contributions (1957–67)		
1.	Rabies		28
2.	Influenza		51
3.	Other Respiratory Viruses		75
4.	Intestinal Viruses		80
5.	Vaccinia		90
6.	Trachoma Inclusion Conjunctivitis Agents		93
7.	Herpes Simplex		94
8.	B Virus		96
9.	Measles Virus		97
10.	SV_{40} Virus		97
11.	Tissue Culture Methods		98
12.	Cholera		100
13.	Enteric Fevers		113
14.	Epidemic Diarrhoea		115
15.	Syphilis		115
16.	Diphtheria		117
17.	Tuberculosis		119
18.	Leprosy		120
19.	Snake Venoms		120
20.	Publications, 1907—57		121
	1957—67		127
IV.	To-Day and Tomorrow		129

(K) VETERINARY ASSISTANT SURGEONS

Dr. S. Divakaran, B.V.Sc. (8–8–1959 to 30–3–'63).
Dr. R. Raghavan, B.Sc., B.V.Sc. (6–5–1963 to 22–6–'64).
Dr. G. V. J. A. Harshavardhan B.Sc,. B.V.Sc. (Since 24–2–1964).
Dr. A. Gajanana, B.Sc., M.V.Sc. (Since 13–7–1964).

(1) HEAD CLERKS

Sri J. Gnanapragasam. (1907 to 29–10–'36).
Sri K. S. Parameswara Iyer. (30–10–1936 to 31–3–'46).
Sri R. Subramanian, B.A., B. Com. (1–4–1946 to 27–4–'59).
Sri V. Nagarajan, B.A. (Since 1–5–1959).

FOREWORD

The Pasteur Institute of Southern India, Coonoor, started functioning on 6th April 1907 and was officially opened on 25th April 1907 by His Excellency Sir Arthur Lawley, Governor of Madras. This Souvenir which is being brought out on the occasion of the Diamond Jubilee of the Institute is a humble tribute to all those who have guided its destinies and to the workers to whose unremitting endeavours it owes its position in the scientific world to-day.

N. VEERARAGHAVAN.

3. TRAINING

1956

Under a fellowship awarded by the World Health Organization and at the request of the Government of India, Mr. Ghulam Nabi Ghafur and Mr. Abdul Matin Taib, technicians from the Central Public Health Laboratory, Afghanistan, underwent training for a period of six months from 21–4–1956 in general laboratory techniques relating to the manufacture of antirabies vaccine.

1957

Sri T.C. Tripathy, Laboratory Assistant, State Vaccine Institute, U. P. Patwadnagar, underwent training in the techniques of manufacture and testing of antirabies vaccine and the diagnosis of rabies from 15-11–1957 to 16–12–1957.

Sri R. Shantha Kumar Prabhu worked as a voluntary worker from 26–8–1957 to 9–5–1958.

1958

Under a fellowship awarded by the World Health Organization, Mr. Mohammed Khan and Mr. Baghar Shah Sarwary, technicians at the Vaccine Institute of Afghanistan, Kabul, underwent training for a period of six months from 22–3–1958 to 22–9–1958 in laboratory techniques relating to the manufacture of antirabies vaccines.

Under a fellowship awarded by the World Health Organization, Mr. Gulam Nabi Mateen of Central Public Health Laboratory, Kabul, Afghanistan, underwent training for two months from 17–7–1958 to 13–9–1958 in the Pathology and Rabies Research Departments of the Institute.

Sri Jacob Kuruvilla, Valparai, underwent a course of training in clinical laboratory methods from 1–2–1958 to 28–2–1958.

Dr. V.R. Vivek, B.V. SC. & A.H. worked as a voluntary worker from 1–9–1958 to 23–7–1959.

1959

Under a fellowship awarded by the World Health Organization, Mr. Lutfullah Mohamadizia of the Central Public Health Laboratory, Kabul, Afghanisthan, underwent training for a period of six months from 17–2–1959 to 5–8–1959 in general laboratory techniques.

Under a W.H.O. travel fellowship in control of quarantineable diseases, Dr. Eva Levi Jovovic, Yugoslavia, underwent training for a week from 4–4–1959 in the laboratory aspects of rabies, influenza and adenoviruses.

Dr. G.P.C. Lal of the State Vaccine Institute, Patwadnagar, U.P., underwent training from 5–14 March, 1959 in the methods of manufacture of antirabies vaccine.

Dr. D. N. Sen Gupta, Assistant Professor of Microbiology, All India Institute Hygiene and Public Health, was trained in tissue culture techniques for a period of one month from 10–5–1959.

1960

Under an I.C.M.R./R.F. Fellowship Dr. (Kumari) S. Balaya, Demonstrator in Bacteriology, Lady Hardinge Medical College, New Delhi, underwent higher training in virology from 7–12–1959 to 6–8–1960.

Dr. Vasant Pandit, M.B.B.S., worked as a voluntary worker from 1–3–1960 to 31–12–1960.

1961

Dr. J.C. Gupta, M.D., Assistant Professor of Pathology, M.G.M Medical



SHRI JAWAHARLAL NEHRU
Photograph of the Painting in the Auditorium

College, Indore, underwent training in virology from 5–3–1961 to 14–5–1961.

Dr. (Kumari) K. Tara Bai, M.B.,B.S., and Dr. V.R. Kalyanaraman, M.B.,B.S., Assistant Surgeons in the Madras Medical Service were posted at the Institute to undergo training for the M.SC. degree in Bacteriology of the Madras University. They worked at the Institute from 1–7–1961 to 1–4–1963.

Sri K.N. Venkatesan. B.SC., Special Officer (Guts), Coonoor, underwent training in bacteriological techniques from 1–9–1961 to 30–11–1961.

1963

Dr. E.R.B, Shanmugasundaram, Professor of Biochemistry, University of Madras, studied specialized techniques from 14–5–1963 to 8–6–1963.

Smt. Padma Krishnaswami, M.SC., Coonoor, worked as a voluntary worker from 8–7–1963.

Sri A.V. Rangarajan, Assistant, Agricultural College and Research Institute, Coimbatore, was given training in the method of maintaining a mouse colony from 10–2–1964 to 14–2–1964.

1965

Sri A.K. Taneja, M. Pharm., Research Student in the Department of Biochemistry, Indian Institute of Science, Bangalore, studied tissue culture procedures as applied to the cultivation of vaccinia and influenza viruses from 19–4–1965 to 4–5–1965.

Dr. M.K. Subramaniam, M.A., D.SC., F.A.SC., Assistant Professor, Cytogenetics Laboratory, Indian Institute of Science, Bangalore, studied tissue culture and cytological techniques from 26–4–1965 to 5–5–1965.

Dr. C. K. Jayaram Panicker, M.B., B S., M.SC., Assistant Professor of Bacteriology, Medical College, Kottayam, studied the working of intestinal and respiratory virus units from 17–5–1965 to 31–5–1965.

Dr. C.L. Sehgal, Assistant Director, National Institute of Communicable Diseases, New Delhi, underwent training in the testing and standardization of antirabies vaccine from 2–8–1965 to 2–9–1965.

Dr. G.K. Trivedi, M.B.,B.S., D.P.H., M.P.H., (U.S.A.), Medical Officer of Health, Aliabada (Gujarat State) underwent training in the manufacture and testing of antirabies vaccine from 13–8–1965 to 2–9–1965.

Dr. K. Sivaramakrishnan, Assistant Research Officer, Department of Pathology, Stanley Medical College, Madras, underwent training in fluorescent techniques from 15–11–1965 to 30–11–1965.

HAFFKINE INSTITUTE
LIBRARY
BOMBAY



4. POST-GRADUATE QUALIFICATIONS

Dr. I.G.K. Menon qualified for the PH.D., degree of the Madras University in 1956 for his thesis entitled 'Tropical eosinophilia and false positive serological reactions'.

The following medical graduates obtained the M.SC. degree of the Madras University.

Dr. A. Balasubramanian	(Virology)
Dr. D.S. Chandrasekhar	(Serology)
Dr. (Kumari) K. Tara Bai	(Virology)
Dr. V.R. Kalyanaraman	(Virology)
Dr. Vasant Pandit	(Virology)

5. ADVISORY WORK AND MEETINGS

The senior members of the staff are periodically called upon to serve on various Advisory Committees, scientific meetings etc. Some of them are given below.

Dr. N. VEERARAGHAVAN

WHO

Member, Expert Advisory Panel on Rabies, since 1952.

Member, Expert Committee on Rabies, 1953, 1956, 1965.

Chairman, Expert Committee on Rabies, 1959.

Member, Special meeting on rabies research held at Paris in May 1961.

Participant, International Rabies Symposium organized by the International Association of Microbiological Societies, held at Lake of Annecy in May 1965.

Government of India

Member, Indian delegation to the 'Conference on diseases in countries with hot climate' organized by the Ministry of Health, U.S.S.R. at Tashkent in September 1961.

Indian Council of Medical Research

Member, Governing Body, 1959–60; 1965–66.

Member, Scientific Advisory Board, 1962–65.

Member, Virus Diseases Advisory Committee, 1957–62.

Chairman, Virus and Arthropod-borne Diseases Expert Group, 1963–65.

Member, Virus and Arthropod-borne Diseases Expert Group 1966

Member, Reviewing Committee, 1966.

Armed Forces Research Committee

Member since 1967

Madras State Research Committee

Member since 1947.

Madras Government

Member, Dr. U. Krishna Rau Committee appointed by the Government of Madras in connection with the reorganization of the King Institute, Guindy. (1960).

Section I

PRESIDENT, MEMBERS OF THE CENTRAL COMMITTEE AND ASSOCIATION AND HONORARY SECRETARY AND TREASURER AS ON 1st SEPTEMBER 1967

PRESIDENT

Dr. (Kumari) A. B. Marikar, M.D., D.G.O.

EX-OFFICIO MEMBERS OF THE CENTRAL COMMITTEE

1. The General Officer Commanding, Madras Area, Madras, 4
2. The Collector of the Nilgiris, Ootacamund.
3. The District Medical Officer of the Nilgiris, Ootacamund.
4. The Director of Medical Services, Madras.
5. The Dean, Government General Hospital and Madras Medical College, Madras.
6. The Director, King Institute, Guindy, Madras.
7. The Officer commanding, Military Hospital, Wellington.
8. The Chairman, Municipal Council, Coonooor.
9. The Director, Pasteur Institute, Coonoor.
10. The Director of Public Health in Mysore, Bangalore.
11. The Hon'ble Minister for Health, Kerala State, Trivandrum.
12. The Hon'ble Chief Minister, Government of Andhra Pradesh, Hyderabad.
13. The Director of Public Health, Madras.
14. The Superintending Engineer, Coimbatore Circle, Coimbatore.
15. The Assistant Director of Medical Services, Headquarters, Madras, Mysore and Kerala Area, Madras, 16.
16. The Surgeon to His Excellency the Governor of Madras.
17. The Deputy Director of Medical Services, Madras.
18. The Officer Commanding, Military Hospital, Avadi, Madras.
19. The Chief Medical Officer, Southern Railway, Madras.
20. The Deputy Director, Pasteur Institute, Coonoor.
21. The Collector of Madras, Madras.
22. The Commissioner of the Corporation of Madras, Madras.
23. The Director of Animal Husbandry, Madras.
24. The Director of Medical Services, Andhra Pradesh, Hyderabad.
25. The Director of Public Health, Andhra Pradesh, Hyderabad.
26. The Superintendent, King George Hospital, Visakhapatnam.
27. The Deputy Secretary to Government, Education, Health and Local Administration Department, Andhra Pradesh, Hyderabad.
28. The Director of Animal Husbandry, Andhra Pradesh, Hyderabad.

ELECTED MEMBERS OF THE CENTRAL COMMITTEE

1. Dr. T. R. Rangaswami, M.B.,B.S., B.S. Sc.
2. Sri V. K. Aravindaksha Menon.
3. Col. M. S. Joshi, F.R.C.S., I.M.S. (Retd.).
4. Mrs. Mary Clubwala Jadhav.
5. Dr. P. V Cherian, F.R.C.S., D.L.O.
6. Sri R. Ramanathan Chettiar.

Indian Cancer Research Centre

Member, Staff Structure Committee (1964).

Dr. I.G.K. MENON

W H O

Member, Expert Advisory Panel on Virus Diseases, 1952–59.
Member, Expert Committee on Respiratory Virus Diseases, 1958.
Represented India as a delegate to the VII International Congress for Microbiology held at Stockholm in August 1958.

Indian Council of Medical Research

Member, Virus Advisory Committee 1957, 1958.

Dr. P.V. KURIAN

Indian Council of Medical Research

Member, Venereal Diseases Sub-Committee 1961, 1962.
Secretary, Microbiology Expert Group, 1963–65.
Participant, Tissue Culture Seminar organized by the M.S. University of Baroda from 21–28 January 1965.

Dr. A. BALASUBRAMANIAN

Indian Council of Medical Research

Member, Microbiology Expert Group, 1966.

7. Dr. E. Somasekhar, F.R.C.S. (Edin.), L.R.C.P., D.P.H.
8. Mrs. Rajam Ramaswami, J.P.
9. Sri P. S. Subramanian, F.C.A.
10. Sri D. C. Kothari.
11. Sri L. Gonsalves, B.A., B.L.
12. Dr. R. V. Rajam, M.S., F.R.C.P., F.R.S.
13. Sri T. Purushotham, M.L.C.
14. Sri V. T. Rangaswamy Aiyangar.
15. Dr. (Mrs.) M. C. Mathew, L.M. & S.
16. Dr. V. R. Thayumanaswami, M.S., F.R.C.S., M.ch. (Ortho.).
17. Dr. S. R. Pandit, B.A., M.B.,B.S., B.S.Sc., D.B., F.A.Sc.
18. Sri Rai Bahadur C. Balchand.
19. Sri A. Varadappa Chetty.
20. Sri Rao Bahadur T. S. Kachapikesa Mudaliyar, B.A.
21. Dr. Y. S. Narayanan Rao, M.B.,B.S., D.T.M. D.B. (Lond.).
22. Major General S. C. Alagappan.
23. Sri S. Chellapandian, B.A.,B.L.

LIFE MEMBERS OF THE ASSOCIATION

1. Messrs. John Taylor & Sons, Oorgaum.
2. Messrs. Spencer & Co., Ltd.
3. The Raja of Pudukkottai.
4. The United Planter's Association of Southern India, Coonoor.
5. The Coimbatore District Tuberculosis Sanatorium Society, Coimbatore.

HONORARY LIFE MEMBER OF THE ASSOCIATION

Lt. Col. M.L. Ahuja, M.D., D.P.H.

MEMBERS OF THE ASSOCIATION

1. Mr. Ramsay Unger.
2. The Treasurer, U. L. C. Mission, Guntur.
3. M/S Stanes & Co., Ltd., Coimbatore.
4. The Chief Medical and Sanitary Officer, The Kolar Gold Fields Undertakings, (Medical Establishment), Champion Reefs, K.G.F.
5. Dr. C. A. Martin.
6. Major T. D. Jeevarathnam.
7. Sri P. V. Rajamannar.
8. Sri S. V. Ramanujam Chetty.
9. Mrs. Mary Gilbert.
10. Miss. C.M.P. Finch.

HONORARY SECRETARY AND TREASURER

Dr. N. Veeraraghavan, M.B.,B.S., D.Sc.

Section III

SCIENTIFIC CONTRIBUTIONS (1957–'67)

INTRODUCTION

The contributions of the Institute during the first 50 years have been presented in the Souvenir brought out on the occasion of the Golden Jubilee of the Institute celebrated on April 2, 1957. In this review a brief account is given of the work done during the past 10 years. These have been published regularly in scientific journals and in the Annual and Scientific Reports of the Institute. Here the attempt has been made to present a bird's eye view of the main lines of work. For this reason reference has been made to the more important contributions without going into details. Each of the major subjects on which the Institute has been interested has been dealt with separately. As the account deals only with the work of the Institute, references to the contributions of other workers in the field have been limited to events of historical importance.

1. RABIES

In this section is presented a brief and perforce sketchy account of the contributions made by the Institute on rabies. The matter is arranged under different headings with a view to emphasize the work on various aspects of the disease.

(1) HISTORICAL

From 1881–88 Pasteur, by a series of brilliant researches, proved the constant occurrence of rabies virus in the central nervous system of diseased animals. He showed that other animals could be infected with it by direct inoculation upon the surface of the brain. He offered to the world a method by which the disease could be prevented by the inoculation of the rabbit fixed virus in various stages of attenuation, and thereby placed the coping stone upon a life work which has transformed the whole face of medical theory. Up-to-date no discovery concerning the disease is on a par with the classical work done by him.

A few years following Pasteur's discovery the treatment for rabies was started by several institutes in different parts of the globe. The first institute to be started for this purpose in our country was the Pasteur Institute of India, Kasauli, in 1900. This was followed by the Pasteur Institute, Coonoor, which started functioning in April 1907 under the Directorship of Capt. J. W. Cornwall.

(2) EPIDEMIOLOGY

Incidence of rabies in human beings

In India more than 3,00,000 persons bitten by rabid animals undergo antirabies treatment annually. In the States of Madras, Andhra Pradesh, Mysore and Madhya Pradesh, which are served by the Pasteur Institute, Coonoor, over 1,00,000 persons are treated every year.

It will be seen from Table 1 that in the Madras State alone 23,962 persons have died of hydrophobia during the 52 years from 1913–65. Of these, 5,369 were from the urban areas and 18,593 from the rural areas.

Incidence of rabies in animals

It is found that 60–70 per cent of brains of animals suspected to have died of rabies received for examination at the Institute from various parts of South India are positive for rabies. In a study of an unselected series of 100 animals suspected to have died of rabies consisting of 92 dogs, 1 jackal, 2 cats, 1 cow, 3 calves and 1 goat, it was found that 66 per cent of the animals were rabid.

2. MEMBERS OF THE STAFF AS ON 1st SEPTEMBER 1967

PASTEUR INSTITUTE

DIRECTOR

Dr. N. Veeraraghavan, M.B., B.S., D. Sc.

DEPUTY DIRECTOR

Dr. P. V. Kurian, M.B., B.S., D.T.M., M.S.

HEAD CLERK

Sri V. Nagarajan, B. A.

OTHER STAFF

Sri M. Rajagopala Menon.
Sri K. Velayudhan.
Sri N. Moracha.
Sri M. Velu.
Sri A. M. Rozario.
Sri William John.
Sri F. J. Jesudoss.
Sri M. Nathan.
Sri S. Sreedharan.
Sri P. Girijasankaran.
Sri T. Thimma.
Sri T. Kuppuswamy.
Sri G. Wesley.
Sri K. M. Hallan.
Sri J. S. Samuel Watson.
Sri Y. N. Bellie.
Sri H. T. Bellie.
Sri S. T. Thandayuthapani.
Sri T. Giriya.
Sri Mallan.
Sri D. Sunnan.
Sri S. Chennan.
Sri K. M. Raju.
Sri Stanislass.
Sri T. Chenniah.
Sri Kempa.
Sri R. Somu.
Sri Angamuthu.
Sri Boran.
Sri C. Mallan.
Sri Marian.
Sri Byran.
Sri K. Muthu.
Sri K. Raju
Sri David.
Sri Muthuswamy.
Sri Ramaswamy.
Sri Kuppuswamy.
Sri Thangamuthu.
Sri Aboo Arabi.
Sri P. V. Raghavan.
Sri M. Javanan.
Sri A. Kunnikannan.
Sri M. Natarajan.
Kumari C. R. Susheela.
Sri A. C. Idicula.
Sri Michael Peter Gass.
Kumari S. Padma.
Smt. Z. Mariamma.
Sri R. Kitto.
Sri R. Devaraj.
Sri A. Kadirvelu.
Sri R. Munuswamy.
Sri Rajan Samuel.
Sri Mohamud Ibrahim.
Sri M. Govindan.
Sri Chellan.

TABLE 1

Deaths from Hydrophobia in the Madras State During 1913–'65.

Year		Town	Rural area	Total
1913	...	38	102	140
1914	...	26	48	74
1915	...	19	46	65
1916	...	17	9	26
1917	...	26	11	37
1918	...	30	44	74
1919	...	34	28	62
1920	...	47	50	97
1921	...	41	48	89
1922	...	62	158	220
1923	...	31	251	282
1924	...	85	279	364
1925	...	64	421	485
1926	...	100	388	488
1927	...	76	560	636
1928	...	71	493	564
1929	...	76	343	419
1930	...	100	356	456
1931	...	128	533	661
1932	...	115	423	538
1933	...	109	355	464
1934	...	110	302	412
1935	...	112	434	546
1936	...	115	551	666
1937	...	98	728	826
1938	...	149	960	1,109
1939	...	242	1,064	1,306
1940	...	145	1,134	1,279
1941	...	186	1,289	1,475
1942	...	145	1,273	1,418
1943	...	130	1,115	1,245
1944	...	170	906	1,076
1945	...	120	511	631
1946	...	118	321	439
1947	...	109	279	388
1948	...	108	215	323
1949	...	134	283	417
1950	...	131	271	402
1951	...	137	142	279
1952	...	119	102	221
1953	...	65	55	120
1954	...	65	37	102
1955	...	159	118	277
1956	...	98	90	188
1957	...	139	111	250
1958	...	116	168	284
1959	...	160	195	355
1960	...	107	139	246
1961	...	116	139	255
1962	...	140	133	273
1963	...	129	162	291
1964	...	103	193	296
1965	...	99	227	326
Total	...	5,369	18,593	23,962

Note: 1913–1952 - - Composite Madras State inclusive of Andhra area.
1953–1965 — Madras State exclusive of Andhra area.

The nature of biting animals

Cornwall (1923) in his classical paper on statistics of antirabies inoculations in India reported that in the old Madras State the bite was inflicted in most instances by dogs (95.4%), and only rarely by jackals (2.7%). He considered that in other parts of India the proportions were different, a much larger number being infected by jackals.

It is surprising how these figures correspond closely to those presented in the Annual Report of the Institute for the year 1965. It will be seen that out of 4,32,275 persons who underwent a complete course of treatment, 4,24,624 (98.43%) were bitten by dogs, 9,022 (2.08%) by jackals and the remaining by other animals.

Infectivity of biting animals

Cornwall (1923) has summarised the results of his study on this subject extending over several years as follows:

'Certain dogs, proved rabid by laboratory tests, have bitten many people without infecting any of them. Other dogs, also proved rabid by laboratory tests, have infected the majority of the people whom they bit. Probably different strains of the virus of rabies differ in infectivity.

When the criterion of the infectivity of the biting animal is the death from hydrophobia of one of the persons bitten, the risk run by the other persons, bitten by the same animal, who have received no treatment can be estimated. A number of such instances have been collected by the Pasteur Institute of Southern India. The bitten persons had received no treatment.

Number of persons bitten	...	423
Death from hydrophobia	...	148
Mortality per cent	...	35

So it may be concluded that in Southern India about 35 per cent of

3. MEMBERS OF THE STAFF AS ON 1st SEPTEMBER 1967

ENQUIRIES SPONSORED BY THE GOVERNMENT OF INDIA, MINISTRY OF HEALTH

ASSISTANT DIRECTOR

Dr. A. Balasubramanian, M.B., B.S., M.Sc.

RESEARCH OFFICER

Dr. Vasant Pandit, M.B., B.S., M.Sc.

MEDICAL OFFICERS

Dr. J. Sampath Kumar, M.B., B.S.
Dr. Mathew Francis, M.B., B.S.
Dr. (Smt.) Mary Chakko, M.B., B.S.

OTHER STAFF

Dr. G. V. J. A. Harshavardhan, B. Sc., B. V. Sc.
Dr. Gajanana, B.Sc., M.V.Sc.
Kumari N. Remadevi, M.A., M.Sc.
Sri M. R. Dharmarajan, M.Sc.
Smt. V. K. Visalakshi, B. A.
Sri P. Vijayan, M.Sc.
Smt. Rohini Gideon, M.A.,M.Sc.
Dr. C. Rajkumar, B.V.Sc.
Sri U. S. Bheemarao, B.A.
Sri Jayaprakash Narayan, B.Sc.
Smt. Susan Cherian, B.Sc.
Sri K. K. Nanjundan, B.Sc.
Kumari K. V. Vasantha, B.Sc.
Sri H. K. Krishnan, B.A.
Sri Ravoof, B.Sc.
Sri P. Venugopal, B.Sc.
Sri J. Raman, B.Sc.
Sri B. Jogheesan, B.Sc.
Sri A. P. Mathew, B.Sc.
Sri James William, B.Sc.
Sri R. Joghee.
Sri K. S. Kullan.
Sri S. Bellie.
Sri K. J. Joghee.
Sri B. Subramaniam.
Sri M. P. Ramachandran.
Sri K. Nannurao.
Sri P. Rangaraj.
Sri S. Joghee.
Sri K. Siddhan.
Sri A. Chandran.
Sri K. Govinda Panicker.
Srl Y. B. Sevenah.
Sri R. Devaraj.
Sri K. J. Kari.
Sri K. S, Sarojini.
Sri M. Raju.
Sri J. Samuel Sadrach.
Sri K. Rajubettan.
Sri H. Lingan.
Sri J. M. Pearl.
Kumari C. Saraswathi.
Kumari P. M, Kunjithi.
Kumari C. N. Anandavally.
Sri J. Menon.
Sri M. S. Ramakrishnan.
Sri C. T. Mohanan.
Sri K. Vasudevan.
Kumari R. Saroja.
Smt. B. A. Sundari.
Sri C. N. Natarajan.
Sri L. Sivananjan.
Sri K. Balakrishnan.
Sri M. Rajamuthu.
Smt. Agnes Appaiah.
Sri M. Govindarajan.
Kumari T. S. Anandavally.
Sri Gopalakrishna Pookad.

persons bitten by rabid dogs which are actually infective will, if untreated, develop hydrophobia and die. No account is taken in this estimate of the position, number or severity of the bites inflicted'.

A similar study has been in progress at the Institute since 1946. It was found that out of 180 persons who were bitten by proved infective animals and who refused to have treatment 100 died of hydrophobia giving a mortality rate of 55.25%.

Veeraraghavan *et al.* carried out an investigation to determine (a) the percentage of animals that showed the virus in the brain in a group suspected to have died of rabies, (b) the number that contained the virus in the submaxillary glands, (c) the proportion that showed the presence of Negri bodies, and (d) the correlation, if any, between the occurrence of Negri bodies and the presence of virus in the brain and submaxillary glands. An unselected group of 100 animals consisting of 92 dogs, 1 jackal, 2 cats, 1 cow, 3 calves and 1 goat was studied.

It was found that out of 100 animals examined only 66 per cent proved to be rabid. Among these, 39 (59.1%) had virus in the submaxillary glands. These results indicate that 39% of animals suspected to be rabid and 59% of animals proved to be rabid contain virus in their salivary glands and are capable of transmitting the disease in nature.

Reservoirs of infections in wild life

It is well known that the wolf, fox and jackal serve as chief reservoirs of infection in many countries in the world. It has been reported that the species of striped and spotted skunks are a reservoir of rabies infection in Iowa and other parts. An outbreak of rabies attributed to the Indian mongoose (*Herpestes javanicus*) has been reported from Puerto Rico. In this outbreak there was transmission of the infection to dogs, cattle, goat and swine. Recent studies in wild life rabies in Texas indicate a close association between Mexican free tailed bats and certain carnivores, especially the racoon.

In India the jackal would appear to be the main reservoir of rabies infection in wild life, particularly in the hill districts and areas adjoining forests. Unfortunately, we do not have any information regarding the incidence of the disease in other wild life. For instance, we do not know what role the Indian mongoose plays in the spread of the disease in India.

The epidemiology of rabies in wild life is a very complex problem for the simple reason that we know very little about the ecology of most forms of wild life. We do not know the factors responsible for the appearance of an epizootic of rabies in wild life as well as those which lead to its disappearance. For instance, there was an epizootic of rabies among jackals in the Nilgiris in 1943. This was preceded by a considerable increase in the jackal population. During the epizootic we isolated several strains of virus. After a few months the jackal population decreased considerably and this was followed by the waning of the epizootic. The Nilgiris was almost free from rabies till July 1956. During 1955 there was an enormous increase in the jackal population and in July 1956 a severe epizootic of rabies broke out among the jackals and dogs in the hills. There has not been a serious outbreak of rabies in the district since 1956.

Bat rabies

The first case of hydrophobia following bat bite in India has been reported by the Institute. A pointsman working in the Bendi gate railway station in Srikakulam district of Andhra Pradesh saw a group of boys pelting stones at a bat which was lying on the ground unable to fly. He chased the boys away and picked it up with a view to replace it on the branch of a nearby tree. The bat bit

STAFF

im viciously in the forearm near the wrist. The man developed hydrophobia three months later and died. There was no history of the man having come in contact with any other rabid animal.

Bats were collected from the area soon after the incident and examined for evidence of rabies infection. Negri bodies and the virus were not demonstrable in the brains of the bats examined.

In 1967 a bat was received for examination from Madurai. The history was that it was paralysed and found crawling towards an infant lying in a cradle. The examination of the brain and salivary glands did not show any evidence of rabies infection.

An analysis of 129 cases of hydrophobia

During the period 1931 to August 1964, 129 cases of hydrophobia reported at the Institute. An analysis of the particulars regarding the cases and the biting animals have been made.

It was found that the shortest incubation period of 10 days was seen in persons bitten on the scalp, forearm, hand, finger and leg. This indicated that the incubation period did not depend on the part of the body *per se* where the bites were inflicted or their distance from the central nervous system. The na ure of the tissue involved appeared to be more important.

The incubation period varied from 2 weeks to 3 months in a majority of the cases. The longest incubation period was 11 months in a case bitten on the leg.

The duration of illness varied from less than a day in one case to 12 days in another. Ordinarily it ranged from 1 to 6 days.

Hydrophobia among persons bitten by apparently healthy dogs

Recently there have been reports of hydrophobia occurring among persons bitten by apparently healthy dogs. This has been explained by a subclinical infection in the animal with transient salivary excretion of the virus followed by recovery.

Only three such instances have been brought to our notice during 1939–64 indicating the extreme rarity of the condition. The particulars regarding the cases were as follows:

(i) A boy, aged 6 years, bitten on the right thigh by his own dog on 16–9–1950, developed hydrophobia on 8–12–1950 and died. The dog was alive and well when the boy developed the disease.

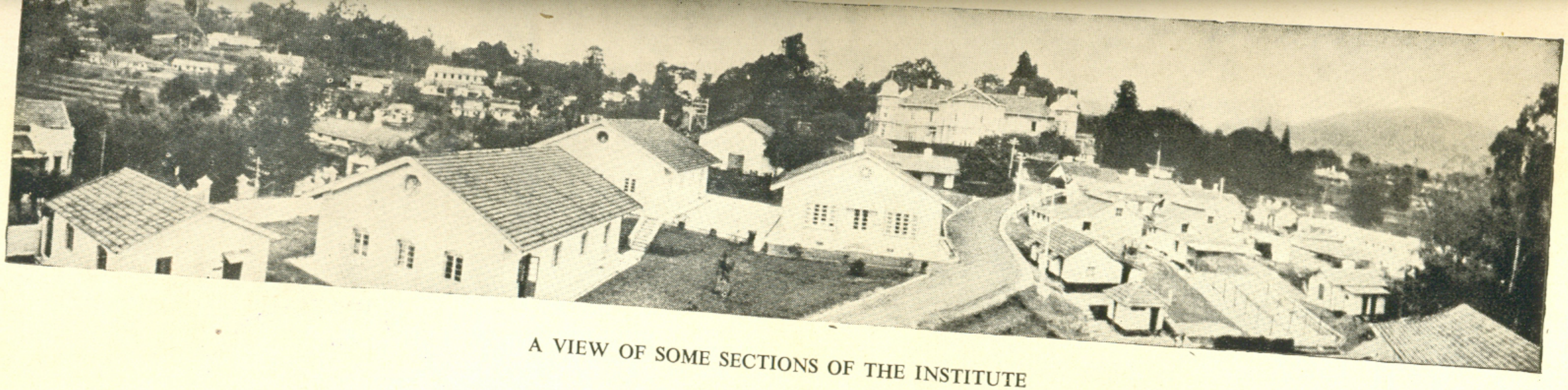
(ii) A boy, aged 17 years, bitten on the face and foot during May 1964, developed hydrophobia on 6–6–1964. The dog was alive and well when the boy developed the disease.

(iii) Three children aged 4, 6, 7 and 12 years were bitten on the lower limbs by a domestic pup in October 1964. All of them received 4 doses of antirabies vaccine after which the treatment was discontinued as the pup was alive and well on the tenth day after inflicting the bites. All the children developed hydrophobia around 4 months after the date of bite. The pup was alive when the children developed the disease.

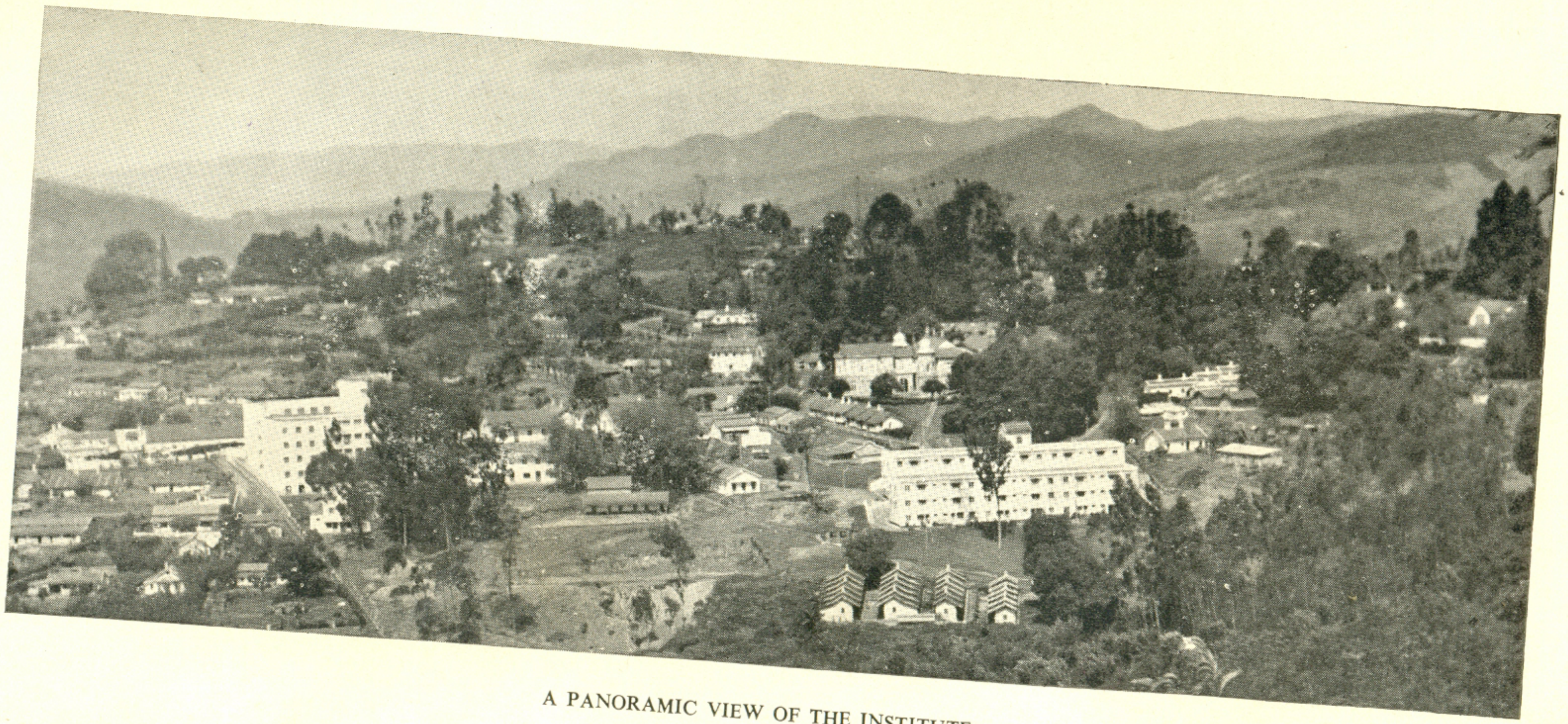
In a country like India where rabies is highly endemic the chance of infection from another source has always to be borne in mind. But, this possibility was unlikely in the cases referred to above.

In all these cases attempts to get the dogs for investigation failed as the animals were destroyed as soon as the bitten persons developed the disease.

In December 1965, another case of hydrophobia following the bite of an apparently healthy dog was reported by Dr. Kodandarama Rao, a private practitioner at Surandai in Tirunelveli District. The particulars regarding the case were as follows.



A VIEW OF SOME SECTIONS OF THE INSTITUTE



A PANORAMIC VIEW OF THE INSTITUTE

On 2–11–65, Sri Murugan, a boy aged 14 years of Surandai village, was standing on a road roller and the dog in question was lying on the ground by the side of the roller. He was asked to get down from the roller and, while doing so, stepped on the dog which bit him on the left leg. As the animal was alive and well for seven days antirabies treatment was not given.

On 16–12–1965, 44 days after bite, the boy developed high temperature with headache and pain all over the body. The condition was diagnosed as influenza and treated. He developed symptoms of hydrophobia on the 46th day and died two days later.

The dog did not bite any other person or animal and did not manifest any change in behaviour before or after biting the boy. Also, there was no evidence of rabies among dogs in the village at that time.

Carrier state in rabies

On receipt of information of the death of the boy at Surandai a team from the Institute was sent to the village about 600 km away to bring the dog for observation and study. The dog was brought to Coonoor on 1–1–1966 and has been under observation ever since.

From 5–1–1966, the saliva of the dog is being collected daily and examined by the fluorescent antibody technique and animal inoculation.

Out of 358 samples examined till 1–1–1967, virus was isolated on 13 occasions during the period 16–1–1966 to 1–5–1966. There was no regular periodicity in the excretion of the virus.

The smears and sections of the brains of the animals examined by the fluorescent antibody technique showed the presence of rabies virus antigen.

Neutralization tests with three isolates showed that antirabies serum neutralized greater than log 4.2 to log 5.6 LD_{50} of the virus indicating that they were rabies virus.

A cross neutralization test was performed using the dog virus (DV), CVS and a strain of rabies street virus and the sera raised against DV and CVS in guinea-pigs. It was found that DV was neutralized by CVS antiserum and that DV antiserum neutralized CVS as well as the street virus indicating that DV was rabies virus.

An interesting finding was the absence of specific neutralizing antibody in the dog which is known to excrete rabies virus periodically.

These studies, which are being continued, establish the possibility of a carrier state among dogs. Its occurrence, however, should be considered very exceptional.

Abortive rabies in dogs

(i) With a view to determine whether dogs could suffer from abortive rabies infection, 101 apparently normal stray dogs in the city of Madras were examined.

The brains of all the animals were negative for rabies by smear, section and biological test. In 3 animals smears of the brains were positive by the fluorescent antibody test. It was possible that they were incubating the disease.

The salivary glands of all the animals were negative by biological test and fluorescent antibody reaction indicating that there was no evidence of salivary excretion of rabies virus among them.

Examination of the non-deactivated sera indicated the presence of neutralizing antibody in 58 of them. Titration of the antibody content of the above sera

Sri Nachimuthu.
Sri Nanjan.
Sri Ponnan.
Sri Chellan.
Sri Nanjan.
Sri Chickkaiah.
Sri M. Ramappan.
Sri N. Muthuswamy
Sri A. Yesudoss.
Sri Sundaram.
Sri S. Shanmugam.
Srl V. Doss
Sri B. Basuvan.
Sri C. Lingan,
Sri N. Lakkan.
Sri V. Chellan,
Sri S. Philip.
Sri Ethirajulu,
Sri C. Raju.
Sri M. Karuppaswamy.
Sri Kolandaswamy.
Sri T. M. Muthuswamy.

4. MEMBERS OF THE STAFF AS ON 1st SEPTEMBER 1967

I. C. M. R. ENQUIRIES UNDER DR. N. VEERARAGHAVAN

Sri R. Rangasami.
Sri N. Radhakrishnan.
Sri M. Mariasusai.

HAFFKINE INSTITUTE
LIBRARY
BOMBAY

showed the presence of specific neutralizing antibody in a dilution of 1 in 10 in only 4 animals.

(ii) A similar study was carried out in respect of the stray dog population at Delhi. Thirty dogs were examined.

The brains and salivary glands of all dogs were negative by the fluorescent antibody test and the biological test.

Examination of the non-deactivated sera indicated the presence of neutralizing antibody in two animals. Titration of the antibody content of the 30 sera after deactivation showed the presence of neutralizing antibody in only one animal.

These studies indicate that the occurrence of abortive rabies infection among stray dogs with salivary excretion of the virus is very rare.

Possibility of virus mutation

As Kaplan (1952) points out, bat rabies suggests the important possibility that through natural host-parasite adaptation it may be possible for other species of animals to harbour the rabies virus and transmit it for long periods without manifesting any clinical signs themselves. It is quite likely that continued exposure of many forms of natural life to rabies infection might result in the establishment of enzootic reservoirs in animal species more resistant to the rabies virus than the bat. It cannot be ruled out that biological chance and time have already created the reservoirs.

This problem has been under continuous study at the Institute and so far no evidence of a reservoir of infection in other than the commonly known animals has been found.

(3) RABIES VIRUS

Cultivation of rabies virus

Fixed virus

Extensive studies on the cultivation of rabies virus *in vitro* have been in progress at the Institute continuously since 1937.

Several attempts were made to cultivate the virus on the chorioallantoic membrane of the developing egg without success. Next attempts were made to propagate the virus in tissue cultures using plasma media containing chicken embryo brain covered with Tyrode-serum. Modified Maitland techniques were also employed. With the above methods, although it was possible to demonstrate the multipication of the virus, the concentrations obtained in cultures were not high.

Attempts were made to cultivate the virus in synthetic media. After extensive trials it was possible to demonstrate evidence of multiplication of rabies fixed virus in a medium containing 2.5% of a sample of glycine obtained from the British Drug Houses, 0.15% peptone, 2% sheep serum and 1.5% steamed sheep brain extract. With the above medium it was possible to obtain in cultures at least five times the maximum concentration of virus obtained in the brains of infected sheep used in the manufacture of antirabies vaccine. Subsequent work showed that tryptophane, thiamine hydrochloride,nicotinic acid, pyridoxine hydrochloride, calcium pantothenate, riboflavin and biotin concentrate had growth stimulating properties on cultures of rabies virus *in vitro*. With the addition of all the above factors it was possible to dispense with the use of steamed sheep brain extract from the medium and obtain concentrations of virus nearly 3,000 times that ordinarily obtained in the brains of infected sheep used in the manufacture of antirabies vaccine.

The glycine (B.D.H.) used in all the experiments was obtained in 1939. When this stock was exhausted in 1947, fresh supplies were obtained from the same firm. Experiments showed that it was not possible to reproduce the results with the new sample of glycine. The results were equally unsatisfactory with samples of glycine obtained from other manufacturers. The unusually high concentration (2.5)% that had to be used in the medium for satisfactory

Section II

1. PROGRESS DURING 1957–67

The Pasteur Institute of Southern India, Coonoor, was registered under the Societies Act of 1860 on 2nd April 1907. It started functioning on 6th April and was officially opened on 25th April 1907 by His Excellency Sir Arthur Lawley, Governor of Madras.

Golden Jubilee Celebrations

The Golden Jubilee of the Institute was celebrated on 2nd April 1957. The Central Committee of the Institute had decided earlier that the best way to celebrate the occasion was to (i) construct additional laboratories and animal houses to be named 'The Golden Jubilee Block' to enable the Institute to undertake work on respiratory and intestinal viruses and thereby increase its sphere of usefulness to the country, (ii) bring out a souvenir giving an account of the origin, development and scientific contributions of the Institute, and (iii) revise the scales of pay of the staff and bring them into line with those obtained in other institutions in the country.

The construction of the laboratories and animal houses covering a floor space of 6,000 sq. ft. was started in January 1956 and completed by the end of March 1957. The old natural runs near the club room were demolished and in that area a storeyed building was put up. It consists of five animal houses and a post-mortem room on the ground floor facing one side, and a set up six rooms with an enclosed verandah on the top floor facing the opposite side and opening at ground level on the lawn. The laboratories are provided with arrangements for filtration of incoming air, ventilation by exhaust fans and sterilization by high temperature of the outgoing air likely to be contaminated with highly infective viruses. These were furnished and fitted up with a generous grant from the Ministry of Health, Government of India. The building named 'The Golden Jubilee Block' was declared open by the Hon'ble Rajkumari Amrit Kaur, Union Minister for Health, on 2nd April 1957.

A souvenir dealing with the origin, development and scientific contributions of the Institute and the new lines along which it could expand its activities and serve the country was brought out on the occasion.

Proposals regarding the revision of scales of pay were considered by the Committee earlier and approved. The President of the Association announced at the inaugural meeting that they would be given effect to from 1st April 1957.

A memorable event on the occasion was the magnificent gesture made by the staff of the Institute by donating a superb 400 watt mercury vapour lamp with fittings costing about Rs. 1,000 to illuminate the area around the Golden Jubilee Block.

The achievement of the Institute during the first 50 years of its existence has been presented in the Golden Jubilee Souvenir. Its progress during the past 10 years are briefly dealt with below.

Routine Work

Since 1957 the routine activities of the Institute have been steadily increasing.

The main function of the Institute is to produce antirabies vaccine for the treatment of persons bitten by rabid animals. The Institute now supplies vaccine to the Governments and private practitioners in the States of Madras, Mysore, Andhra Pradesh and Madhya Pradesh and the railways. Over 1,20,000 persons were treated with the vaccine supplied by the Institute during 1966 compared with 34,000 patients treated during 1957.

results together with the fact that solutions of the old sample were straw coloured suggested that the good results obtained were not due to the glycine *per se* but to some impurity or impurities present in it.

Since 1947, work has been in progress to determine the nature of the impurity. Based on the information from the manufacturers that glycine was produced by three synthetic methods during the years 1938–39, several compounds have been tried as possible impurities. Apart from this, the possibility of other factors being necessary for growth as well as the presence of some toxic components in the constituents of the medium now employed have been investigated. Since 1947 over 400 compounds comprising of proteoses, peptones, polypeptides, amino acids, trace minerals, metabolic intermediates, purines, pyramidines, nucleic acids, carbohydrates, organic acids, hormones and other substances have been tried in various concentrations and combinations as possible factors essential for growth. The results of these studies have been presented in the Scientific Reports of the Institute. None of the compounds tried had any beneficial effect on the multiplication of the virus *in vitro*. These studies are being continued.

Egg adapted Flury strains

Since 1960, studies have been in progress to cultivate the low and high egg passage Flury virus *in vitro*. A method has been developed for the cultivation of these viruses in chick embryo tissue cultures employing a synthetic medium without serum. The viruses can be subpassaged serially. Titres ranging from $10^{4.5}$ to $10^{6.0}$ per ml. with low egg passage virus and from $10^{5.0}$ to $10^{6.5}$ per ml. with high egg passage virus can be obtained by this method.

Effect of salts

Wallis and Melnick (1962) reported the stabilization of polioviruses by $MgCl_2$. $MgCl_2$ has been found to enhance the inactivation of measles virus at all temperatures, while $MgSO_4$ and $Na_2 SO_4$ protected the virus against thermal inactivation.

The stability of rabies fixed virus when mixed with various salts and heated at 56°C. for 15 minutes was investigated. It was found that only $Na_2 SO_4$ and KCl appeared to afford some protection to the virus against thermal inactivation.

Reduction of nitrocompounds by rabies fixed virus

It is known that chloramphenicol has no value against rabies infection. It was considered possible that the ineffectiveness of the antibiotic might be the result of the reduction of the nitro group to an amino group and the formation of an inactive substance.

Preliminary studies were carried out with nitrobenzene and m-nitrobenzene and infected guinea-pig brain tissue. It was found that these compounds were reduced to the amino compounds, the formation of which could be studied spectrophotometrically.

Amine formation was greatly affected by the pH, and the formation of two peaks in the pH curve suggested that the reaction might be enzymatic. The one at lower pH might be xanthine oxidase which appeared constantly in large quantities in the rabies virus infected brains. The largest quantity of amine was formed at alkaline pH above 9.0 with glycine buffer compounds containing the–SH group like thioglycollic acid, mercaptoethylamine and cysteine and sodium chloride facilitated amine formation. Among purines and pyramidines, it was found that some compounds favoured while others inhibited amine formation.

The demand for antirabies vaccine for the treatment of exposed animals (cattle, dogs, etc.) has also increased. The Institute now supplies about 5,000 courses of vaccine for their treatment.

The Institute serves as a first class medical diagnostic laboratory in the area. It undertakes all bacteriological, serological and biochemical investigations for the hospitals and practitioners in the Nilgiris and surrounding districts. The work in this section has enormously increased during recent years.

The Institute helps the State Government and the local public health authorities by storing and distributing vaccines. The facilities at the Institute are made available to the health authorities during epidemics.

Research work

In view of its chronic financial difficulties the Institute has depended to a large extent on financial assistance from other sources for its research programmes. From 1953–59 an enquiry on rabies sponsored by the Indian Council of Medical Research was functioning at the Institute. Also, from 1956 two enquiries, one on cholera and the other on syphilis, were undertaken on behalf of the Madras State Research Committee. The Government of India Influenza Centre, which was started in 1950, continues to function at the Institute.

The Institute has always believed in developing its work around men. It has been its policy to select workers with ability and initiative, give them the basic training and allow them to specialize in a field in which the Institute was interested. After specialization they were given all facilities to develop the particular line of work and in turn train other workers.

Dr. P. V. Kurian, who had received special training for two years in tissue culture and the adeno group of viruses at Michigan, returned in July, 1956. Dr. A. Balasubramanian, who was sent to Canada under a Colombo Plan Fellowship, returned in December 1956 after specialization in the intestinal group of viruses and the tissue culture techniques used in the preparation of poliomyelitis vaccine.

With the availability of the above trained personnel and the opening of the Golden Jubilee Block specially designed for the purpose, it was possible to start work on respiratory and intestinal viruses. The Respiratory and Intestinal Viruses Unit financed by the Indian Council of Medical Research started functioning at the Institute from April 1957. Also, an enquiry on influenza supported by the Indian Council of Medical Research started working under the late Dr. I. G. K. Menon during the year.

The pioneering work undertaken by the Institute in the fields of influenza and intestinal viruses proved to be very valuable. The services rendered by the Institute to the country during the pandemic of influenza in 1957, the epidemic of encephalitis in Delhi in 1958, encephalitis epidemic in Nagpur and surrounding areas in 1959, the polio epidemic in Andhra Pradesh in 1961 and the outbreaks of polio in Palghat and Coimbatore in 1964 and in Dhalavoipuram in 1965, are well known.

On 28th March 1958, our revered Prime Minister the late Shri Jawaharlal Nehru visited the Institute and took a keen interest in its activities. He very kindly took up with the Ministry of Health, Government of India, the question of providing regular financial assistance to the Institute.

As a result, from 1st July 1959, the Ministry of Health took over the financial commitments in respect of all the research activities of the Institute including those formerly sponsored by the Indian Council of Medical Research and the Madras State Research Committee. They sanctioned the payment of a capital grant for

(4) DIAGNOSIS

Fluorescent antibody technique

The Institute was the first to employ the fluorescent antibody technique for the diagnosis of rabies infection in the country. Studies on this important subject have been in progress since 1961.

Till 1964, lissamine rhodamine B 200 was the fluorochrome used to conjugate hyperimmune antirabies serum for use in the test. Since 1965, fluoroscein isothiocyanate has also been employed. Comparative studies showed that there was no significant difference between lissamine rhodamine B 200 and fluorocein isothiocyanate when used as fluorochromes to conjugate antirabies serum in the diagnosis of rabies.

(ii) In a study to assess the value of flluorescent antibody technique in the diagnosis of rabies, smears of the brains of animals suspected to have died of rabies infection were examined by fluorescent microscopy and the results compared with those obtained by stained smears (Seller's method) and section (Mann's method) of the brains. It was found that out of 3,577 specimens examined 2,364 (66.1%) were detected by fluorescent antibody technique, while 1,717 (48.0%) were detected by smear or section.

The results of examination by the fluorescent antibody technique were compared with the results of animal inoculation tests in 101 untreated guinea-pigs inoculated with rabies street virus. The fluorescent antibody test was positive in 73% of the brains, while the biological test proved to be positive in 59%. Both the tests were negative in 23% of the brains.

Out of 798 experimental guinea-pigs, which died in spite of treatment with various vaccines and antirabies serum, the brains of 353 (44%) animals were positive by the fluorescent antibody while 268 (33%) were positive by the biological test. The brains of 189 (23%) animals were negative by both the tests.

It appeared that in the cases of smears from treated animals fluorescent antibody technique revealed also non-infective antigenic material. It was found that smears from uninfected animals, whether treated or not, were uniformly negative.

An attempt was made to study the incidence of salivary gland excretion of the virus in experimental guinea-pigs. A total of 2,408 animals was studied. Of these, 1,878 had received different vaccines with or without antirabies serum, and 81 had received antirabies serum alone. The number of untreated controls was 449.

It was found that rabies virus could be demonstrated by the fluorescent antibody technique in the salivary glands of 13% to 43% of animals treated with pooled phenolized vaccine, 50% to 60% of animals treated with duck embryo vaccine, and 45% to 73% of animals treated with high egg passage (HEP) Flury vaccine (Lederle). The percentage was only 10% to 29% in animals treated with low egg passage (LEP) Flury culture vaccine, and 22% to 7% in those treated with HEP Flury culture vaccine. About 57% of animals given antirabies serum alone showed the presence of virus in the salivary gland compared with 31% of the untreated controls.

(iii) Recently reports have appeared as to the possibility of non-specific immunofluorescence when higher concentrations of the dye are used for conjugation or when the excess of unconjugated dye is not completely removed. Also, newer methods of conjugation as well as removal of the excess fluorochrome have been described.

Systematic studies were undertaken to determine whether the specificity and the sensitivity of the fluorescent antibody technique could be improved by (a)

the expansion of the laboratories and a recurring grant for the following research programmes: (i) Studies on the value of antirabies serum and its production; (ii) Studies on rabies; (iii) Studies on influenza; (iv) Studies on respiratory and intestinal viruses; (v) Studies on vaccinia and smallpox; and (vi) Studies on cholera. All the above enquiries started functioning under the Director with effect from 1st July 1959 and are being continued.

The research work of the Institute during the past 10 years is presented elsewhere in the souvenir. Some of the important contributions are dealt with below.

Rabies

The Institute is internationally recognised for its research work on rabies and is now serving as the International Reference Centre on Rabies of the WHO. It has been actively collaborating with the WHO in its research programmes on rabies since 1952 and with the Indian Council of Medical Research since 1953.

From its inception it has been studying the epidemiology of the disease in the country. Since 1961, newer methods of diagnosis like the fluorescent antibody technique have been introduced and are being evaluated. A continuous attempt is being made to improve the antirabies treatment in man. As a result of these studies it has been possible to produce a vaccine of uniform potency by pooling several batches of antirabies vaccine. This method, which was introduced at the Institute in 1958, is being adopted now by all the centres producing the vaccine. Work is in progress to determine whether the immunizing value of this vaccine could be further improved by substituting inactivating agents like β-propiolactone in the place of phenol used at present. The incidence of post-treatment complications with the vaccine supplied by the Institute is very low (1 in 16,713) and it is found that patients with serious involvement could be saved by the prompt administration of corticosteroids. Nevertheless, attempts are being made to produce a vaccine free from the paralytogenic factor which could be used with safety among patients allergic to nerve tissue vaccine. Experimental work indicates that a vaccine prepared from infected suckling mouse brains might prove useful for this purpose. An evaluation of the duck embryo vaccine extensively used in the United States indicated that the protection conferred by it was inferior to that produced by pooled 5% sheep brain vaccine.

Extensive investigations carried out at the Institute established the value of antirabies hyperimmune serum in the treatment of severely exposed persons. It is found that serum by itself is not of much value. But, given in small doses of 5 international units per pound of body weight, as an adjunct to vaccine, it affords excellent protection in severely bitten persons. The optimum conditions for the administration of serum have been worked out and it is being regularly used in the treatment of persons at grave risk since 1956.

With a view to improve the present vaccine work has been in progress since 1937 to grow the rabies virus in tissue culture. As a result of these investigations it is now possible to grow in chick embryo tissue cultures the egg adapted Flury strains of rabies virus employing a synthetic medium without serum. These tissue culture vaccines, which contain live virus, have been found to be excellent immunizing agents in dogs. An important finding has been that one dose of the high egg passage Flury tissue culture virus together with a dose of antirabies serum gives excellent protection in guinea-pigs comparable to that conferred by 14 doses of pooled 5% sheep brain vaccine and serum. Since the high egg passage Flury virus can be administered to man this observation suggests the possibility of treating man with one dose of tissue culture virus and a dose of serum instead of the 14 doses of large

decreasing the concentration of fluorochrome used, (b) adopting different techniques for conjugation, and (c) eliminating the unconjugated dye by various methods. As a result of these investigations it has been found possible to improve the specificity of the technique for the diagnosis of rabies.

(6) ANTIRABIES VACCINE

Strains of the rabies fixed virus

The Kasauli strain of rabies fixed virus was employed at the Institute from 1907 to 1930 for the preparation of antirabies vaccine. Following the observation that the Paris strain was antigenically superior to the Kasauli strain the Institute started using the Paris strain of fixed virus from May 1931.

Animals

Rabbit has been the animal of choice ever since Pasteur's work and was used at the Institute for the preparation of vaccine till the beginning of 1930. As demands for antirabies vaccine increased and since it was not possible to obtain rabbits readily in large numbers the Institute started using sheep for the manufacture of antirabies vaccine during 1930 and it continues to be the animal of choice even to-day.

Types of vaccine

Glycerinated cord. Cornwall started antirabies treatment at the Institute on 6th April 1907. He considered that Pasteur's dried cord method had numerous disadvantages and that Hogyes dilution method was almost from every point of view the best in vogue at that time. As the number of patients expected during the first year was small and as Hogyes method entailed the use of at least one rabbit daily, a start was made by Cornwall using glycerinated material. The cords were preserved in glycerine in the ice box till required for use. There was thus little waste of material. Glycerinated cord was used till 15th November 1908 and 404 persons were treated by that method.

Hogyes dilution method. This method was started from 16th November 1908 when the patients became more numerous. Cornwall considered that there was no good reason for following the complex and troublesome scheme adopted by Hogyes. He states: 'There appeared to be no good reason for following the complex scheme adopted by Hogyes which was based on the almost equally complex scheme of Pasteur. There was no real justification for the different doses in Pasteur's scheme. He was dealing with a material he knew very little about and he might just as well have drawn up a simple scheme at once. Hogyes followed him, making the complex more complex.' Cornwall was of the opinion that Hogyes dilutions were too high, and his course of treatment too long. He, therefore, employed a scheme with a shortened period and lower dilutions. Good results were being obtained when an unfortunate failure occurred after treatment in a person who had only been licked. This death was somewhat surprising and it was even suggested that he had been given the disease by the treatment. It later transpired that the patient had been bitten by a rabid dog several weeks before the lick and that he had been advised by a physician to proceed to Coonoor for treatment which he neglected to do. But the damage had been done and the dosage had to be reduced from 15th November 1910. This treatment was continued till 31st January 1912 and whether by accident or not the results of failures during this period doubled viz., 0.69% compared with 0.30% obtained with the higher dosage schedule. It was Cornwall's opinion, based on considerable experimental work, that though the possibility of inducing rabies by Hogyes method could not be denied, yet the results obtained by the method, not only in India but in Europe, were good and that living virus was a better immunizing agent than dead virus.

quantities of vaccine now being administered.

Attempts are regularly being made for improving the method of testing the potency of antirabies vaccine and a protection test in guinea-pigs has been developed which, while simulating natural conditions, is found to give reliable results.

Recently there have been reports of persons bitten by apparently healthy dogs developing the disease and the possibility of dogs suffering from inapparent rabies infection with transient viraemia has been postulated. Extensive investigations carried out on a dog, (brought from a village named Surandai in Tirunelveli district on 1st January 1966) which was responsible for the death of a boy from hydrophobia, have revealed that apparently healthy dogs can excrete the rabies virus. But, this should be considered as very exceptional since studies on a large number of stray dogs in the cities of Madras and Delhi did not reveal the presence of salivary excretion of the virus among them.

Influenza

The Institute is the main laboratory for work on influenza in the country. The Government of India Influenza Centre was established at the Institute in 1950 with the financial support from the Government of India and with a gift of valuable equipment from the WHO. Influenza viruses were isolated for the first time in India at the Centre by the late Dr. I.G.K. Menon, type A prime virus in 1950 and type B virus in 1951. The Indian Council of Medical Research supported an enquiry on the antigenic analysis of Indian strains of influenza virus at the Centre for 3 years from 1952–55. The valuable information obtained from this enquiry as well as the epidemiological studies led to the setting up of a pilot plant for the production of influenza virus vaccine in 1954 Dr. Menon was the first to isolate the Asian virus in the country from the throat gargles of the first cases of Asian influenza who reached Madras harbour on 16th May 1957 from Malaya by the ship s.s. *Rajula*. His extensive and thorough studies on the various aspects of the Asian pandemic of influenza which swept throughout the country during a brief period of 12 weeks constitute a classical contribution on the subject. His investigations on the antiviral properties of iodine led to the use of Mandl's solution containing iodine for painting throats of susceptible persons as a measure of prophylaxis against influenza as well as the administration of collosol iodine in the treatment of cases with serious manifestations. With the possibility of the second wave of influenza striking the country looming large, the Ministry of Health desired that the Institute should produce sufficient vaccine at least to protect essential personnel. Over 50,000 doses of vaccine were produced and distributed to the various State Governments and administrations.

Since 1959, an attempt is being made to study the incidence of the disease in the Nilgiri district by virus isolation throughout the year from cases showing evidences of respiratory infection. These investigations indicate that the two- or three-year cycle usually regarded as typical of influenza type A epidemics does not occur. A remarkable finding has been the total disappearance of the earlier virus with the arrival of an antigenically different strain. Generally, there is an increased incidence of the disease in the Nilgiris in the second half of the year particularly in the months September to December with a strain which has established itself in the community. But, a totally new strain against which the population has no antibody could appear and cause extensive outbreaks even during the hottest part of the year. While the isolation of type A viruses varied from year to year it has been possible to isolate type B viruses from the community every year if attempts are made throughout the year. The biological characteristics of all the

Carbolized vaccine. In 1911 Semple published his work on carbolized vaccine and the Institute started using carbolized 1% rabbit brain vaccine from 1st February 1912. During the years 1930-31, carbolized 1% sheep brain vaccine was employed. Based on reports that an increase in the concentration of the brain tissue in the vaccine gave better results the Institute tried out 1, 2 and 5% sheep brain vaccine during 1932. Since 1933, only 5% sheep brain vaccine has been used.

Potency tests

The guinea-pig protection test (Veeraraghavan) was developed at the Institute during the course of investigations on the efficacy of treatment with serum and vaccine. It was found that 7 daily doses of 0.3 ml. of a potent vaccine administered for 7 days before peripheral challenge with rabies street virus conferred excellent protection.

Several experiments were carried out to determine the relative values of the guinea-pig protection test and the NIH, Habel and Nylar tests, in assessing the potency of antirabies vaccines.

It was found that vaccines which passed the guinea-pig protection test gave very good results in post-infection treatment of guinea-pigs. This test had the advantage that the challenge procedure simulated natural infection, and that, on a weight basis, the amount of vaccine administered was not unduly large, being only twice as much as that corresponding to a class III schedule used in human treatment.

In the NIH test, a challenge over 5 LD_{50}, very often proved to be overwhelming. But, the results of the test, whenever the end points could be determined, were satisfactory.

An interesting observation was that higher protection values could be obtained in the Habel test if a strain of fixed virus antigenically similar or close to the CVS fixed virus was used in the preparation of the vaccine.

The Nylar test appeared to be a good test for determining the potency of LEP and HEP Flury vaccine produced in embryonated chicken eggs or in chicken embryo tissue culture. The test could also be used for testing inactivated vaccines like Semple vaccine.

(7) ANTIRABIES TREATMENT

The Pasteur Institute, Coonoor, was until 1922 the only place in South India where patients could receive antirabies treatment. In 1922, the Institute was the first to decentralise antirabies treatment in India by opening a few subsidiary treatment centres. This was possible as a result of extensive investigations carried out by Cornwall (unpublished data) on the keeping qualities of the vaccine. These findings were later confirmed by Gloster *et al.* (1925). The results obtained were so successful that a policy of further decentralisation was pursued, with caution up to 1938. In 1939 the issue of antirabies vaccine was extended to registered medical practitioners for the treatment of their private patients. With this step decentralisation of antirabies treatment was complete. Large numbers of hospitals, dispensaries and registered medical practitioners in the States of Madras, Andhra Pradesh, Mysore and Madhya Pradesh receive their supplies of vaccine from the Institute. The Institute now issues yearly more than 1,00,000 courses for the treatment of human beings. A satisfactory organization exists for the collection of statistical data.

Results of treatment, 1907–65

During the years 1907 to the end of 1965 the total number of patients treated at the Institute and the subsidiary treatment centres was 10,14,157. Of these, complete case records are available for 5,96,705 patients, i.e., 58.8% of cases treated. An analysis of the data based

isolated strains of influenza as well as their antigenic analysis are being investigated regularly. It has been found that there has been an antigenic shift in the recently isolated type A and type B viruses.

Different methods in vogue for the production of influenza vaccine from infected eggs have been compared and a simple method has been evolved for obtaining a relatively pure virus for making a better vaccine. As a result of extensive studies a method has been developed for the cultivation of influenza viruses in chick embryo chorioallantoic membrane tissue cultures using a synthetic medium. This method which yields pure virus in high titres is found to be highly economical and well suited for the production of large quantities of vaccine in an emergency.

Respiratory viruses

The Institute is the main centre for the study of the other respiratory viruses in the country. The investigations during the past 10 years have revealed the widespread incidence of adenoviruses in the community, particularly among children These viruses, while they do not generally cause mortality, are responsible for a considerable morbidity among the population. It is found that about 30% of all sera received at the Institute for various investigations contain antibodies against these viruses indicating their wide prevalence. During 1961–63 there were extensive outbreaks of respiratory illness among recruits in a military establishment nearby. Investigations undertaken by the Institute revealed that the outbreaks were caused by adenovirus type 21. Apart from adenovirus infections, those caused by the parainfluenza and the syncitial group of viruses and mycoplasma are being studied. The infections due to these viruses, though not very common, are found to occur in small outbreaks occasionally.

Intestinal viruses

The Institute is now serving as an important centre for research on intestinal viruses.

The role of enteroviruses in the occurrence of encephalitis among children is under constant study. The Institute investigated the epidemic of encephalitis among children in Delhi in 1958 and that in Nagpur and surrounding areas in 1959. It has also studied minor outbreaks in Madras, Mysore and the Nilgiris.

Studies on poliomyelitis have been in progress at the Institute continuously since 1957. It investigated the polio epidemic in Andhra Pradesh in 1961 and the outbreaks of polio in Palghat and Coimbatore in 1964 and in Dhalavoipuram in 1965. All these outbreaks were due to type 1 poliovirus and affected children mostly in the age groups 6 months to 4 years. Since 1960 basic data are being collected at Coimbatore on the incidence of poliomyelitis, the types of viruses prevalent, the age at which the children come in contact with the virus as judged by the appearance of antibodies, and the other enteroviruses prevalent among them. These investigations indicate that the susceptible age to infection with poliovirus type 1 is upto 7 years and that with types 2 and 3 upto 6 and 8 years respectively. The children appear to be most susceptible from 6 months to 2 years of age.

In view of the increasing incidence of polio among children, the Ministry of Health, Government of India, decided to start the production of poliovaccine in the country. They considered that live poliomyelitis vaccine (Sabin) would be the most suitable for the purpose and requested Dr. A. B. Sabin to visit the different laboratories in the country and recommend an institution where his vaccine, which contains live virus and is recognized to be a difficult one to make, can be produced. Dr. Sabin, who was so impressed with the laboratory facilities

upon the strain of rabies virus used, the animal employed for the preparation of the vaccine, and the concentration of brain tissue in the vaccine, is presented in Table 2.

TABLE 2

Results of antirabies treatment at the Pasteur Institute, Coonoor, and its centres from 1907–'65

Year	Fixed virus	Type of vaccine	Animal	Completely treated		Incompletely treated	
				No. treated	Mortality %	No. treated	Mortality %
1907–08 ...	Kasauli	Glycerinated cord	Rabbit	522	0.96	...	...
1909–12 ...	,,	Hogyes'	,,	3,665	1.20	...	...
1913-29 ...	,,	Semple 1%	,,	60,877	0.99	1,015	1.38
1930–31 ...	Paris	,, 1%	Sheep	15,421	0.60	2,067	0.77
1932	,,	,, 1-5%	,,	9,770	0.30	1,457	0.75
1933–55 ...	,,	,, 5%	,,	2,36,391	0.11	83,814	0.26
1956–65 ...	,,	,, 5%	,,	1,09,942	0.06	71,764	0.08

It will be seen from these data that among the different types of vaccine used at the Institute during the 59 years the best results have been obtained with carbolized 5% vaccine prepared from the brain of sheep inoculated with the Paris strain of rabies fixed virus.

Evaluation of the results of antirabies treatment

Recently considerable amount of work has been reported on the immunizing value of various types of antirabies vaccine using the mouse test developed by Webster and Habel. The results of these experiments and the studies on the active immunization of experimental animals make some workers doubt the efficacy of phenolized vaccines used in the treatment of persons bitten by rabid animals. Ordinarily an evaluation of the statistical data relating to the mortality among those treated should give a clear indication as to the value of treatment. But, in rabies the position is complicated by the important observation that certain animals, proved rabid by laboratory tests, bite many people without infecting them while others infect a majority of persons whom they bite. This may be due to variation in the virulence of the strains of rabies virus or the virus not being present in the saliva of the rabid animal during its biting period. It is, therefore, extremely difficult to estimate correctly in any particular instance the degree of risk run by a bitten person as well as the chances of his developing the disease. This uncertainty leads to a large number of persons, who probably are not at risk, being given antirabies treatment. Consequently, the figures for mortality from rabies in treated persons is extraordinarily low.

In these circumstances the only way of estimating accurately the influence of prophylactic treatment in preventing the onset of rabies is that advocated by Cornwall (1923). He suggested that for a correct assessment of the value of treatment it was necessary to study the fate of a number of similar groups of persons bitten by rabid animals, the infectivity of each animal at the time of biting being proved by the subsequent death from hydrophobia of at least one of the persons bitten by them. An evaluation of the mortality rate among the treated and untreated in these groups would then give an accurate index of the value of treatment. Cornwall considered such data difficult to obtain. Since 1946, these data are being collected by Veeraraghavan. The mortality rates among the treated and untreated in 348 groups of persons bitten by proved infective

at the Institute and the pioneering work already done by it in the field, recommended to the Ministry of Health that the Pasteur Institute, Conoor, was ideally suited for the purpose. As desired by the Ministry of Health, the Institute agreed to produce the vaccine for the protection of 50 to 60 million susceptible children in the country. As it was for a very noble cause the Institute agreed to prepare it and hand it over to the Ministry of Health for distribution to the various State Governments at cost price.

With generous assistance from the Ministry of Health, the laboratories for the purpose have been built and furnished. The imported as well as the indigenously available equipment required for the work are provided by the USAID Mission, New Delhi, while other locally available items of equipment, staff and other expenses are met by the Ministry of Health.

The Polio Vaccine Unit was inaugurated by the Hon'ble Dr. Sushila Nayar on 2nd July 1966. The Hon'ble Sri M. Bhaktavatsalam, Chief Minister of Madras, presided over the function.

The three types of seed viruses to be used in the production have been prepared and tested at the Institute. Through the good offices of the WHO, the Medical Research Council Laboratories, London, have retested the batches and declared them as suitable. Since the receipt of the final report in June 1967, the actual production of polio vaccine has commenced. As a measure of abundant precaution the first batches of vaccine are being retested by the Institute of Poliomyelitis and Viral Encephalities, Moscow, and the Haffkine Institute, Bombay.

Vaccinia

A considerable amount of work has been done on vaccinia virus and a method has been developed for the cultivation of the virus in sheep kidney cell cultures. Good concentrations of the virus are obtained and the culture virus is found to be highly immunogenic. This should prove useful in vaccination against smallpox.

Trachoma

Studies are in progress on trachoma. Methods have been standardized for the isolation of trachoma virus from infected cases and attempts are being made to adapt the strains to different cell cultures with a view to produce a vaccine against this common condition which leads to blindness.

Other viruses

Investigations are also in progress on herpes simplex virus, measles and other simian viruses.

Tissue culture

During the past 10 years the Institute has developed excellent facilities for tissue culture work. It has improved the methods of cultivation, maintenance and storage of cell cultures obtained from kidneys and other organs of different animals. It also maintains a variety of standard cell lines used in virological work.

Bacterial diseases

In addition to the study of virus diseases considerable amount of work is being done on bacterial infections. These include cholera, enteric fevers, syphilis, diptheria, tuberculosis and leprosy. The investigations on cholera include the development of better methods for the isolation and identification of the vibrio and the pathogenesis of the disease. All available evidence indicates that pentamethylenediamine, a chemical elaborated by the cholera vibrio acting on the mucous membrane of the intestine, may play an important role in the production of symptoms in

animals during the past 20 years have been studied. The results could be summarized as follows:

Number of persons bitten ...	998	
Number completely treated ...	738	
Deaths from rabies among the completely treated ...	57	(7.7%)
Deaths 15 days after the completion of treatment (treatment failures) ...	28	(3.8%)
Number incompletely treated ...	61	
Deaths from rabies among the incompletely treated ...	6	(10.0%)
Number who died during treatment ...	18	
Number untreated ...	180	
Deaths from rabies among untreated ...	100	(55.3%)
Number untreated and those who died during treatment ...	198	
Deaths from rabies among the untreated and those who died during treatment ...	118	(59.6%)

Taking into consideration the mortality rate of 55.3% among the untreated, it would appear that the 5% Semple vaccine used at the Institute saves 14 out of 15 persons bitten by proved infective animals.

Cornwall considered that a less accurate, but still useful way of arriving at a valuation of antirabies treatment was to take only those instances in which a presumably rabid dog has bitten several persons, of whom some accept treatment and some refuse. If both groups are observed for several months it should be possible to assess the value of treatment.

Cornwall (1923, 1924b) following these lines of enquiry for over 12 years (1912–24) obtained the following data which are unique in the literature on antirabies treatment. As Otten (1941) points out 'they are the only extensive and exact investigations made in the field, which certainly deserve a better fate than to lie buried in the Annual Reports of the Pasteur Institute of Southern India at Coonoor'.

Cornwall found that out of 2,217 persons bitten by presumably rabid animals, 829 received treatment. Among them 24 (2.9%) died of hydrophobia while among the 1,388 untreated, 88 (6.3%) died. He also showed that in Southern India about 35% of persons bitten by rabid dogs, which were actually infective, died of rabies if untreated. He came to the conclusion that treatment with the 1% Semple vaccine then in use was able to save one out of every two persons who would otherwise have died of the disease.

An enquiry on these lines has also been in progress at the Institute since 1946. The main object of the enquiry is to determine whether there is any variation in the mortality rates as a result of treatment with 5% Semple vaccine instead of the 1% vaccine used by Cornwall. The mortality rates among the treated and untreated bitten by presumably rabid animals have been reported by Veeraraghavan.

The results could be summarized as follows:

Number of persons observed ...	17,569		
Number completely treated ...	15,633		
Deaths among completely treated ...	57	(0.31%)	
Deaths 15 days after the completion of treatment (treatment failures) ...	28	(0.18%)	
Number incompletely treated ...	1,301		
Deaths among incompletely treated ...	6	(0.46%)	
Number who died during treatment ...	18		
Number untreated ...	616		
Deaths among untreated ...	100	(16.23%)	
Number untreated and those who died during treatment ...	634		
Deaths among the untreated and those who died during treatment ...	118	(18.61%)	

cholera. In syphilis, the fluorescent antibody technique has been developed for a reliable diagnosis of the condition.

Buildings and improvements

The construction af the Golden Jubilee Block was started in January 1956 and completed in March 1957. The cost of building with a total floor space of about 6,000 sq. ft. was Rs. 73,243. The water supply, drainage and septic tank cost Rs. 5,388 and the room to house the gas plant Rs. 1,130. The charges for the electrification of the building together with that of exhaust fans etc., was Rs. 12,022, while the amount spent on laboratory furniture was Rs. 6,535. The Ministry of Health made a generous grant of Rs. 80,230 for equipping these laboratories.

A building meant to serve as Post Office and garage was constructed near the rear gate of the Institute at a cost of Rs. 7,300 in 1957. The Post Office started functioning on 2nd January 1958.

The rooms in the main building vacated by the Nutrition Research Laboratories were reconditioned, furnished and fitted up during 1958. The cost of re-laying the flooring with linoleum was Rs. 3,826, while that of rewiring the rooms and the purchase of electrical fittings amounted to Rs. 1,562. A part of the expenditure in connection with the redoing of the laboratories was met from a grant of Rs. 6,000 kindly made available by the Government of Madras.

The large hall used as the museum by the Nutrition Research Laboratories was completely renovated by re-laying the floor with mosaic tiles, repairs to the roof, and repainting of the ceiling, doors and windows during 1959. The expenses incurred in this connection were met from a grant kindly made available by the Government of Madras.

The construction of the new laboratories at the Institute, which was started during the end of 1960, was completed by the beginning of 1963. The laboratories are designed for the routine production of live poliovirus vaccine and influenza or any other egg vaccine. They consist of sub-basement and basement floors, ground floor, first floor and second floor. In addition, provision has been made for the construction of a third floor at a later date. It has an auditorium in the ground floor and two museum halls, one in each of the first and second floors. The laboratories have been completely fitted up. There are 6 hot rooms, 4 cold rooms and 3 deep-freeze (-20°C.) rooms. An electric lift has been provided to serve the various floors. A generator set has been installed exclusively for the laboratories to ensure continuous supply of electricity in the event of power failure.

The expenditure for the construction and fitting up of the laboratories under various heads were as follows: (i) general builders works, Rs. 8,95,992.31; (ii) sanitary installations, Rs. 90,278.10; (iii) electric installations, Rs. 1,46,908.84; (iv) electric lift, Rs. 37,916.31; (v) 6 hot rooms, 4 cold rooms and 3 deep-freeze rooms, Rs. 1,78,124.32; (vi) laboratory work tables, Rs. 15,835.22; (vii) steel furniture, Rs. 27,871.25; (viii) generator set (inclusive of the cost of construction of generator room), Rs. 22,933.55 and architect's fee, Rs. 66,955.08. The total cost amounted to Rs. 14,81,814.98,which was met from a generous grant made available by the Ministry of Health, Government of India.

The construction of the monkey house to quarantine 100 monkeys at a time for use in the production of poliovaccine was started in January 1964 and completed in August 1965. The cost of the entire construction, inclusive of water supply, sanitation and electrification amounted to Rs. 64,270.69 and was met from a generous grant made available by the Ministry of Health.

In view of the ever increasing demand for antirabies vaccine, the room in the

It will thus be seen that the treatment failures in this study are only 0.18% compared with 2.9% reported by Cornwall for a similar group. This would indicate that the results of treatment with 5% Semple vaccine used now at the Institute are superior to those obtained with the 1% Semple vaccine used by Cornwall during the years 1912–24.

Post-treatment complications

(i) Mild reactions

The reactions following antirabies treatment were carefully studied at the Institute during the years 1907–18 and were described by Cornwall (1918). The main reactions observed by him in the order of frequency were:

(a) A local cutaneous and subcutaneous tissue reaction between the 6th and 11th days.

(b) General dermal reaction — an urticarial or papular rash.

(c) General systemic reaction in the form of malaise.

(d) A reaction of the central nervous system, also between the 6th and 11th days, in the form of temporary heart failure, occipital pain, nausea or vomiting or perhaps involuntary micturition. Thirty such cases were observed by him among 14,000 patients.

(ii) Neuroparalytic accidents

Such complications were not encountered during the years 1907–12 when the glycerinated-cord or Hogyes' method of treatment was employed; nor did they occur during the years 1913–31, when the Kasauli strain of rabies fixed virus was used and the vaccine was a phenolized 1% suspension of infected rabbit or sheep brain. There was no case of neuroparalytic accident during the year 1932, when there was change over to the Paris strain of fixed virus and trials were being made with 1%, 2% and 5% sheep brain vaccine. The first case of post-treatment complication occurred in 1933, when the Institute started using 5% sheep brain vaccine regularly. Among the 5,01,400 patients who received a complete or incomplete course of treatment during the period from 1933 to the end of 1965, and for whom complete case records are available, 30 patients developed post-treatment complications, giving a rate of incidence of 1 in 16,713. The details are given in Table 3.

TABLE 3

Post-treatment complications

Year	Fixed virus	Type of vaccine	Animal	Number treated	Post-treatment complications
1907–08 ...	Kasauli	Glycerinated cord	Rabbit	522	nil
1909–12 ...	,,	Hogyes	,,	3,665	nil
1913-29 ...	,,	Semple 1%	,,	61,892	nil
1930–31 ...	Paris	,, 1%	Sheep	17,508	nil
1932 ...	,,	,, 1–5%	,,	11,227	nil
1933–65 ...	,,	,, 5%	,,	5,01,400	30 (1 in 16,713)

In 29 cases the accident occurred between 11 and 37 days after the commencement of treatment, and in 1 case 74 days after. One case occurred among those receiving 2 ml. of vaccine daily, and 20 among those receiving 5 ml. daily. Only 9 cases were reported among those receiving 10 ml. doses. The various types encountered were: encephalomyelitic, 2; dorso–lumbar, 16; Landry's, 11 and neuritic, 1. Three cases with dorso-lumbar involvement and 9 with Landry's



INSTITUTE GATE DECORATED FOR GOLDEN JUBILEE



RAJKUMARI AMRIT KAUR ARRIVING TO INAUGURATE
GOLDEN JUBILEE CELEBRATIONS

paralysis died. It is interesting to note that both the encephalomyelitic cases occurred among patients who had a second course of antirabies treatment. The interval between the first and second course was 10 months in one case and 7 years in the other.

It will thus be seen that the occurrence of neuroparalytic accidents is sharply limited to the period when 5% Semple vaccine was used for treatment. Fortunately, this rate of incidence is very small and compares favourably with those reported from other parts of the world.

(iii) Treatment with antihistaminics, ACTH and cortisone

Antihistaminics have been used in treating cases of neuraparalytic accidents. According to recent reports very good and, sometimes, even dramatic response may be obtained among patients with most serious involvement by the timely administration of ACTH or cortisone. One case of dorso-lumbar paralysis treated with pyribenzamine recovered. Also, one case of dorso-lumbar involvement treated with ACTH and five treated with cortisone made complete recovery.

(8) STUDIES ON THE IMPROVEMENT OF ANTIRABIES VACCINE

An important line of work in progress at the Institute since its inception is to improve the antirabies vaccine used in treatment. Antirabies vaccines at present in use are crude suspensions of the brain from sheep or rabbits in the late stage of infection with rabies fixed virus. The vaccine commonly used in India is a 5% suspension of fixed virus sheep brain in carbol-saline prepared according to the method introduced by Semple. The chief defects with the vaccine are: (a) it contains a large amount of nerve tissue which is unlikely by itself to have any antigenic value, and (b) the vaccine is administered in large doses over long periods (upto 10 ml. daily for 14 days), and it is, therefore, cumbersome from the point of view of the patient. Also, the injection of large quantities of nerve tissue might be responsible for the occurrence of neuroparalytic accidents. The important problem in rabies is to evolve a refined vaccine which can be given in smaller doses over a shorter period of time and which will be free from possible bye-effects such as paralytic accidents.

Semple vaccine prepared in distilled water

It was reported by D'Silva *et al* (1951) that when distilled water was substituted for normal saline in the preparation of 5% Semple vaccine the antigenicity of the vaccine was considerably increased. With a view to assess this claim the Institute supplied 5% Semple vaccine prepared in distilled water from 1 March 1954 to 31 October 1954. In view of the comparatively high incidence of paralytic accidents following treatment with vaccine prepared in distilled water its use was discontinued from 1 November 1954. The mortality among those treated with this vaccine and those who refused treatment was investigated. These included groups of persons bitten by infective, proved or presumably rabid animals. The results did not appear to be strikingly different from those obtained among persons treated with vaccine prepared in normal saline.

It was also not possible to demonstrate in animals that vaccine prepared in distilled water immunized more quickly and gave better protection in the post-infection treatment of animals compared with vaccine prepared in normal saline.

Pooled antirabies vaccine

Investigations at the Institute indicated that there was considerable variation in the antigenicity of batches of antirabies vaccine even when the different variables were controlled. For example, batches of vaccine prepared from sheep, inoculated with an equal quantity of the same virus suspension and having the same



RAJKUMARI AMRIT KAUR INAUGURATING GOLDEN JUBILEE CELEBRATIONS

incubation period and duration of illness, were found to vary not only in virus content but also in immunizing potency. It was also found that the antigenic value was not always related to the virus content of the brain from which it was prepared. The antigenicity of some of the batches were very good in comparison with the reference vaccine, while that of others were poor. In countries like India, where rabies is widely prevalent and where it is not uncommon for an Institute to use 75–100 sheep a week to meet the demands for antirabies vaccine, it would obviously be impracticable to test the antigenic potency of each and every batch of vaccine prepared from a sheep brain, even if facilities for doing so should exist. It was, therefore, obvious that a method should be evolved by which a vaccine of uniform potency could be readily prepared.

It was found that a vaccine of reasonably uniform potency was obtained when 12 batches of vaccine were pooled before being put into ampoules.

The result of pooling a dozen batches of vaccine prepared from a dozen sheep were interesting. While the individual batches of vaccine were better than, equal to or poorer than the reference vaccine in their antigenic value, the pooled vaccine was consistently superior to the reference vaccine as well as to most of the individual batches tested. It was also found that the immunizing potency of different pools was fairly uniform and that it was invariably better than that of reference vaccine. These findings indicated that in practice it was possible to obtain a vaccine which was fairly uniform in its antigenic value and was at least 3 to 4 times as potent as the reference vaccine.

The results of post-infection treatment with pooled vaccine in guinea-pigs challenged with strains of street virus were particularly interesting. Webster (1939) in a critical review of the immunizing potency of antirabies vaccine pointed out that (i) all workers, save Fermi, had failed to demonstrate a significiant protective effect of vaccination following experimental exposure to rabies virus by any route; and (ii) vaccine, virulent or non-virulent, given before exposure had generally been found effective only when (a) the test virus was given peripherally rather than centrally into the nervous system and in amounts fatal to less than 100% of the controls, and (b) the vaccine employed was given in multiple doses and in large amounts, at least 1% of the body weight. Investigations at the Institute showed that with doses of pooled vaccine comparable to those administered to human beings it was possible to confer solid protection to experimental animals against virulent strains of street virus provided the treatment was started 7 days before challenge. With larger doses of vaccine the mortality could be reduced considerably if the treatment was started only 3 days before infection. Even when treatment was started after infection, there was evidence of some survival among animals given the vaccine corresponding to the human dose in 14 instead of 7 doses. Better results could be obtained by increasing the quantity of vaccine administered about threefold. It was, therefore, obvious that pooled vaccine therapy should give excellent protection in human beings in whom the incubation period was generally much longer than that obtaining in experimental animals.

Based on these findings pooled vaccine has been issued from the Institute since 1958. This method is now being adopted by other institutions in the country and abroad.

Lyophilization of phenolized antirabies vaccine

Employing a phenolized antirabies vaccine made from the brains of sheep infected with the Metchnikov Institute strain of fixed virus, Selimov *et al.* (1959) reported that the immunogenic properties of the vaccine were completely preserved on lyophilization from the frozen state.



INAUGURATION OF THE WHO INTERNATIONAL TRAINING COURSE BY HIS HIGHNESS GOVERNOR OF MYSORE

In Habel tests to determine the potency, lyophilized vaccines gave protection indices of 1000 and over, in 14 experimental series out of 15. Both the lyophilized and liquid vaccines protected 86% to 95% of mice against street virus challenge, whereas there was 100% mortality in the untreated controls. In view of the possible value of a stable, dry, antirabies vaccine which could be stored and transported with ease, it was considered desirable to confirm the findings. With this object in view the method of production of lyophilized, phenolized pooled vaccine was standardized. A series of investigations showed that lyophilized phenolized vaccines possessed excellent antigenicity.

Also, it was found that lyophilized vaccines were remarkably stable and retained their potency at 4°C. for periods upto 2 years.

Duration of immunity

There is no real basis to suggest present recommendations regarding the re-treatment of patients who have been exposed to the risk of rabies infection for a second time after they have once been exposed and have had a course of antirabies treatment. Studies were therefore undertaken to determine (a) the resistance of guinea-pigs which had survived a moderate challenge as a result of treatment with serum and vaccine to subsequent challenge with homologous and heterologous strains of street virus, administered at different intervals of time after the first challenge, and (b) the value of booster doses of vaccine in re-treatment.

It was found that even with large groups of animals treatment with serum and vaccine saved nearly 70% against challenges of about 100 LD_{50}. The animals which survived such treatment and challenge continued to have a considerable degree of immunity to re-challenge even 15 months after the first treatment. There was no advantage in giving two booster doses of vaccine during this period. The immune status of rechallenged guinea-pigs seemed to depend primarily on the original treatment rather than on the strain of virus used for the first challenge. Guinea-pigs, which survived an earlier challenge as a result of treatment, fared better against the later challenge than fresh groups of treated but not challenged animals given the same challenge.

Inactivation with β-propiolactone

Investigations carried out at the Institute indicated that β–propiolactone (BPL) might be a better inactivating agent than phenol. Studies were undertaken to determine whether the antigenicity of sheep brain vaccine could be improved by employing BPL instead of phenol.

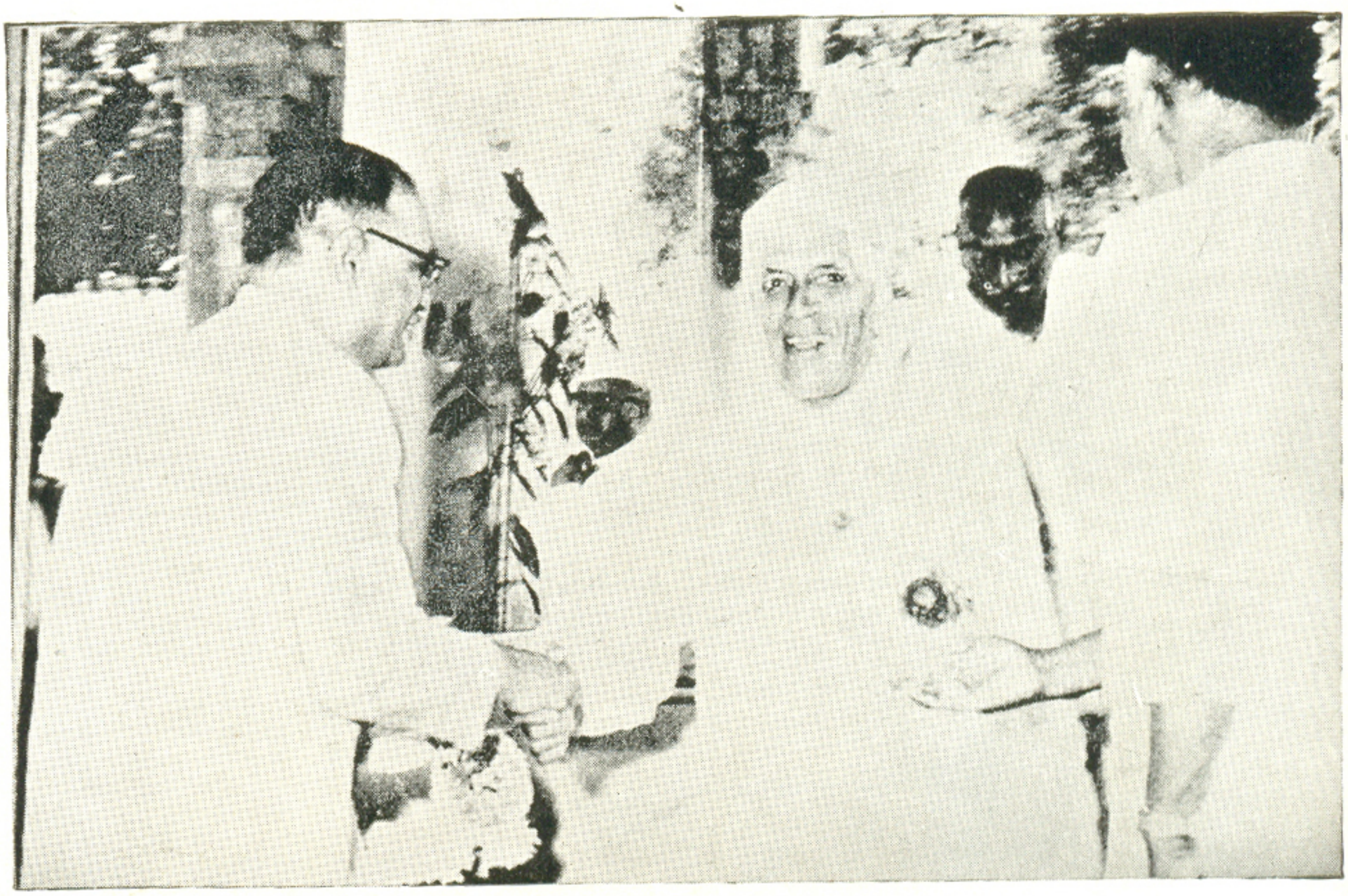
It was found that a 10% or 20% suspension of the brain of sheep infected with the Paris strain of rabies fixed virus was rendered avirulent by 1% phenol after incubation at 37°C. for 44 hours. They were inactivated by a 1 in 3000 concentration of BPL when kept at 4°C. for 24 hours.

Potency tests showed that the ED_{50} by the NIH method was generally lower and the LD_{50} protection in the Habel test was always higher when BPL was used for inactivation. In post-infection treatment of guinea-pigs challenged with street virus the results were similar.

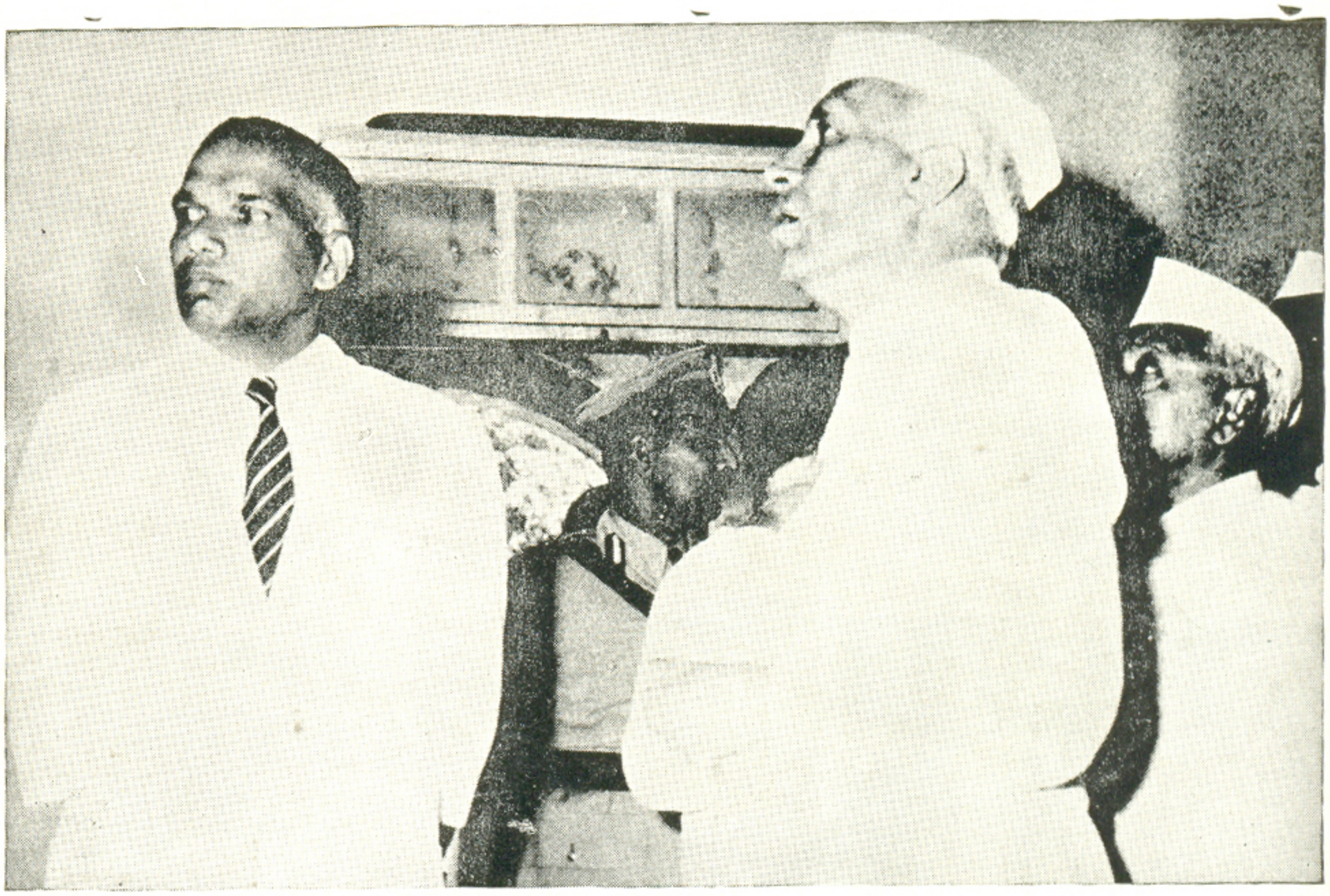
Suckling mouse brain vaccine

Recently, it has been reported that vaccines prepared from suckling mouse or rat brains, apart from being relatively free of the paralytogenic factor, were highly immunogenic. Investigations were undertaken to determine whether the antigenicity of antirabies vaccine could be improved by (a) using infected suckling mouse brain instead of sheep brain, and (b) employing BPL for inactivation of the virus instead of phenol.

It was found that a 20% suspension of infected suckling mouse brain was



PRIME MINISTER SHRI JAWAHARLAL NEHRU'S VISIT TO THE INSTITUTE



PRIME MINISTER SHRI JAWAHARLAL NEHRU'S VISIT TO THE INSTITUTE

rendered avirulent in less than 6 hours by 1% phenol at 37°C., while it was inactivated by a 1 in 1000 concentration of BPL in 12 hours at 4°C.

The ED_{50} by the NIH method was lower and the LD_{50} protection in the Habel test was higher when BPL was used for inactivation. The LD_{50} protection in the Habel test was always higher than log 3.5 .

The protection conferred by sheep and suckling mouse brain vaccines inactivated with phenol or BPL in guinea-pigs was investigated. The results indicated that the immunizing value of sheep or suckling mouse brain vaccines inactivated with BPL was superior to that obtained with those inactivated with phenol. It was interesting to note that a 1% suspension of suckling mouse brain vaccine inactivated with BPL conferred the same protection as a 5% vaccine. An important finding was that the 5% sheep brain vaccine inactivated with BPL afforded as good a protection as the 5% suckling mouse brain vaccine inactivated with BPL.

Duck embryo vaccine

Powell and Culberston (1950) reported the successful cultivation of rabies fixed virus in embryonated duck's eggs. It was found that the material derived from duck embryos was substantially free from encephalomyelitic factors. The virus grown in duck embryo could be inactivated with β-propiolactone and the inactivated vaccine was found to be antigenic in mice. Also, in treated animals and volunteers, serum antibodies appeared early and persisted. The early appearance of antibodies, the reduced risk of neuroparalytic accident and conformity to the standards of potency led to the acceptance of the duck embryo vaccine in human treatment in the U.S.A.

No studies were reported on the value of duck embryo vaccine in the post-infection treatment of experimental animals when given alone or with anti-rabies serum. Investigations were, therefore, undertaken to determine its value in the pre- and post-infection treatment of guinea-pigs under standard conditions.

Post-infection treatment of guinea-pigs with duck embryo vaccine in a dose corresponding to that recommended for human treatment gave very poor results whether the vaccine was given alone or with serum.

Even when the dose of duck embryo vaccine was raised to the same level as that recommended for pooled vaccine, the results with the vaccine given alone or with serum were inferior to those obtained with pooled vaccine given under identical conditions.

In groups given the vaccine before infection, pooled vaccine gave results significantly superior to those given by the duck embryo vaccine.

Potency tests also demonstrated the better antigenicity of pooled vaccine compared to that of duck embryo vaccine.

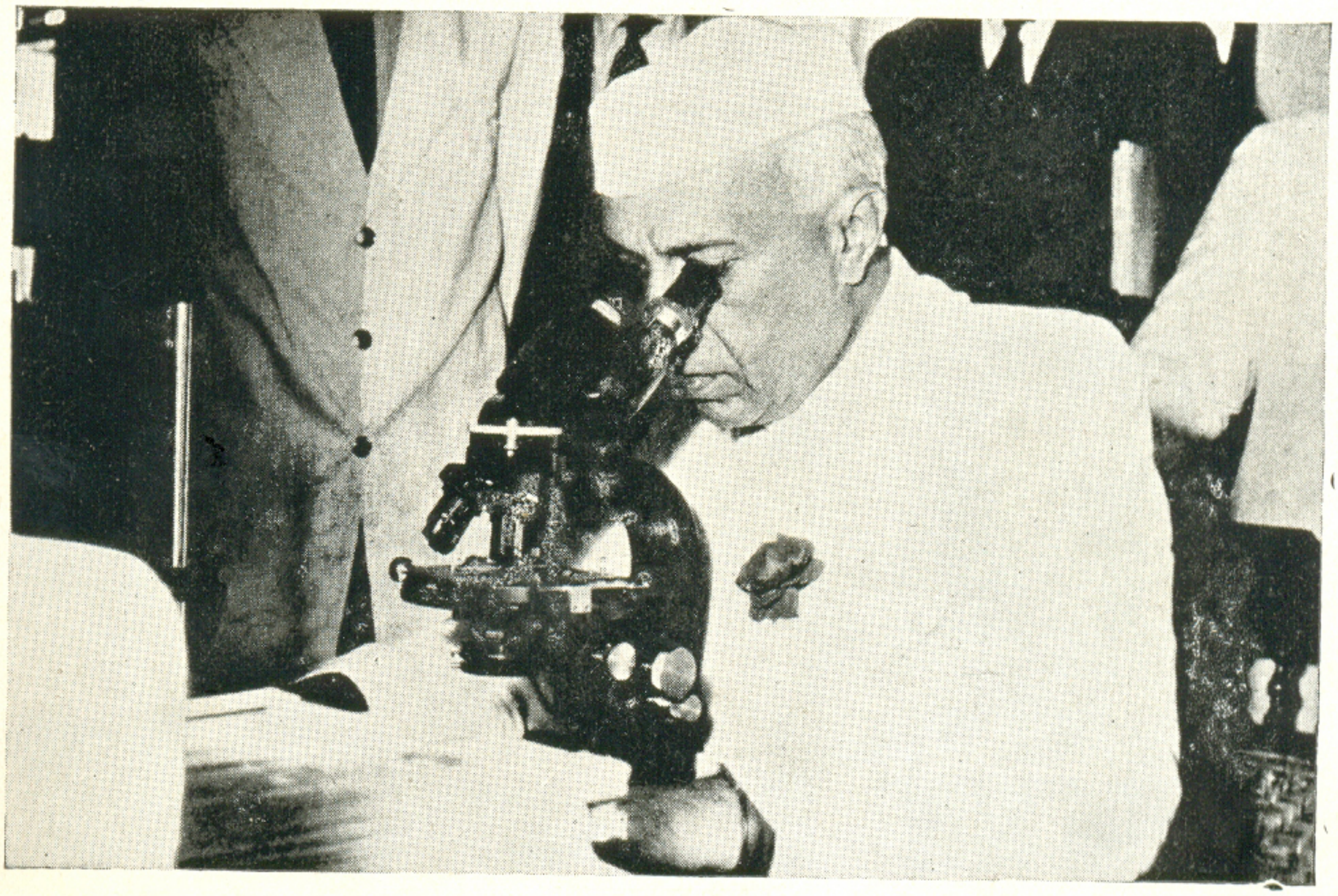
In spite of these findings, duck embryo vaccine given in adequate doses will be very useful in the treatment of exposed persons allergic to nervous tissue vaccine.

Egg adapted Flury live virus vaccines

Koprowski and Black (1954) found that 1 out of 4 animals challenged with the NYC strain of street virus 9 days after immunization with the egg adapted Flury strain (75th egg passage) escaped death. When challenged 12 days after inoculation with the Flury virus, 3 animals survived out of 4, and of those challenged 18 days after vaccination all survived. They considered that there was no major antigenic difference between the low and high egg passages of the Flury virus and reported that a single administration of the latter induced immunity in dogs, calves, monkeys and chimpanzees. They observed that one injection of Flury virus



PRIME MINISTER SHRI JAWAHARLAL NEHRU'S VISIT TO THE INSTITUTE



PRIME MINISTER SHRI JAWAHARLAL NEHRU'S VISIT TO THE INSTITUTE

alone given 24 hours after exposure to street virus had either negligible preventive action or none at all. In marked contrast they found that combined treatment with a batch of antiserum of horse origin and Flury virus showed good protective action in guinea-pigs and dogs. Based on these findings they felt that, once the antigenic dose of the Flury strain was determined for man, definite consideration should be given for the use of combined treatment with antiserum and Flury strain in human prophylaxis.

Investigations were undertaken to determine the protection conferred by the low egg passage (LEP) and high egg passage (HEP) Flury viruses against Indian strains of street virus under different conditions.

HEP Flury virus grown in embryonated chicken eggs

The HEP Flury virus was produced in embryonated chicken eggs according to the standard method, tested for potency, and used in these experiments.

Very good results were obtained in guinea-pigs given the HEP Flury vaccine 12 days before infection. The vaccine administered 7 days before challenge conferred very little protection compared with the excellent results obtained with the phenolized vaccine given in doses corresponding to human treatment. These findings indicated that HEP Flury vaccine was of no value against rabies when given after infection.

When serum was given 24 hours after infection and HEP Flury vaccine 48 hours after infection with the NYC strain of street virus, the results were fairly good. On the other hand, the combined treatment with serum and HEP Flury vaccine conferred no protection against an Indian strain of street virus, in marked contrast to the good results obtained with serum and phenolized vaccine against both the NYC and Indian strains. These findings suggested that combined post-infection treatment with serum and HEP Flury vaccine might not be of much value against fairly virulent strains of street virus similar to those prevalent in India.

In another experiment the HEP Flury vaccine was given post-infection to guinea-pigs in doses corresponding to that of phenolized vaccine with and without serum. It was found that its performance in the protection experiments, whether given alone or with serum, was too poor to warrant its use in the post-infection treatment of rabies.

LEP and HEP Flury viruses grown in chick embryo tissue culture

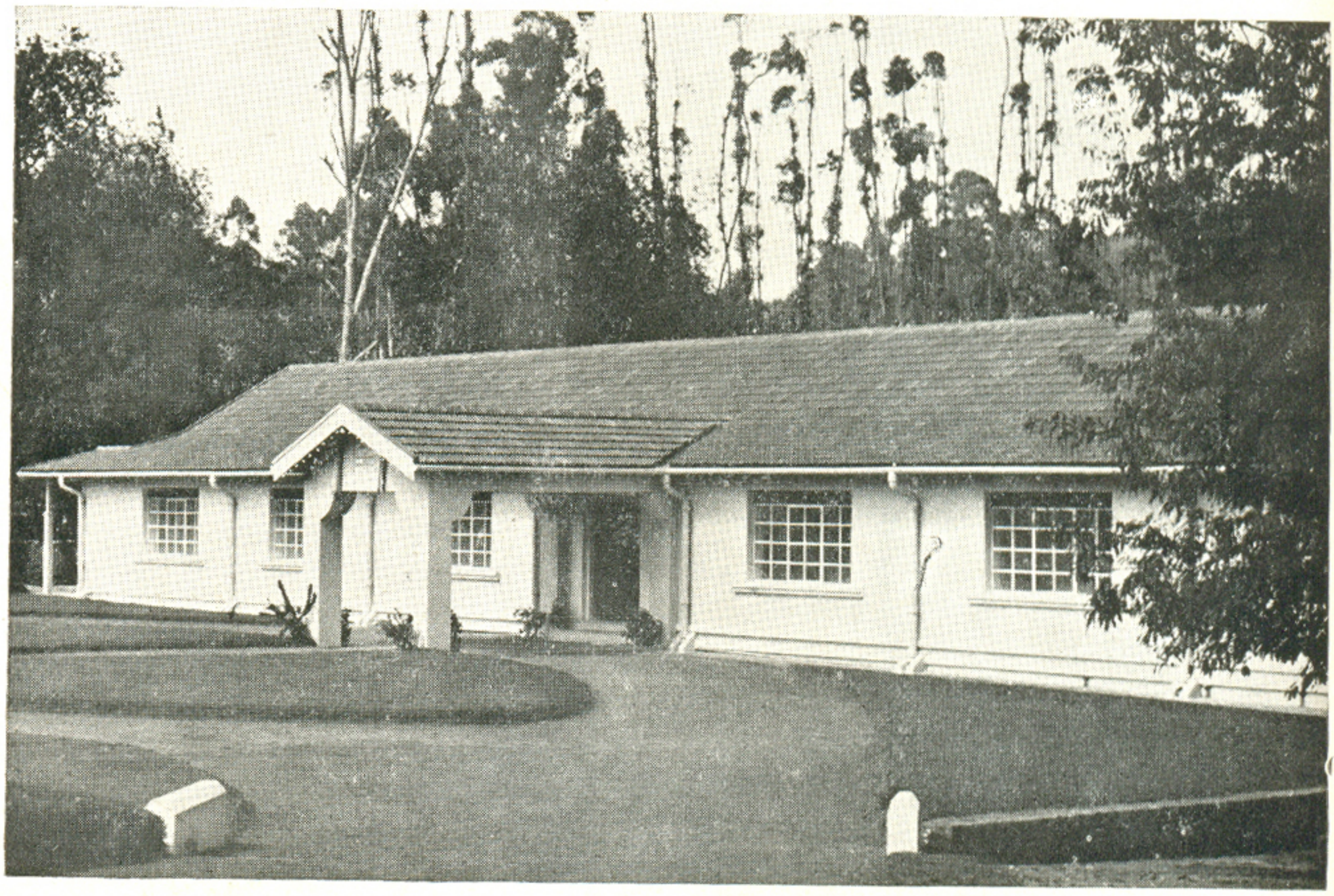
As mentioned earlier a method has been developed for the cultivation of LEP and HEP Flury viruses in chick embryo tissue cultures employing a synthetic medium without serum.

Several large scale experiments have been carried out in guinea-pigs to determine the value of LEP and HEP viruses grown in tissue culture, when given alone and with serum. The HEP Flury chick embryo vaccine (Lederle) and the pooled phenolized vaccine administered under identical conditions served as controls.

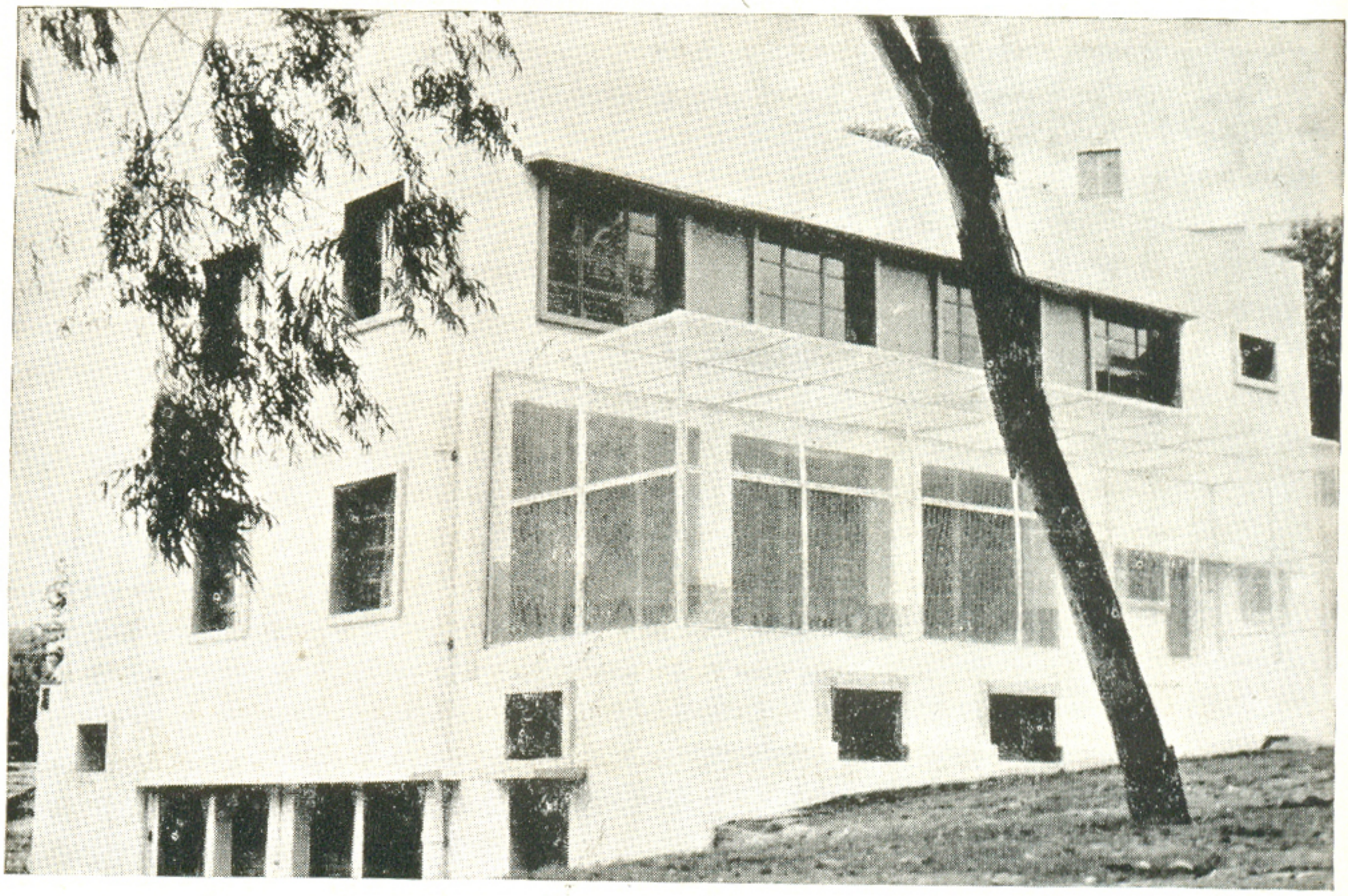
The HEP Flury vaccine (Lederle) did not give significant protection in post-infection treatment of animals when given alone or with serum.

All the different doses of LEP and HEP Flury culture vaccines and pooled vaccine started 7 days before challenge gave significant protection. In the pre-infection treatment with LEP and HEP Flury culture vaccines, the effective log LD_{50} of virus was less than 3.48 and 5.50 respectively.

In the post-infection treatment of guinea-pigs the LEP and HEP tissue



THE GOLDEN JUBILEE BLOCK



MONKEY HOUSE

culture vaccines given in a single dose appeared to confer the same protection as 14 doses of phenolized vaccine. With LEP vaccine the effective log LD_{50} without and with antirabies serum was 3.99 and 3.80 respectively. With HEP vaccine alone, the effective log LD_{50} was 5.76, while that with serum was 5.67.

The survival rates were better when serum was used in addition to LEP and HEP culture vaccines and pooled vaccine.

The effective LD_{50} of HEP tissue culture virus required to protect guinea-pigs was about 100 times greater than that of LEP virus. The results were similar in the Nylar test.

The effective LD_{50} of these viruses as determined by the Nylar test was the same as that obtained in guinea-pig protection experiments.

These findings indicate the possibility of using the LEP and HEP strains of Flury virus grown in tissue culture in the post-infection treatment of animals and man respectively, by giving them either alone or with antirabies serum as an adjunct.

Immunization of dogs. The immunizing value in dogs of the LEP and HEP Flury viruses cultivated in chick embryo tissue cultures using a synthetic medium without serum was investigated. This study was undertaken with the co-operation of the National Institute of Communicable Diseases, Delhi. The inoculation of the dogs and the collection of pre- and post-vaccination sera were done at the National Institute of Communicable Diseases, while the determination of the neutralizing antibody content of the sera was done at the Pasteur Institute, Coonoor.

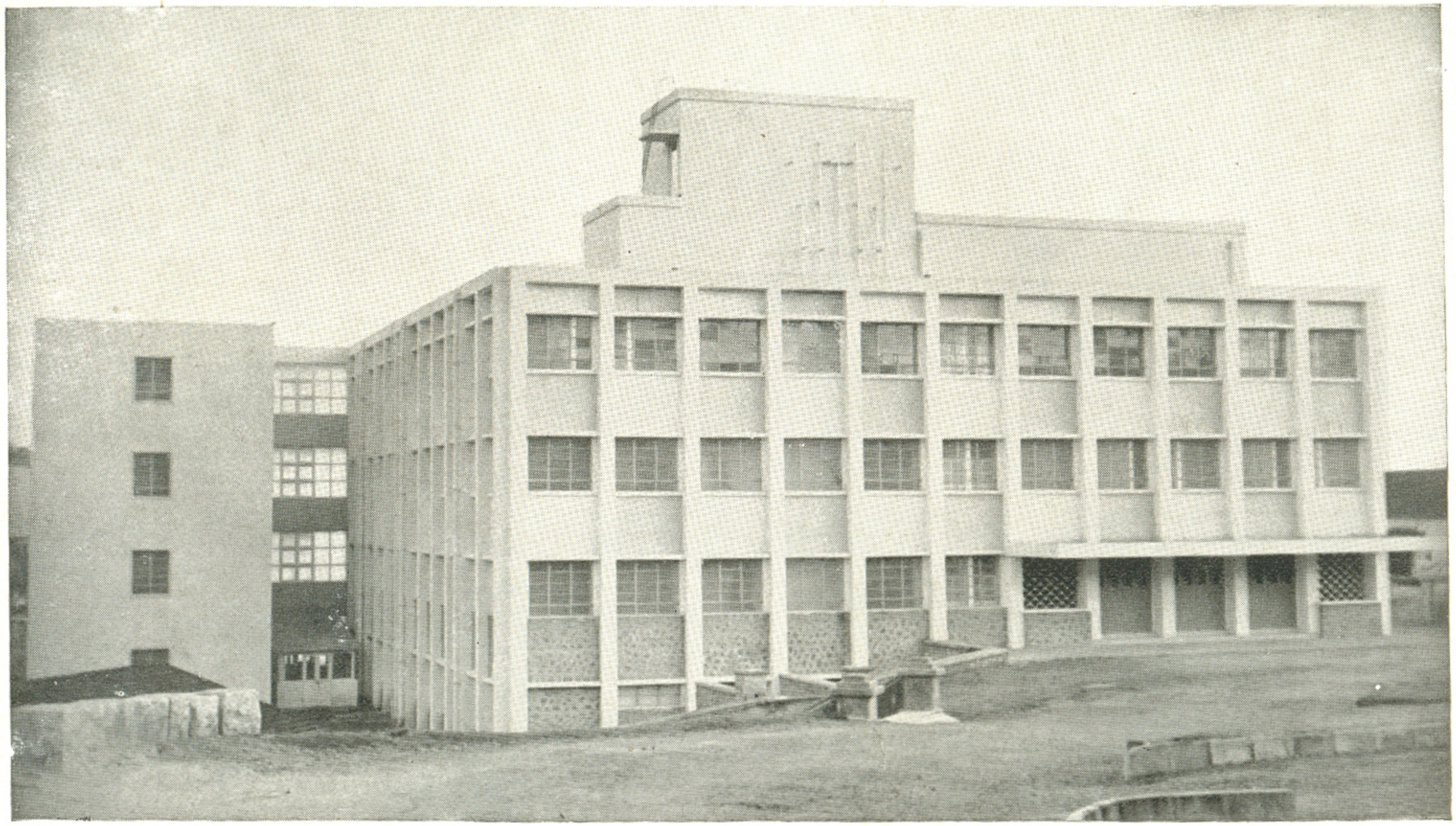
It was found that the LEP and HEP Flury culture vaccines did not produce any untoward reactions in dogs when given intramuscularly in a single dose of 3 ml. Both the LEP and HEP vaccines produced demonstrable neutralizing antibody against rabies virus in dogs.

(9) ANTIRABIES SERUM

Ever since the work of Babes and Lepp in 1889, the protective power of antirabies serum has been studied by many workers but with conflicting results. This is not surprising as most of the experimental work reported so far on antirabies serum suffers from one or more of the following drawbacks: (i) The strains of fixed and street virus used for challenge have varied from worker to worker. (ii) The challenge material obtained from different passages or different species of animals have been employed by the same worker. (iii) Few workers, except Koprowski, have used infective canine salivary gland material for challenge purposes. (iv) The route of challenge has varied from worker to worker. (v) The data regarding the severity of challenge used in terms of LD_{50} doses of the virus are not available. (vi) The fixed virus used for the production of hyperimmune serum has also been employed for challenge. (vii) The source of hyperimmune serum, the quantity given, as well as the route of administration have varied from worker to worker and in the majority of cases have no relation to the conditions obtained in human treatment. (viii) The potency of sera used in terms of the challenge virus is not available. (ix) The period of observation of animals treated with hyperimmune serum has not been long enough.

In the large scale experiments on antirabies serum carried out at the Institute an attempt was made to avoid or over come most of the drawbacks listed above. The results of these experiments are summarized below.

In all the experiments no significant saving could be demonstrated among guinea-pigs challenged with street virus and treated with serum before or after



NEW LABORATORIES

infection. Antirabies serum alone, while considerably prolonging the incubation period, had no saving effect. Although the conditions of the experiments were similar to those employed by Koprowski *et al.* (1950) and the strain of challenge virus was the same it was not possible to demonstrate any protection with serum alone. The fact that serum administered before infection in large doses intramuscularly and intracerebrally could not protect the animals emphasized the inefficacy of serum therapy alone. These findings, together with the observation that sera containing a high concentration of neutralizing antibody obtained from solidly immune animals confer no protection, while the animals themselves can stand severe challenges, throw doubt on the validity of the practice of evaluating protective power only in terms of neutralizing antibody level in blood.

An interesting observation in this study was that good protection could be obtained when serum homologous to the particular strain of challenge virus was administered. This finding would explain the good results obtained by Habel (1945), Ishi (1952) and others, who used fixed virus for challenge and sera raised against the same or antigenically similar strains of fixed virus for treatment. The protection afforded by even very small quantities of homologous serum was striking compared with the consistent failure following treatment with massive doses of heterologous serum, even when administered before infection.

Further, the considerable prolongation of the incubation period in guinea-pigs given sera from guinea-pigs raised against even heterologous strains of virus indicated that the value of immune serum from the same species of animal was superior to that obtained from other species.

It would, therefore, appear that for best results it is esential that the serum should be homologous to the strain of virus as well as the species of animal. Such a possibility would rule out the use of serum alone in human treatment in view of the obvious difficulty of obtaining immune serum raised in man against the particular strain of virus introduced by the bite of a rabid animal.

Combined therapy with serum and vaccine in animals

Habel (1945) and Berke and Turkay (1956) reported that the results with serum prophylaxis when used against street virus were better when combined with vaccine treatment, while Koprowski *et al.* (1954) found that vaccine treatment of exposed animals seemed neither to enhance nor to decrease the protective power of serum. Our results showed that combined serum and vaccine therapy was of considerable value under certain conditions.

Antirabies serum alone administered locally 1 hour after challenge conferred no protection.

Results of post-infection treatment with vaccine against varying challenges indicated that, when vaccine alone was given, a dosage of 14 x 0. 15 ml. per pound of body weight — corresponding to a class III (severe exposure) dosage of 140 ml. for a person weighing 70 pounds or more — was necessary.

With combined therapy it was found that when the challenge was mild, about 10 LD_{50}, the administration of serum in addition to vaccine did not appear to be of advantage. When the challenge was about 50 LD_{50} the protection conferred by combined therapy with serum and vaccine was better than that obtained with vaccine alone, though not always statistically significant. These results suggested that there was no advantage in giving serum when the challenge was mild as vaccine alone gave good protection in such cases.



When the challenge was about 100 LD_{50} the protection conferred by combined therapy was very good and markedly superior to that obtained with vaccine treatment alone. Against challenges ranging from 100 to 200 LD_{50} the results of combined therapy were moderately good while vaccine treatment was of no value. When the challenge was about 300 LD_{50} the protection with combined therapy was still significant, while at higher challenges no treatment was of any value.

The dosage of serum (25 international units per pound of body weight), recommended for human treatment is based on the work of Koprowski *et al.* (1954). In our experiments, using a dosage of vaccine corresponding to the human treatment and serum it was found that the best results were obtained when one-fifth of the dosage recommended i.e., 5 I.U. per pound of body weight, was employed. The results were poorer with larger as well as smaller doses suggesting that an optimum relation between the dosage of serum and vaccine has to be maintained for good results.

Atanasiu *et al.* (1956) from a study of rabies neutralizing antibody response to different schedules of serum and vaccine inoculations in non-exposed persons, reported that the evidence was inconclusive with respect to the early presence of antibody from the antiserum possibly affecting the later antibody response caused by the phenolized vaccine. The poorer results obtained by us with larger doses of serum, the mortality among animals which received concentrations of serum and vaccine before infection and the poor antigenic value of *in vitro* mixtures of serum and vaccine suggest the possibility of interference between the antigen in the form of vaccine and antibody in the form of serum. The observations of Habel (1956) support this view.

The best results were obtained when serum and vaccine were started as early as possible. There was no advantage in giving serum first and following it 24 hours later with vaccine as suggested by Koprowski *et al.* (1954). Protection was considerably less when vaccine treatment was started 6 days after serum, as recommended by Habel (1945).

The experiments suggested that combined serum and vaccine therapy could be useful when started upto 6 days after infection. After this period the administration of serum did not appear to be of much value. Thus, the delay in starting treatment resulted in the loss of the advantage conferred by serum in combined therapy.

An important observation was that with the doses of serum and vaccine employed it was not possible to obtain 100% protection against any of the challenges tested.

Combined therapy with serum and vaccine in man

An enquiry has been in progress at the Institute since 1956 to determine the value of antirabies serum given as an adjunct to vaccine therapy.

Patients considered to be at grave risk following deep and/or extensive injuries on the head, face, neck or fingers, and reporting at the Institute within 72 hours after bite, were chosen for this study. On admission each patient was tested for serum sensitivity by administering a small intradermal dose of serum. Two doses of serum were employed. For adults the dosage administered was 700 international units while that for children weighing less than 70 pounds was 350 units. In each patient, the regular treatment with 5% Semple vaccine was started immediately after administering serum. The condition of the treated patients was ascertained 6 months after treatment.

A total of 758 patients received combined serum and vaccine therapy till the end



BUILDING FOR INOCULATION AND DISSECTION OF SHEEP



DR. ALBERT B. SABIN
ADDRESSING THE
MEDICAL ASSOCIATION

of 1965. In the case of 44 patients, the biting animal was alive and well at the end of the 10-day observation period, and therefore, the treatment was discontinued. In 117 cases, vaccine treatment could not be completed after the serum and one or more doses of vaccine had been given, as the patients failed to report. One patient, who received serum and a full course of treatment, died due to other causes. One death due to hydrophobia was reported among the remaining 597 patients, giving a mortality rate of 0.17%.

(10) OTHER LINES OF TREATMENT

Periodically claims are being made by practitioners of indigenous medicine in the country that they have an effective remedy for the treatment of rabies. The Institute has always been interested in evaluating the claims in experimental animals under standard conditions and several preparations have been tried with negative results. One of the remedies tested was an oil extracted from the flesh of a white mongoose.

ACTH. The value of ACTH in experimental rabies infection and in a case of hydrophobia has been studied. The administration of ACTH did not appear to be of any value in animals which were given the challenge virus intracerebrally. It did not alter the course of the disease in animals which had started showing signs of rabies. It appeared to be of some value when the virus was given peripherally in animals already under the influence of ACTH.

In view of the recent reports that rabies virus may be reactivated in the tissues of a host by ACTH, the effect of administration of ACTH to the survivors of guinea-pigs inoculated with rabies street virus and treated with antirabies serum and/or different vaccines, as well as those which were not treated, was investigated. It was interesting to note that reactivation of rabies virus with ACTH occurred only in animals which were untreated or which received antirabies serum alone. None of the animals given LEP or HEP Flury virus or phenolized vaccine, alone or with serum, showed evidences of reactivation of the virus. This study suggested the danger of persons, exposed to the risk of rabies infection and not undergoing treatment, developing the disease on the administration of ACTH for the treatment of some other ailment.

In the case of hydrophobia in which it was tried the clinical impression was that there was marked deterioration in the condition of the patient after the administration of ACTH.

β-phenylserine. Hosley (1955) reported that β-phenylserine inhibited the multiplication of rabies virus in cultures of minced mouse brain. Pons and Preston (1961) found that the daily intraperitoneal administration of 10 to 15 mg. of β-phenylserine protected rats from death from lethal doses of rabies virus, when treatment was started 24 hours before infection. In view of these reports the compound was tried in an early case of hydrophobia.

The patient was a female aged 50 years. She was bitten on the right arm about a month earlier. There were two bites. They were not cauterized and the patient did not undergo antirabies treatment. She noticed pain at the site of bite on the night of 21-5-1963 and inability to drink water on the next morning. She was brought to the Institute by midday. She was restless and was talking rationally but had an anxious look. She was given 1 gm. of β-phenylserine, intramuscularly, at 12-30 P.M. followed by ¼ gr. morphia. At 7.00 P.M. patient was very restless and was getting severe spasms of all the muscles. She was given a second dose of 1 gm. of β-phenylserine and ½ gr. of morphia. She died at 4-30 a.m.

Thus, the patient died within 24 hours after manifesting definite signs of hydrophobia. The duration of illness was unduly short and might have been due to the influence of the drug.



DR. ALBERT B. SABIN VISITING THE LABORATORIES



SRI M. BHAKTAVATSALAM AND DR. SUSHILA NAYAR ARRIVING FOR INAUGURATION OF THE POLIO VACCINE UNIT

ANTIRABIES TREATMENT OF ANIMALS

The Institute started issuing antirabies vaccine for the treatment of animals in 1923. Upto the end of 1965, the number of animals treated was 81,353. Of these, 50,352 were dogs and 24,317 cattle. Although indents for the prophylactic treatment have been steadily increasing. the main demand still continues to be for the post-infection treatment of animals. The extent of the demand is indicated by the fact that 4,815 animals were treated during 1965. Of these, 2,608 were dogs. This steady increase in the demands for vaccine is due to the rabies conciousness among the public and the fact that the treatment for animals is made available free of charge by the Government.

The vaccine used for treatment of animals is the same as that used for human treatment. Only the dosage is different. The Institute recommends multiple doses of vaccine for the prophylactic as well as post-infection treatment of animals. The results of investigations carried out at the Institute indicating that the same quantity of vaccine given in 7 or 14 doses gives much better immunity than a single dose emphasize the value of multiple injections advocated by the Institute.

In view of the general belief that post-infection treatment of dogs, cattle and other animals is of no value it was considered desirable to investigate the problem. A careful study to determine the efficacy of post-infection treatment of animals bitten by proved or presumably rabid animals has been in progress since 1955. The results obtained so far can be summarized as follows:

Dogs. The fate of 3,391 dogs which were treated with phenolized 5% vaccine, post-infection, has been followed up. Of these, 96 out of 1,199 (8.01%) bitten on the head, 18 out of 533 (3.38%) bitten on the neck, 19 out of 530 (3.58%) bitten on the trunk, 22 out of 689 (3.19%) bitten on the limbs, and 19 out of 440 (4.32%) bitten on more than one site, died of rabies. The overall mortality was 174 out of 3,391 (5.10%). If, however, the animals which died within 15 days after the completion of treatment are not considered as treatment failures, the overall mortality rate would be 90 out of 3,307 (2.72%).

Cattle. The fate of 5,200 heads of cattle which were treated with phenolized 5% vaccine has been studied. Of these, 198 out of 2,922 (6.78%) bitten on the head, 11 out of 354 (3.11%) bitten on the neck, 23 out of 541 (4.25%) bitten on the trunk, 20 out of 825 (3.52%) bitten on the limbs, and 22 out of 558 (3.94%) bitten on more than one site, died of rabies. The overall mortality was 283 out of 5,200 (5.44%).

If, however, animals which died within 15 days after the completion of treatment are not considered as treatment failures, the overall mortality rate would be 124 out of 5,041 (2.46%).

Studies at the Institute have shown that in this part of the country about 66% of animals suspected to be rabid prove positive. Of these, about 59% contain the virus in the salivary glands. This would mean that about 39% of presumably rabid animals are capable of transmitting the infection. Considering that dogs and cattle are very susceptible to rabies infection, the mortality rates among them would suggest that post-infection treatment of these animals is of value.

REFERENCES

Sci. Rep. Pasteur Institute, Coonoor. 1947–66.

ATANASIU, P. *et al.* (1956) *Bull. Wld Hlth. Org.*, 14, p. 593.

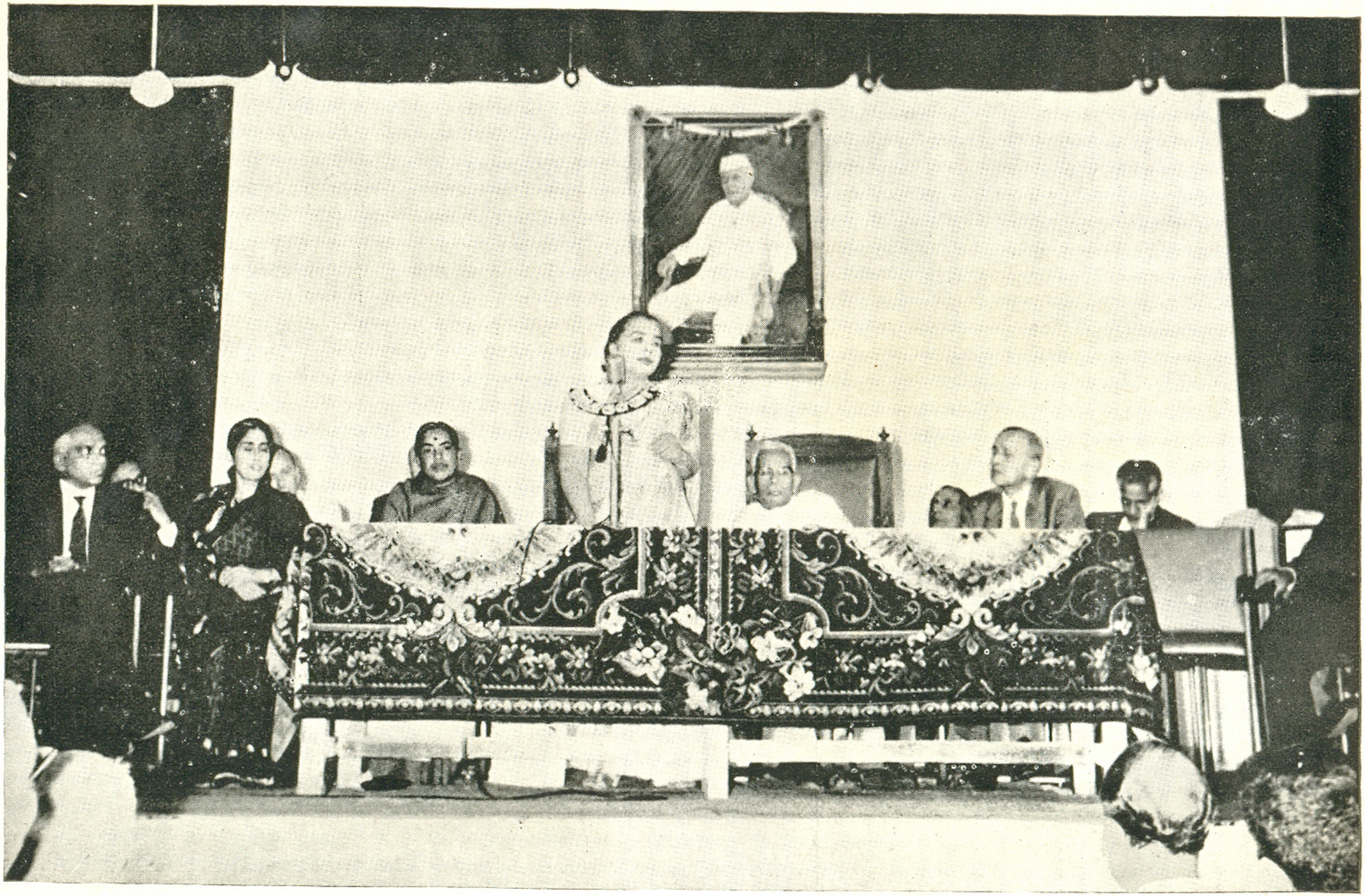
BABES, V. and LEPP (1889) *Ann. Inst. Pasteur.*, 3, p. 385.

BERKE, Z. and TURKAY, N. (1956) *Turk ij.tecr. Biyol. Derg.*, 15, p. 317.

CORNWALL, J.W. (1918) *Ind. Jour. Med. Res.*, 6, p 237.

Idem. (1923) *Brit. Med. J.*, 2, p. 298.

Idem. (1924) *Ann. Rep. Pasteur. Inst., Coonoor.*



DR. SUSHILA NAYAR INAUGURATING THE POLIO VACCINE UNIT

D'SILVA, C.B. *et al.* (1951) *Ind. Jour. Med. Res.*, 39. p. 423.

HABEL, K. (1945) *Pub. Hlth. Rep.*, 60. p. 545. *Idem.* (1956) *Bull. Wld Hlth. Org.*, 14, p. 613.

HOSLEY, R. (1955) *Thesis. University of Michigan.*

ISHI, K. (1952) *Jap. J. Exp. Biol. Med.*, 5, p. 159.

KOPROWSKI, H. *et al.* (1950) *Amer. J. Med.*, 8, p. 412.

KOPROWSKI, H. and BLACK, J. (1954) *J. Immun.*, 72, p. 79.

OTTEN, L. (1914) *Med D.V.G.*, 30, p. 127.

PONS, M. W. and PRESTON, W. S. (1961) *Virology*, 15, p. 164.

POWELL, H.M. and CULBURTSON, C.G. (1950) *Pub. Hlth. Rep.*, 65, p. 400.

SELIMOV, M. A. *et al.* (1959) *Unpublished working document* WHO Rabies 114.

VEERARAGHAVAN, N. (1957) B*ull. Wld Hlth. Org.*, 17, p. 937. *Idem.* (1959) *Bull. Wld Hlth. Org.*, 20, p. 381.

VEERARAGHAVAN, N. and BALASUBRAMANIAN, A., (1958) *Ind. Jour. Med. Res.*, 46, p. 514.

VEERARAGHAVAN, N. and SUBRAHMANYAN, T.P, *Ind. Jour. Med. Res.*, 46, p. 518. *Idem.* (1960) *Bull. Wld Hlth. Org.*, 22 p. 381. *Idem.* (1961) *Bull. Wld Hlth. Org.*, 25, p. 115. *Idem.* (1963) *Bull. Wld Hlth. Org.*, 29, p. 323. *Idem.* (1963) *Bull. Wld Hlth. Org.*, 29 p. 511.

VEERARAGHAVAN, N., BALASUBRAMANIAN, A. and RANGASAMI, R. (1958) *Bull. Wld Hlth. Org.*, 18, p. 469.

VEERARAGHAVAN, N., BALASUBRAMANIAN, A. and SUBRAHMANYAN T. P. (1957) *Bull. Wld Hlth Org.*, 17, p. 943.

WALLIS and MELNICK (1962) *Virology*, 16, P. 122.

WEBSTER, L. T. (1939) *Amer. J. Hyg.*, 30. p. 113.

2. INFLUENZA

(1) EPIDEMIOLOGY

India is known to have been visited by the influenza pandemics of 1781, 1889 and 1918, apart from that of 1957. The first of these is supposed to have originated there in September 1781. According to the report of the Sanitary Commissioner to the Government of India for 1918, the pandemic of that year affected 70 millions, i.e., 30% of the population at risk. The case-fatality rate varied in different areas from 1% to 50%, with an average of 10%, while 20% of the deaths occurred in the age-group 20–40 years. There were 7 million deaths in the area under survey, which represented only half of India. Despite its obvious importance as a public health problem, the information available on the behaviour of sporadic and epidemic influenza, as distinct from the pandemic type, is scanty.

Feeling that many of the problems concerning influenza could be solved only by international collaboration, the World Health Organization set up the World Influenza Centre in London, England, and the Strain Study Centre for the Americas in Brooklyn, N. Y., U. S. A., and invited all Member States of the Organization to co-operate in the international programme by setting up regional laboratories that would serve as listening posts. The main functions of these laboratories were to study the problem of influenza in the area continuously, to isolate strains of virus and determine their antigenic pattern, and to warn the World Influenza Centre if a new variant of the virus appeared.

As part of this programme and with the assistance of the Government of India, an influenza centre was organized at the Pasteur Institute of Southern India, Coonoor, in 1950 under the late Dr. I. G. K. Menon and designated as the Government of India Influenza Centre. Dr. Menon was responsible for developing the Centre as the main laboratory for work on influenza in the country and directed its activities with distinction till his premature death in May 1959.

The first problem that faced the Centre at its inception in 1950 was that of collecting reliable information on the incidence of the disease in the area. It was felt that the data based entirely on

LIBRARY
HAFFKINE INSTITUTE
BOMBAY-400 012.



SHRI M. BHAKTAVATSALAM ADDRESSING THE GATHERING



PART OF THE GATHERING

clinical diagnosis unsupported by laboratory findings might be of no value. It was therefore decided to collect epidemiological information from the Nilgiri District and correlate the data with laboratory findings. Four institutions from three townships were selected for the purpose. Data on the number of cases of influenza seen every month, as well as on the total number of all cases attended to in out-patient departments for the same periods, were gathered. These have been presented regularly in the Scientific Reports of the Institute. It was found that a marked increase in the number of cases diagnosed clinically as influenza was generally associated with the isolation of the virus, indicating that the collection of epidemiological data based entirely on clinical findings was of some value.

The approach followed from 1950 to the beginning of 1959 was to concentrate on the isolation of virus whenever the epidemiological data mentioned above showed a marked increase in clinically diagnosed cases of influenza, the aim being to establish the identity of the virus responsible for the outbreak.

Since 1959, with the co-operation of the medical officers, an attempt has been made to study the incidence of the disease in the district by carrying out virus isolations throughout the year.

As part of this programme the number of throat gargles examined during 1959–66 and the number of influenza viruses isolated are given in Table 4.

The influenza virus isolations at the Coonoor Centre each year since its inception are given in Table 5.

The distribution of influenza virus isolations in different months of the year are given in Table 6.

Type A viruses

Isolations. The first isolation of influenza virus in the country was done by the late Dr. Menon on 5 August 1950. Between that date and December 1966, 159 strains of type A virus have been isolated.

It will be seen from Table 6 that outbreaks of influenza confirmed by isolations of type A–prime (A1) virus occurred in the district during the following periods: August 1950; August to November 1951; December 1952; November 1953; September to November 1954; June 1955; January to March 1956; and January 1957.

With the appearance of the 1957 pandemic strain in the country, outbreaks due to Asian (A2) virus occurred in the district during the following periods: May, June, September, November and December 1957; January, February, July and October 1958; May, July, August, October and December 1959; January, March, September and October 1960; April and September to December 1961; February, May and July to September 1962; April and August to October 1963; November and December 1964; January 1965 and February and March 1966.

Antigenic pattern. The antigenic analysis of the strains by Menon *et al.* showed that the A1 strains isolated in India during the years 1950–54 were related to the Liverpool subtype, isolated from an outbreak associated with heavy mortality in December 1950, in Liverpool, England. They found that the strains isolated in any year were closely related to each other, but were slightly different from those isolated in the other years.

In July 1955, a strain of virus was isolated from the throat gargle of a patient which on antigenic analysis was found to be related to A/Eire/55.

In January 1956, there was a sudden outbreak of influenza in the district. The outbreak spread rapidly, attaining peak figures in March and subsided by the middle of April. Throat gargles were collected from five different

main building used for the preparation and filling up of the vaccine was redone during 1964, It was partitioned into two air-conditioned rooms with an ante-room. The floorings and walls of these rooms were tiled. The cost of remodelling the room amounted to Rs. 9,348.46. Also, the room used for the dissection of sheep was demolished and rebuilt. Two suites of three rooms each, one for inoculation of sheep and the other for dissection, were constructed. The floorings and walls were tiled. The cost of redoing amounted to Rs. 16,145.79.

In view of the difficulty experienced by the staff in getting living accommodation at Coonoor, the Central Committee decided to utilise all its available resources for the construction of residential quarters. The construction of the first block comprising of a three-storeyed building consisting of 18 flats, which was started during August 1962, was completed in September 1963. Each flat measures 26 ft. x 26 ft. and comprises of 2 bed rooms, a living room, a kitchen, a bath room and an enclosed verandah. The cost of the entire construction inclusive of water supply, sanitation, electrification, side revetments and approach road, amounted to Rs. 2,66,326.05.

The construction of the second block of quarters similar to the above was started in December 1963 and completed in March 1965. The cost of the entire construction inclusive of water supply, sanitation, electrification, side revetments and approach road amounted to Rs. 2,47,498.38.

With a view to augment the water supply to the Institute a well was constructed in 1959. The expenditure amounting to about Rs. 15,000 incurred in connection with the construction of the well and a 6,000 gallons capacity storage tank nearby was met from a grant kindly made available by the Government of Madras.

Owing to the expansion of the activities at the Institute it was found increasingly difficult to manage with the low tension electric supply. A 150 K.V.A. transformer was installed at a cost of Rs. 24,726 in 1957. The expenditure was met from a generous grant from the Ministry of Health.

During 1964 the overhead electric lines within the Institute compound were rearranged to conform to the latest regulations. The cost of rearranging the overhead electric lines and remodelling the control switch board amounted to Rs. 8,966.48.

Equipment

The excellent laboratory facilities at the Institute have been possible because of the generous assistance reeeived from the Ministry of Health and various international agencies.

When the Government of India Influenza Centre was started in 1950, the WHO made a gift of valuable equipment consisting of a –20°C. deep-freeze, a freeze-drying plant, an electric incubator with a capacity of 1,440 eggs and a high speed centrifuge, all of which are still in use.

During 1956-58, the Technical Co-operation Mission of the United States made a generous gift of the following valuable items of equipment: (i) Spinco Model E, Analytical ultra-centrifuge costing about Rs. 85,000; (ii) a –30°C. deep-freeze cabinet costing about Rs. 4,250; (iii) a –70°C. deep-freeze cabinet costing about Rs. 28,000; (iv) a special portable interference electrophoresis apparatus costing about Rs. 22,000 and (v) two centrifugal freeze-dryers.

In connection with the poliovaccine production programme, the USAID Mission, New Delhi, has during 1964–66 made a generous gift of specialized imported equipment worth $ 79,137 and indigeneous equipment worth Rs. 3,57, 150.

TABLE 4

Place	1959		1960		1961		1962		1963		1964		1965		1966	
	No. of throat gargles examined	No. Positive	No. of throat gargles examined	No. Positive	No. of throat gargles examined	No. Positive	No. of throat gargles examined	No. Positive	No. of throat gargles examined	No. Positive	No. of throat gargles examined	No. Positive	No. of throat gargles examined	No. Positive	No. of throat gargles examined	No. Positive
Coonoor ..	69	10	67	4	312	13	319	5	306	11	409	9	370	4	390	4
Kotagiri ...	...	...	14	1	...	...	...	...	...	...	...	...	...	...	...	...
Ootacamund ...	9	9	22	6	236	5	149	4	97	3	163	3	159	...	189	...
Wellington ...	48	15	137	12	182	7	213	9	88	5	232	3	233	1	249	1
Coimbatore ..	...	..	18	2	...	...	...	...	...	...	...	...	...	...	...	...
Bangalore ..	5	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Madras ...	...	...	7	...	...	..	...	...	..	...	32	...	29	1	...	...
Kullakamby ...	..	...	..	...	...	...	3	...	..	...	...	...	...	...	...	...
Total ..	131	35	265	25	730	25	684	18	491	19	836	15	791	6	828	5

In connection with the same project the WHO during 1966 has very kindly made available essential equipment, chemicals and replacements worth about $ 15,000.

In addition, the WHO has been making periodical grants for rabies research at the Institute. These have been utilised for purchasing valuable equipment, like the fluorescent microscope, cryostat, etc., chemicals and urgently required replacements.

The Ministry of Health, Government of India has on various occasions made available generous grants for the purchase of equipment. These include: Rs 80,230 in 1956; Rs. 24,773 in 1957; Rs. 80,000 in 1958 and Rs. 2,13,209.33 from 1959–66.

Fellowships

Apart from providing valuable equipment some of the international organizations have actively helped the Institute by providing fellowships for training abroad in new disciplines for some of its staff.

Dr. D. S. Chandrasekhar, who was the recipient of a Colombo Plan Fellowship underwent training in biophysical techniques and blood grouping methods at the Lister Institute of Preventive Medicine, London; London Hospital, London; State Serum Institute, Copenhagen; and Pasteur Institute, Paris, during 1959–60.

Dr. A. Balasubramanian was the recipient of a WHO Fellowship during 1962–63. During the tenure of the fellowship he studied methods of production and testing of live poliovirus vaccine at the Institute of Poliomyelitis and Viral Encephalitis, Moscow, Glaxo Laboratories and the Immunological Products Control Division of the Medical Research Council, United Kingdom. He also visited the Wellcome Research and Pfizer Laboratories, United Kingdom, which are engaged in the production of live poliovirus vaccine.

Dr. P. V. Kurian was the recipient of a WHO Exchange of Research Workers grant for 3 months from January 1966. During the tenure of the grant he studied the latest developments in the isolation and identification of respiratory viruses. He visited the Public Health Laboratory, Manchester, Regional Virus Laboratory, Glasgow, Cold Research Laboratory, Salisbury, State Serum Institute, Copenhagen, and the Pasteur Institute, Paris.

Laboratory facilities for other organizations and workers

The Institute has afforded bench space and laboratory facilities for many outside organizations. An outstanding example is furnished by the 'Beriberi' and later, 'Deficiency Diseases' enquiries under Sir Robert McCarrison that started at Coonoor in a very modest fashion in 1918 and has developed into the Nutrition Research Laboratories of to-day whose contributions to science are well known. The Malaria Investigations Unit of the Rockefeller Foundation that was taken over in 1942 to become the Southern India Branch of the Malaria Institute of India (now the National Institute of Communicable Diseases) is another example. Mention has also to be made of visiting workers from abroad who have enjoyed similar facilities for brief periods like Prof. Kefoid of the University of California, Dr. Lehmann of St. Bartholomew's Hospital, Miss. Cutbush of the Post-graduate School of Medicine, London. and Dr. Kirk from Australia. In India itself hospitality has been extended to investigation teams like the jaundice unit and enteric unit from King Institute, Madras, and the plague investigations unit of the Madras Public Health Department.

Training

An international training course on Public Health Laboratory Techniques for

TABLE 5

Year	A1 strains related to			A 2	B	C	Total
	A/Liverpool/50	A1/Eire/55	A1/Ned/56				
1950	3	...	...	...	...	...	3
1951	6	...	...	...	2	...	8
1952	4	...	...	...	...	...	4
1953	3	...	..	...	2	...	5
1954	7	...	...	...	...	...	7
1955	...	1	...	...	...	...	1
1956	...	4	9	...	...	...	13
1957	...	...	1	31	...	...	32
1958	...	...	...	23	...	...	23
1959	...	...	...	23	10	2	35
1960	...	...	...	24	1	...	25
1961	...	...	...	21	4	.	25
1962	...	...	...	16	2	...	18
1963	..	...	...	16	3	...	19
1964	..	...	...	2	13	...	15
1965	...	...	...	1	5	...	6
1966	...	..	...	2	3	...	5
Total	23	5	10	159	45	2	244

Virus and Rickettsial Diseases sponsored by the WHO was held at the Institute from 8 – 19 December, 1958. This was the second time the Institute served as a venue for an international scientific gathering.

Fifteen participants from 13 countries in the Western Pacific, South East Asia and Eastern Mediterranean Regions and one observer attended the course. The countries represented were Burma, Ceylon, China, Hong Kong, India, Indonesia, Iran, Iraq, Japan, Philippines, Sudan, Thailand and Viet Nam. Three of the faculty members were from the WHO and four from the Institute.

His Highness Maharaja Shri Jaya Chamraja Wadiyar Bahadur, G.C.B , G.C.S.I., Governor of Mysore, inaugurated the course at 11 A. M. on 8th December 1958.

The principal objectives of the course were to promote application of virology and public health and to show the importance and potentialities of the virus and rickettsial diagnostic laboratory as an integral part of the public health service of a country.

The Institute serves as a training centre in rabies, other virus diseases, and general bacteriology for workers sponsored by the WHO, Central and State Governments, research institutions and medical and veterinary colleges in the country. The requests for training have been steadily increasing and the Institute has made it a point to meet these demands as it considers the training valuable for the development of virological work in the country. The list of persons who have undergone training at the Institute is given on page 24.

Post-graduate education

The Institute is recognized by the Madras and Bombay Universities for training medical graduates for post-graduate degrees of M. Sc., M.D , Ph.D. and D. Sc. in bacteriology and virology. The names of those who have acquired post-graduate qualifications working at the Institute are given on page 26.

Advisory work and meetings

The senior members of the staff are periodically called upon to serve on various Advisory Committees, scientific meetings etc. Particulars regarding some of these activities are given on page 26.

Annual Report

The Institute publishes regularly an Annual and Scientific Report. The research work done during the year is published early in the following year. In view of the pioneering work being done by the Institute in various fields in the country the demand for the Scientific Report from all over the world has considerably increased. About 1,200 copies are supplied annually to scientific workers and institutions all over the world on request.

Library

The Institute library covers the general field of medicine and the specialized ones of bacteriology, virology, tropical medicine and pathology and is one of the finest in the country. It subscribes for 98 journals apart from receiving several complementary issues. It has back numbers from 1907, the year in which the Institute started. The total number of volumes at the end of 1966 was 9,776. Its unique feature is that even to-day, it is a gentleman's library where nothing is locked and is open day and night.

Animals

The fine climate of the Institute and the availability of trained animal tenders have been utilised for building up a good healthy stock of experimental animals, including rabbits, guinea-pigs, white mice, rats, poultry and monkeys. Its own demands of animals have increased considerably during the recent years. Nevertheless, the Institute has made it a point to

TABLE 6

Month	A1/strains related to									A2										B										C
	A1/Liverpool/50					A1/Eire/55		A1/Ned/56																						
	1950	1951	1952	1953	1954	1955	1956	1956	1957	1957	1958	1959	1960	1961	1962	1963	1964	1965	1966	1951	1953	1959	1960	1961	1962	1963	1964	1965	1966	1959
January ...	...	...	...	...	...	...	2	...	1	...	8	..	2	...	...	..	...	1	...	...	...	...	1	...	...	...	2	2	...	...
February...	...	...	...	...	..	...	2	...	...	...	3	...	...	...	1	...	...	...	1	...	...	...	...	...	...	...	1	...	..	...
March ...	...	...	...	..	...	...	...	9	...	...	...	...	10	..	...	...	...	...	1	1	...	...	...	...	...	...	1	...	...	...
April ...	...	...	...	..	..	...	...	...	...	...	...	...	...	2	...	...	...	...	...	...	...	1	.	...	..	...	...	1	...	...
May ...	...	...	...	...	...	...	...	...	...	7	...	2	...	...	2	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...
June ...			...	...	...	1	...	...	...	16	...	...	...	..	...	..	...	...	...	...		...	...	...	..	...	...	...	...	...
July ...	...	...	...	...	...	..	...	...	...	...	11	13	..	...	10	...	...	...	...	...		1	...	...	...	...	...	...	..	...
August ...	3	1	...	...	...	...	...	...	...	...	...	1	...	...	2	9	...	...	...	...	..	2	...	2	1	...	...	...	...	...
September	...	3	...	...	1	...	...	...	...	4	...	...	6	3	1	3	...	...	...	...	..	...	...	...	1	2	3	...	1	...
October ...	.	...	...	...	4	...	...	...	...	...	1	4	3	3	...	2	...	...	...	...	...	..	...	1	...	1	3	1	...	...
November	...	2	...	3	2	...	...	...	...	3	...	...	3	3	...	...	1	...	...	1	1	1	...	1	...	...	1	1	...	...
December	..	...	4	..	..	...	...	...	...	1	...	3	...	10	...	...	1	...	...	...	1	5	...	..	...	...	2	...	2	2

help other institutions and workers by meeting their requirements of animals from time to time.

Garden

The ornamental garden with its beautiful lawns and wealth of rare plants and flowers is one of the finest in the Nilgiris, due largely to the keen interest in its development by the late Lt. Col. K.R.K. Iyengar.

It is obvious that progress in so many diverse fields of activity is hardly possible without the enthusiastic co-operation of the entire staff, particularly when it happens to be small in number. Sri A. Kulla retired on 29–7–1964 after putting in 29 years of meritorious service. Sri A. Joghee retired on 7–9–1963 after 30 years of valuable service.

This period was also marked by a tragic occurrence. Dr. I. G. K. Menon, Deputy Director, who joined the Institute in 1943, suddenly passed away on 30th May 1959 at the age of 52 years. In his premature death the Institute lost a distinguished worker of outstanding ability and a loyal and dedicated servant who gave of his best to the service of the Institute and the ideals it stood for.

In a brief and sketchy account like this it is hardly possible to do justice to the varied activities of the Institute during the past 10 years. Its record of achievement during this period is in itself a promise of things to come and reveals its potential for progress on varied fronts in the future. India to-day stands at the cross-roads of Destiny. In this great endeavour of building up a new India, we can confidently look forward to the contributions of the Institute that has, on the occasion of its Golden Jubilee, adopted as its motto the stirring words of Ulysses: "To strive, to seek, to find and not to yield."

episodes. It was found that the strains isolated from patients in the Pasteur Institute estate and in an orphanage nearby fell into one group, while those from patients in a residential school at Coonoor and from patients at the hospital at Ootacamund fell into another group. The World Influenza Centre, to whom typical strains were sent for examination, reported that the strains in the first group were related to A/Eire/55, while those in the second group were closely related to A/Ned/56. Coonoor thus appeared to have had an outbreak of influenza lasting over 46 days in which two widely differing A-prime virus strains were involved. Thus the gradual shift from the Liverpool subtype over the years was replaced in 1956 by sharp antigenic deviations in the strains isolated.

The first isolations made in January 1957 at Coonoor was found to be related to A/Ned/56.

As reported by Menon (1959), the ship s. s. *Rajula*, arriving from Malaya, was quarantined off the port of Madras on 16 May 1957 with 44 active cases of influenza on board. The earliest cases in the city of Madras following the arrival of the steamer were noticed on 19 May. It was therefore surprising to find cases occurring in the Nilgiri district about 640 km. away on 21 May. The subsequent spread of the infection through the district was very rapid and, by the middle of June, the life in the community was disrupted by the high prevalence of the disease. Thirty-one isolations of Asian (A2) virus were made in 1957, 23 in 1958, 23 in 1959, 24 in 1960, 21 in 1961, 16 in 1962, 16 in 1963, 2 in 1964, 1 in 1965 and 2 in 1966.

The antigenic analysis of 29 representative strains of Asian virus isolated during 1957-62 showed that the strains isolated during 1957-60 were antigenically similar to A2/Singapore/57, while there was a variation in the antigenic make up of 1960-62 isolates.

The 16 strains of Asian virus isolated during 1963 resembled the 1962 isolates at Coonoor. Most of them were antigenically similar to the A2/Ned/63 strain. A few of them isolated during the later part of 1963, however, appeared to resemble A2/Eng/64.

The strains of Asian virus isolated during 1964 and 1965 resembled those isolated during 1963 at Coonoor. They were antigenically similar to A2/Ned/63.

The two strains of Asian virus isolated during 1966 generally resembled A2/Eng/64.

Type B viruses

Isolations. The details regarding the type B viruses isolated during the years 1950–66 and their monthly distribution are given in Tables 5 and 6.

It will be seen that the first type B virus to be isolated at the Centre was in March 1951. The second strain was isolated in March 1951, the third in November 1953, and fourth in December 1953. There were comparatively few isolations during this period owing to the fact that many of the outbreaks which were mild in character were not reported.

However, since 1959, when the programme of isolation of influenza viruses throughout the year was started, the number of isolations of type B viruses has considerably increased. During this period 41 strains have been isolated.

The outbreaks due to type B virus occurred in the district during the following periods: April, July, August, November and December 1959; January 1960; August, October and November 1961; August and September 1962; September and October 1963; January to March and September to December 1964; January, April, October and November 1965; and September and December 1966.

2. OFFICE BEARERS OF THE ASSOCIATION AND STAFF OF THE INSTITUTE SINCE 1907

(a) CERTIFICATE OF INCORPORATION

No. 1 of 1907-08.

I hereby certify, pursuant to Act XXI of 1860 of the Governor-General of India in Council entitled 'An Act for the Registration of Literary, Scientific and Charitable Societies 1860' that the Pasteur Institute of Southern India is duly incorporated as a Society under the aforesaid Act.

Madras,
2nd April 1907.

(Signed) V. Sundararamaiya,
Registrar of Joint Stock Companies.

(b) FIRST MEMBERS OF THE COMMITTEE OF MANAGEMENT OF THE PASTEUR INSTITUTE OF SOUTHERN INDIA, COONOOR

Patrons

His Excellency the Governor of Madras.
His Highness the Nizam of Hyderabad.
His Highness the Maharaja of Mysore.
His Highness the Maharaja of Travancore.
His Highness the Raja of Cochin.

Members

The Hon'ble Justice Sir S. Subrahmanya Aiyar, B.L., K.C.I.E., Diwan Bahadur, Judge, High Court, Madras.
M. R. Ry V. P. Madhava Rao, C.I.E., Diwan of Mysore.
The Hon'ble Mr. M. Hammick, C.I.E., I.C.S., Chief Secretary to Government.
M. R.Ry. Diwan Bahadur S. Gopalachari Avl., Diwan of Travancore.
Surgeon-General W. R. Browne, C.I.E., Surgeon-General with the Government of Madras.
The Hon'ble Mr. J. N. Atkinson, I.C.S., Acting Member of the Board of Revenue.
M. R.Ry. N. Pattabirama Rao Avl., Diwan of Cochin.
Maharaja Sir V. Ranga Rao Bahadur, K.C.I.E., Raja of Bobbili.
The Colonel on the Staff Commanding Southern Brigade.
An Assistant Adjutant-General, 9th (Secunderabad) Division.
The Collector and District Magistrate of the Nilgiris.
Lieutenant-Colonel R. Thomson, I.M.S., Sanitary Commissioner, Madras.
Major F. J. Crawford, I.M.S., Acting Principal, Medical College, Madras.
The Sanitary Officer, 9th (Secunderabad) Division.
The Civil Surgeon, Ootacamund.
The District Medical and Sanitary Officer of the Nilgiri District.
The Rev. W. Miller, LL.D., D.D., C.I.E.

episodes. It was found that the strains isolated from patients in the Pasteur Institute estate and in an orphanage nearby fell into one group, while those from patients in a residential school at Coonoor and from patients at the hospital at Ootacamund fell into another group. The World Influenza Centre, to whom typical strains were sent for examination, reported that the strains in the first group were related to A/Eire/55, while those in the second group were closely related to A/Ned/56. Coonoor thus appeared to have had an outbreak of influenza lasting over 46 days in which two widely differing A-prime virus strains were involved. Thus the gradual shift from the Liverpool subtype over the years was replaced in 1956 by sharp antigenic deviations in the strains isolated.

The first isolations made in January 1957 at Coonoor was found to be related to A/Ned/56.

As reported by Menon (1959), the ship s. s. *Rajula*, arriving from Malaya, was quarantined off the port of Madras on 16 May 1957 with 44 active cases of influenza on board. The earliest cases in the city of Madras following the arrival of the steamer were noticed on 19 May. It was therefore surprising to find cases occurring in the Nilgiri district about 640 km. away on 21 May. The subsequent spread of the infection through the district was very rapid and, by the middle of June, the life in the community was disrupted by the high prevalence of the disease. Thirty-one isolations of Asian (A2) virus were made in 1957, 23 in 1958, 23 in 1959, 24 in 1960, 21 in 1961, 16 in 1962, 16 in 1963, 2 in 1964, 1 in 1965 and 2 in 1966.

The antigenic analysis of 29 representative strains of Asian virus isolated during 1957-62 showed that the strains isolated during 1957-60 were antigenically similar to A2/Singapore/57, while there was a variation in the antigenic make up of 1960-62 isolates.

The 16 strains of Asian virus isolated during 1963 resembled the 1962 isolates at Coonoor. Most of them were antigenically similar to the A2/Ned/63 strain. A few of them isolated during the later part of 1963, however, appeared to resemble A2/Eng/64.

The strains of Asian virus isolated during 1964 and 1965 resembled those isolated during 1963 at Coonoor. They were antigenically similar to A2/Ned/63.

The two strains of Asian virus isolated during 1966 generally resembled A2/Eng/64.

Type B viruses

Isolations. The details regarding the type B viruses isolated during the years 1950–66 and their monthly distribution are given in Tables 5 and 6.

It will be seen that the first type B virus to be isolated at the Centre was in March 1951. The second strain was isolated in March 1951, the third in November 1953, and fourth in December 1953. There were comparatively few isolations during this period owing to the fact that many of the outbreaks which were mild in character were not reported.

However, since 1959, when the programme of isolation of influenza viruses throughout the year was started, the number of isolations of type B viruses has considerably increased. During this period 41 strains have been isolated.

The outbreaks due to type B virus occurred in the district during the following periods: April, July, August, November and December 1959; January 1960; August, October and November 1961; August and September 1962; September and October 1963; January to March and September to December 1964; January, April, October and November 1965; and September and December 1966.

The Hon'ble L. A. Govindaraghava Aiyar, Diwan Bahadur.
The Hon'ble M. Krishnan Nayar Avargal.
The Hon'ble Nawab Raza Khan, Khan Bahadur.
The Hon'ble Mr. V. G. Lynn.
Captain J. W. Cornwall, I.M.S., Director, Pasteur Institute of Southern India, Coonoor.
Captain S. R. Christophers, I.M.S., Superintendent, King Institute of Preventive Medicine, Guindy.

(c) PRESIDENTS OF THE ASSOCIATION

The Hon'ble Surgeon-General W. R. Browne, C.I.E, I.M.S. (21-12-1906 to 7-4-'08).
The Hon'ble Surgeon-General P. H. Benson, I.M.S. (8-4-1908 to 20-5-'11).
Acting Surgeon-General H.St.C. Carruthers, I.M.S. (1908 to '09).
The Hon'ble Surgeon-General W. B. Bannerman, M.D., C.S.I., I.M.S. (21-5-1911 to 3-1-'18).
The Hon'ble Surgeon-General G. G. Giffard, C.S.I., I.M.S. (4-1-1918 to 9-5-'23).
Major-General T. H. Symons, O.B.E., I.M.S. (10-5-1923 to 31-3-'26).
Major-General Hutchinson, C.I.E., K.H.S., I.M.S. (1-4-1926 to 2-8-'28).
Major-General J.W.D. Megaw, C.I.E., V.H.S., I.M.S. (3-8-1928 to 22-8-'29 & 22-2-1930 to 21-3-'30).
Lieut.-Col. C.A.F. Hingston, C.I.E., O.B.E., I.M.S. (23-8-1929 to 21-2-'30 & 22-3-1930 to 15-4-'30).
Major-General C. A. Sprawson, C.I.E., V.H.S., I.M.S. (16-4-1930 to 8-10-'31, 9-2-1932 to 13-7-'33 & 4-10-1933 to 11-10-'33).
Lieut.-Col. R.G.G. Croly, M.B., B.Ch., D.T.M., I.M.S. (9-10-1931 to 8-2-'32 & 14-7-1933 to 3-10-'33).
Lieut.-Col. R.E. Wright, C.I.E., I.M.S. (12-10-1933 to 19-11-'33).
Major-General Sir F.P. Connor, Kt., D.S.O., V.H.S., I.M.S. (20-11-1933 to 28-10-'35, 26-3-1936 to 23-4-'36 & 16-11-1936 to 15-9-'37).
Lieut.-Col. Clive Newcomb, I.M.S. (29-10-1935 to 25-3-'36 & 24-4-1936 to 15-11-'36).
Major-General N.M. Wilson, C.I.E., O.B.E., K.H.S., I.M.S. (16-9-1937 to 18-5-'38 & 21-9-1938 to 22-2-'41).
Lieut.-Col. H.E. Shortt, M.D., D.Sc., I.M.S. (19-5-1938 to 20-9-'38).
Major-General H. Stott, C.I.E., O B.E., K.H S., I.M.S. (23-2-1941 to 16-7-'44).
Major-General J.P. Huban, O.B.E., K.H.S., I.M.S. (17-7-1944 to 28-2-'47).
Dr. P.V. Cherian, M.B.E., F.R.C.S., (Edin.). D.L.O., F.R.F.P.S. (29-2-1947 to 14-5-'47).
Colonel S. L. Bhatia. C.I.E., M.C., M.A., M.D. (Cantab.), F.R.C.P. (Lond.), F.R.S. (E). (15-5-1947 to 12–3–'49 & 11–10–1949 to 28–2–'50).
Dr. A.S. Mannadi Nayar, M.B.,B.S., Ph.D. (13–3–1949 to 10–10–'49).
Dr. J.C. David, M.B.,B.S., Ph.D. (Edin.). (1–3–1950 to 27–4–'51).
Dr. K. Vasudeva Rao, M.D., F.R.C.P. (Lond.), T.D.D. (Wales). (28–4–1951 to 10–11–'51).
Dr. P. Arunachalam, M.D., M.R.C.P. (Lond.), T.D.D. (Wales), D.M.R. (Lond.). (11–11–1951 to 14–3–'53).
Dr. M.V. Ramanamurthi, M.B.,B.S., F.R.C.S. (Edin.). (15–3–1953 to 16–9–'53).
Lieut.–Col Sangham Lal, F.R.C.S. (Eng.), D.O.M.S. (Lond.). (17–9–1953 to 18–9–'57).
Dr. V.R. Thayumanaswami, M.S., F.R.C.S., M.Ch. (Ortho.). (19–9–1957 to 27–9–'61).
Dr. (Kumari) A.B. Marikar, M.D., D.G.O. (28–9–1961 to 4–1–'64).
Dr. P.S. Kumaravelu, M.B.,B.S. (5–1–1964 to 1–4–'64).
Dr. (Kumari) A.B. Marikar, M.D., D.G.O. (2-4-1964 to 15–6–'66).
Dr. T. Janardhanan, M.B.,B.S., M.Sc. (16–6–1966 to 13–12–'66).
Dr. (Kumari) A.B. Marikar, M.D., D.G.O. (Since 14–12–1966).

It is interesting to note that type B virus has been isolated during outbreaks due to A-prime and Asian viruses. In November 1953, out of six throat gargles collected from six patients in the same hospital one morning, Al virus was isolated from three and type B virus from one. Similarly, in a residential school at Ketti, nine throat gargles were collected from children one morning in July 1959. On examination, nine strains of virus were isolated; eight of these were of the Asian subtype while one proved to be type B virus. These findings suggest that both type A and B viruses are prevalent in the district and sometimes appear during the same outbreak.

Antigenic pattern The studies on the antigenic analysis of the B strains carried out by Menon *et al.* showed that the Indian type B strains were far removed from the Lee and Paddington strains, and though closely related to the 1946 Crawley strain were not identical with it. Although they showed a certain degree of identity among themselves, the slow antigenic drift of the later strains from the standard strains of the previous years was apparent.

An antigenic analysis of nine representative strains of type B virus isolated during 1951–62 showed that there was a variation in the antigenic make up of those isolated during 1959–62. These were easier to grow and gave better haemagglutination titres. These strains resembled the type B strains isolated during 1963, which were antigenically similar to B/Johannesburg/33/58. The strains isolated during 1964 resembled B/Johannesburg/33/58 and B/Amakusa/64, while those isolated during 1965 were antigenically related to B/Amakusa/64 and B/Singapore/3/64.

Type C viruses

Our attempts to isolate influenza viruses throughout the year resulted in the isolation, from a small outbreak in a village named Jagathala, of two strains which resembled type C virus. The characteristics of these strains were (a) ability to grow in the amniotic cavity only, (b) haemagglutination of fowl red cells at 4°C., (c) sensitivity to normal rat serum inhibitors, and (d) reaction against Taylor 1233 strain antiserum in haemagglutination and complement fixation tests.

Comments

The Nilgiri district is situated in South India, about 76°E. and 11°N., at an altitude varying from 1700 to 2300 m. above mean sea level. Though representative of the country as a whole in respect of factors such as annual rainfall and nature of population, the district differs from the surrounding areas in the range of temperature experienced. Thus the mean temperature for most of the year is about 16°C. with minimum temperatures varying from —1.7°C. to 11.1°C. and maximum from 18.9°C. to 26.7°C.

Records have been maintained of the maximum and minimum temperatures and relative humidity. An analysis of these data indicates that there is no striking correlation between the prevalence of the disease and these factors.

Epidemiological studies during 1950–57 revealed an increased incidence of the disease in the Nilgiri district in the second half of the year, particularly in the months September to December. Between 1950 and the end of 1955, influenza virus was isolated from 28 cases in the area. Of these, 19 were obtained during the months September to December. The exceptions were (a) three strains isolated in August 1950 which were later found to be the newly appearing Liverpool subtype, (b) a comparatively new type B virus isolated in March 1951 and (c) a new strain isolaetd in June 1955 and shown to be related to A/Eire/55. The four isolations resem-

(d) DIRECTORS OF THE INSTITUTE AND HONORARY SECRETARIES AND TREASURERS

Permanent

Lieut.-Col. J. W. Cornwall, C.I.E., M.A., M.D., D.P.H., I.M.S. (11-7-1906 to 28-2-'26)
Lieut.-Col. T. H. Gloster, M.B., D.P.H., I.M S., (1-3-1926 to 9-4-'28).
Lieut.-Col. K. R. K. Iyengar, C.I.E., O.B.E., M.D., D.P.H., I.M.S. (10-4-1928 to 4-7-'38 & 13-2-1941 to 10-1-'46).
Major H. W. Mulligan, M.D., D.Sc., I.M.S. (5-7-1938 to 12-2-'41).
Lieut.-Col. M. L. Ahuja, M.D., D.P.H., I.M.S. (11-1-1946 to 3-5-'47).
Dr. N. Veeraraghavan, M.B., B.S., D.Sc. (Since 4-5-1947).

Acting

Capt. R. Bryson, I.M.S. (19-4-1909 to 7-5-'09).
Major A. G. McKendrick, M,B., Ch.B., D.Sc., F.R.C.P.E., F.R.S.E., I.M.S. (8-5-1909 to 18-7-'09 & 10-9-1911 to 10-10-'11).
Major J. A. Cruickshank, M B., I.M.S. (11-10-1911 to 27-10-'12, 20-11-1912 to 24-2-'14 & 5-7-1923 to February '24).
Capt. R. E. Wright, I.M.S. (28-10-1912 to 19-11-'12).
Major W. S. Patton, M.B., F.E.S., I.M.S. (30-12-1919 to 22-12-'20).
Dr. I. G. K. Menon, M.B.B.S., Ph.D. (27-1-1951 to 25-11-'51).

(e) DEPUTY DIRECTORS

Dr. I. G. K. Menon, M.B., B.S., Ph.D. (1-4-1957 to 30-5-'59).
Dr. P. V. Kurian, M.B., B.S., D.T.M., M.S. (Since 1-10-1962).

(f) ASSISTANT DIRECTORS

Major A. G. McKendrick, M.B., Ch.B., D.Sc., F.R.C.P.E., F.R.S.E., I.M.S. (15-8-1908 to 29-12-'08, 19-7-1909 to 12-1-'10 & 23-3-1910 to 9-9-'11).
Dr. M. Kesava Pai, M D. (31-12-1908 to 18-7-'09 & 13-1-1910 to 22-3-'10).
Major J. A. Cruickshank, M.B , I.M.S. (10-9-1911 to 10-10-'11 & 25-2-1914 to 4-7-'14).
Captain R. E. Wright, I.M.S. (3-11-1911 to 27-10-'12 & 20-11-1912 to 24-2-'14).
Dr. H. M. LaFrenais, I.M.D. (5-7-1914 to 22-7-'16 & 17-10-1918 to 24-5-'24).
Dr. W. A. Beer, I.M.D. (25-5-1924 to 20-3-'37).
Dr. G. L. C. Philipsz, I.M.D. (24-3-1937 to 5-7-'41).
Dr. N. Veeraraghavan, M.B., B.S., D.Sc. (6-7-1941 to 3-5-'47).
Dr. I. G. K. Menon, M.B.B.S., Ph.D. (4-5-1947 to 26-1-'51 & 26-11-1951 to 31-3-'57).
Dr. P. V. Kurian, M.B., B.S., D.T.M., M.S. (3-12-1948 to 26-10-'49, 27-1-1951 to 25-11-'51 & 1-6-1959 to 30-9-'62).
Dr. A. Balasubramanian, M.B., B.S., M.Sc. (Since 1-10-1962).

(g) SENIOR RESEARCH OFFICERS

Dr. P. V. Kurian, M.B., B.S., D.T.M., M.S. (1-4-1959 to 31-5-'59).
Dr. A. Balasubramanian, M.B., B.S., M.Sc. (1-4-1959 to 30-9-'62).
Dr. D. S. Chandrasekhar, B.Sc., M.B.B.S., M.Sc. (1-7-1964 to 31-8-'65).

bling A/Eire/55 during January to March 1956, nine isolation resembling A/Ned/56 in March 1956, and one isolation resembling A/Ned/56 in January 1957 also proved to be new variants. The totally new Asian strain appeared in May 1957, and was isolated in different months during 1957–66. These findings suggest the possibility that the appearance of the disease at unusual periods of the year may be related to the emergence of new strains of virus. When a virus strain is well seeded in the community, resulting in a partial degree of immunity in the population, it may require a combination of factors such as overcrowding and inclement weather for it to cause outbreaks, generally of a localized pattern. On the other hand, when a new variant appears, against which the population has little or no immunity, it does not need the accessory favouring factors necessary in the case of well-established strains, but spreads regardless of climatic or other factors. Needless to say, its spread will be accelerated if favourable factors also coexist at the time of its first appearance.

A study of the occurrence of influenza outbreaks in the district during the past seventeen years indicates that the pattern does not conform to the two- or three-year cycle usually regarded as typical of influenza type A epidemics.

It will be seen from Table 5 that 159 strains of Asian virus were isolated during the period May 1957 to December 1966, but not a single A-prime virus was encountered. The total disappearance of the earlier virus with the arrival of an antigenically different strain is indeed remarkable.

These studies showed that both type A and type B strains were prevalent in the district and sometimes appeared during the same outbreak, and that strains differing appreciably in antigenic and biological characteristics could be obtained in the same outbreak from patients living in contiguous houses.

It was also found that the various strains of each type or subtype differed considerably from each other and from the respective type-strains in their biological characteristics and antigenic make-up.

(2) BIOLOGICAL CHARACTERISTICS OF INFLUENZA VIRUS STRAINS ISOLATED DURING 1950–66

The occurrence of the different types of strains in the same or nearby localities led us to investigate the biological characteristics of the strains isolated at the Centre during 1950–66. The properties studied were (a) reaction to erythrocytes, (b) sensitivity to inhibitors present in normal sera, (c) effect of heat, and (d) effect of ether.

Haemagglutination with erythrocytes from different animal species

Type A viruses. One of the strains (A1/Coonoor/2/52), isolated in 1952 and characterized as unique by the World Influenza Centre, was found to agglutinate all the erythrocytes tested.

Out of the 159 strains of Asian virus isolated during 1957–66, 71 representative strains were selected for study.

It was found that all strains agglutinated fowl, guinea-pig, monkey and horse erythrocytes.

Sheep erthyrocytes were agglutinated by all the 13 strains of Asian virus isolated during 1957–60. Of the 21 strains isolated during 1961, eleven strains did not agglutinate sheep cells, 8 agglutinated them in a low titre, and 2 in good titre. Of the 16 strains isolated during 1962, four strains did not agglutinate sheep cells, 5 agglutinated them in a low titre, and 7 in a good titre. Of the 18 isolates obtained during 1963–64, thirteen agglutinated sheep erythrocytes in a good titre and 5 in a low titre. None of the 3 strains isolated during 1965-66 agglutinated sheep red cells.

(h) RESEARCH OFFICERS

Dr. P. V. Kurian, M.B., B.S., D.T.M., M.S. (1–4–1957 to 31–3–'59).
Dr. A. Balasubramanian, M.B., B.S., M.Sc., (1–4–1957 to 31–3–'59).
Dr. D. S. Chandrasekhar, B.Sc., M.B., B.S., M.Sc. (1–11–1960 to 30–6–'64).
Dr. Y. M. V. Shenoy (22–6–1963 to 31–1–'65).
Dr. Vasant Pandit (Since 1–12–1966).

(i) VETERINARY OFFICER

Dr. P. Subramanyam, B.V.Sc., M.S., Ph.D. (4–9–1959 to 30–4–'61).

(j) MEDICAL OFFICERS

Dr. M. Kesava Pai, M.D. (21–2–1907 to 30–12–'08 & 23–3–1910 to 29–1–'11).
Dr. Kamath. (24–3–1909 to 18–7–'09).
Dr. LaFrenais, I.M.D. (25–5–1914 to 4–7–'14).
Dr. T. Kesava Menon. (11–3–1916 to 4–5–'19).
Dr. S. Ramaswamy Aiyar. (11–2–1907 to 13–2–'17).
Dr. K. Narayana Menon. (8–4–1912 to 3–9–'14, 23–11–1914 to 13–5–'15).
Dr. P. Murugesa Mudaliar. (1–4–1911 to 3–4–'16).
Dr. V. S. Ranganathan. (2–2–1917 to 26–12–'19).
Dr. A. I. Samuel. (29–3–1917 to 3–7–'17).
Dr. S. Sundara Rao. (9–11–1918 to 12–2–'21).
Dr. M. Anandan. (1–12–1919 to 30–12–'21).
Dr. S. Sitarama Sastry. (13–2–1921 to 31–3–'34).
Dr. K. Kannan Nambiar. (1–11–1921 to 30–6–'22).
Dr. M. Raman Nambiar. (1–7–1922 to 6–9–'33).
Dr. T. Vaitheswaran. (7–9–1933 to 3–5–'38).
Dr. K. Varadarajan. (1–4–1934 to 4–11–'35).
Dr. T. P. Balaji Rao. (16–11–1935 to 20–1–'37).
Dr. H. Govinda Gadiyar. (21–1–1937 to 10–8–'37).
Dr. I. P. Sankara Menon. (11–8–1937 to 31–5–'39).
Dr. P. M. Raghavan. (1–6–1938 to 17–4–'39).
Dr. N. Veeraraghavan, M.B.,B.S., D. Sc. (1–8–1937 to 5–7–'41).
Dr. C.B. D'Silva, M.B., B.S. (1–6–1939 to 3–4–'43).
Dr. I. G. K. Menon, M.B.B.S., Ph. D. (27–4–1943 to 3–5–'47).
Dr. P. V. Kurian, M.B.B S., D.T.M., M. S. (10–2–1947 to 31–3–'57).
Dr. A. Balasubramanian, M.B.,B S , M.Sc. (28–9–1947 to 31–3–'57).
Dr. D. S. Chandrasekhar, B. Sc , M.B.,B.S., M. Sc. (21–6–1954 to 31–10–'60).
Dr. C. K. Jayaram Paniker, M.B.,B.S., (17–10–1955 to 16–8–'58).
Dr. C. R. Raghu, B. Sc., M.B.,B.S. (3–11–1958 to 30–6–'59).
Dr. S. Rangaratnam, M.B .B.S., D.P.H. (5–8–1959 to 15–3–'60).
Dr. A. V. Ramana Reddy, B. V. Sc., M.B.,B.S. (27–1–1960 to 21–7–'62).
Dr. Vasant Pandit, M.B.,B.S., M. Sc. (1–3–1961 to 30–11–'66).
Dr. (Kumari) P. Krishna Kumari, M.B ,B S. (I–10–1962 to 30–9–'64).
Dr. (Kumari) S. Visalakshi, M.B.,B.S. (11–5–1964 to 31–8–'64).
Dr. J. Sampath Kumar, M.B.,B.S. (Since 2–3–1965).
Dr. Mathew Francis, M.B.,B.S. (Since 8–3–1965).
Dr. (Mrs) Mary Chakko, M.B.,B.S. (Since 1–12–1966).

Thirteen strains of Asian virus isolated during 1957-60 did not agglutinate calf erythrocytes. Of the 21 strains isolated during 1961, eleven did not agglutinate calf cells, 5 agglutinated them in a low titre and 5 in a good titre. Of the 16 isolates obtained during 1962, eight did not agglutinate calf cells, 6 agglutinated them in a low titre and 2 in a good titre. Of the 16 strains isolated during 1963, seven did not agglutinate calf cells, 8 agglutinated them in a low titre, and only one in a good titre. The strains isolated during 1964 agglutinated calf cells only in a low titre. None of the 3 strains isolated during 1965 and 1966 agglutinated calf erythrocytes.

Type B viruses. Out of 45 strains of type B virus isolated during 1950–66, 35 representative strains were selected for study.

All the strains agglutinated fowl, guinea-pig, calf and horse erythrocytes. But, it was found that agglutination titres obtained with type B viruses isolated during 1964–65 with sheep, calf and monkey cells tended to be lower than those given by the earlier isolates.

Sensitivity to inhibitors of normal animal sera

Type A viruses. The sensitivity to inhibitors of normal animal sera of 71 representative Asian strains isolated during 1957–66 was determined by the haemagglutination-inhibition test.

About 70%–94% of the sera from normal sheep, calf, rabbit, monkey, fowl, guinea-pig and horse contained inhibitors against these strains. They were generally heat-stable. The inhibitors present in 2% of the sera from calves and horses, 7%–8% of the sera from fowls, guinea-pigs and monkeys, 16% of the sera from rabbits and 24% of the sera from sheep were not destroyed by periodate treatment.

Type B viruses. Twenty-five representative strains of type B virus were studied. Inhibitors were generally absent against these strains in fowl sera. About 83%–97% of the sera from sheep, rabbits, guinea-pigs, calves and monkeys contained inhibitors against them. Of these, 60%–100% were heat-stable. The inhibitors present in 47% of the sera from calves, 50% of the sera from guinea-pigs, 60% of the sera from sheep, 80% of the sera from monkeys and 83% of sera from rabbits were not destroyed by periodate treatment.

Action of heat

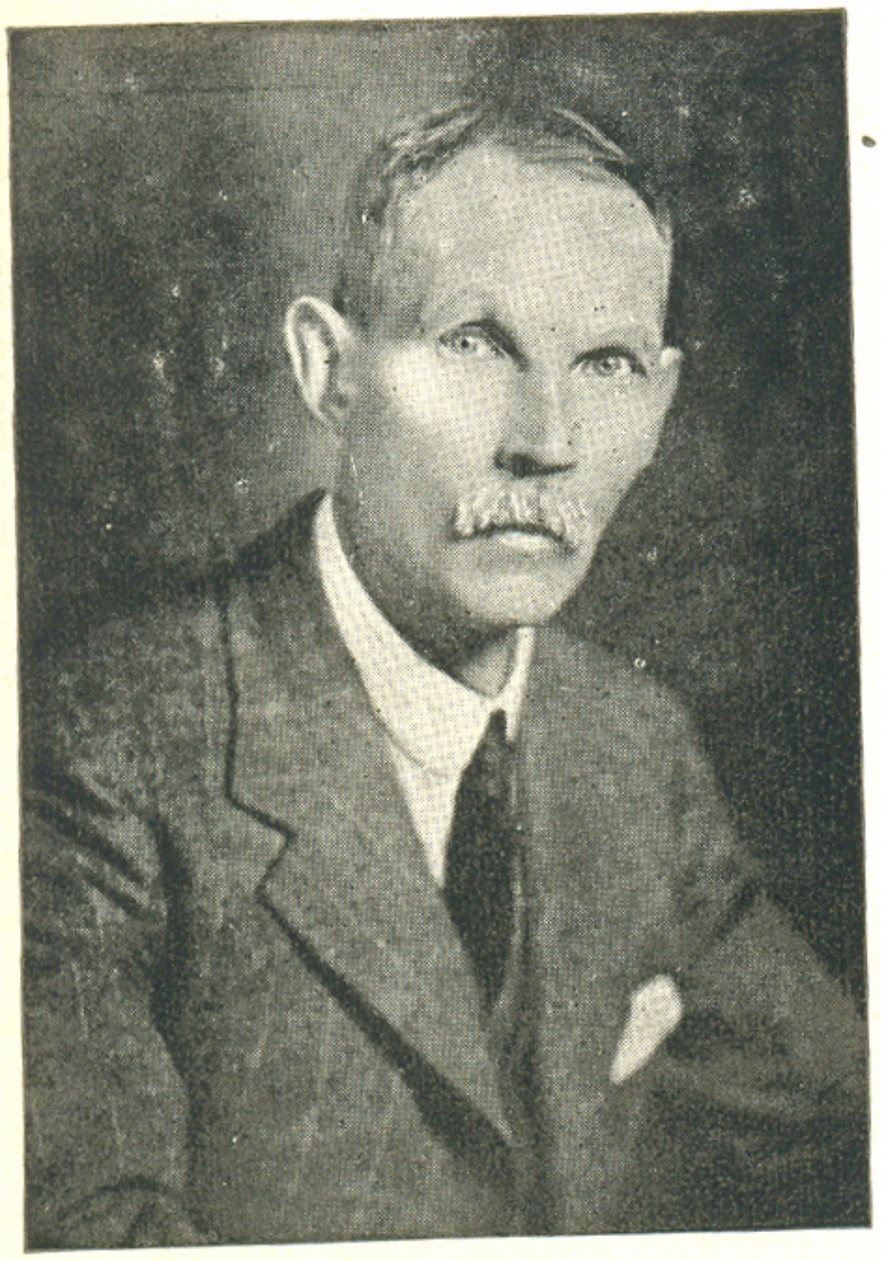
Type A viruses. The effect of heat on haemagglutination by 23 representative strains of Asian virus isolated during 1957–66 was determined. With fowl erythrocytes, HA titres decreased with fourteen, remained unaltered with eight, and increased with one strain. With guinea-pig red cells, the HA titres decreased with sixteen, remained unaltered with six, and increased with one strain.

Type B viruses. Twenty representative strains were tested. With fowl erythrocytes, HA titres decreased with eleven, remained unaltered with seven, and increased with two strains. With guinea-pig red cells, HA titres decreased with eight, remained unaltered with four, and increased with eight strains.

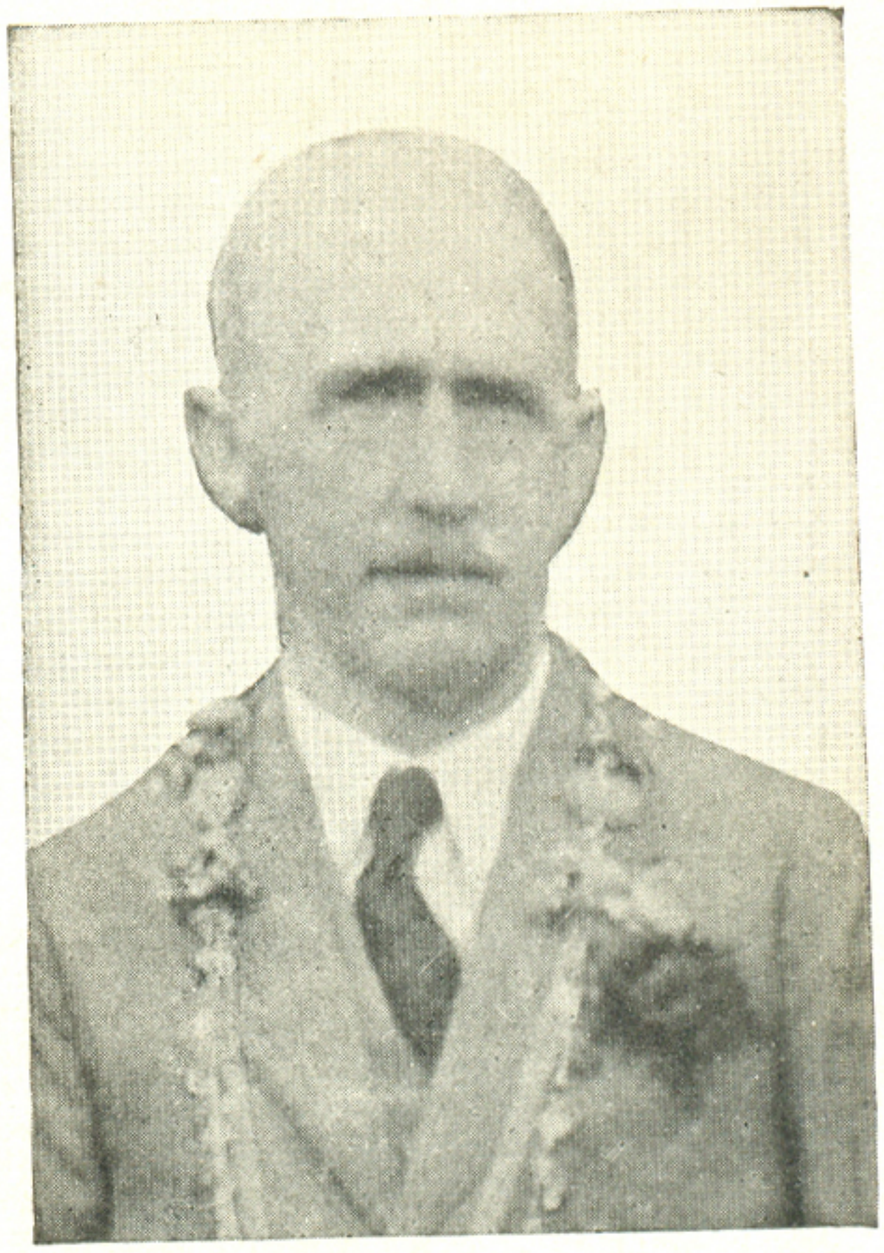
Action of ether

Type A viruses. Twenty-three representative Asian strains were tested. With fowl erythrocytes, HA titres decreased with seven, remained unaltered with eleven and increased with five strains. With guinea-pig red cells, HA titres were unaltered with seven and increased with sixteen strains.

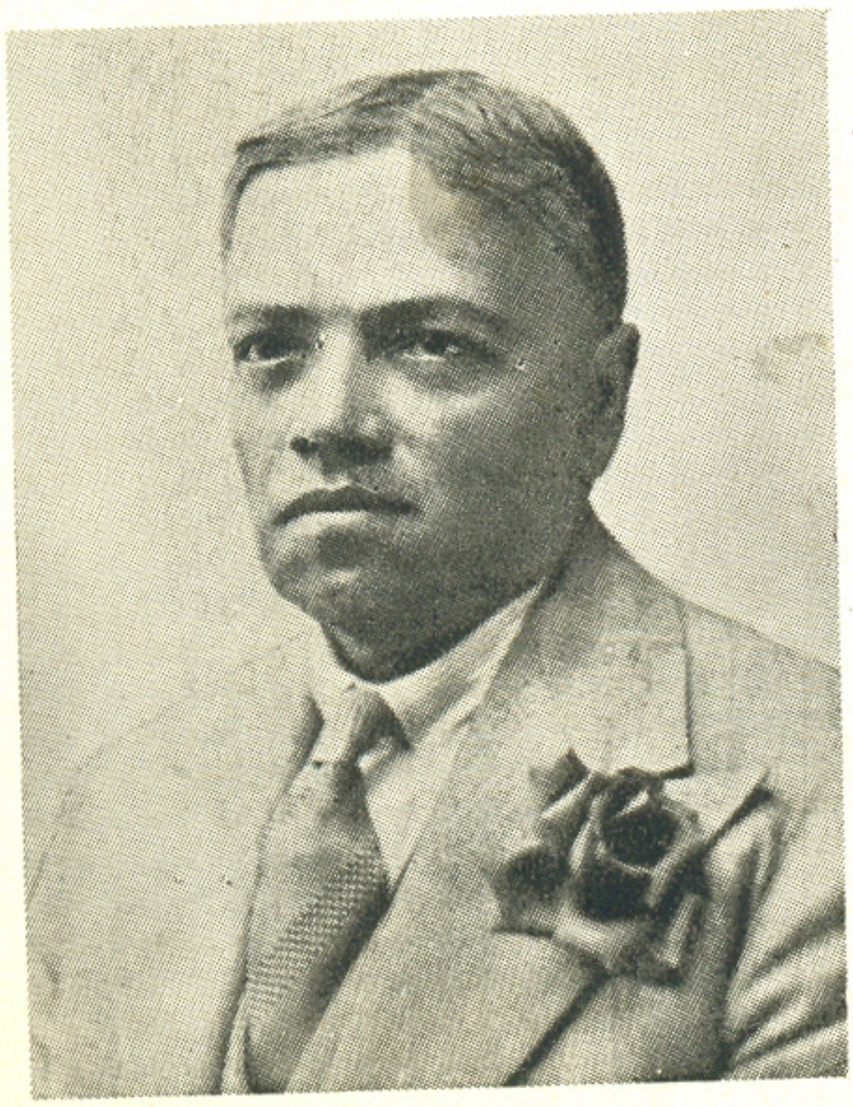
Type B viruses. Twenty-one strains were studied. With fowl erythrocytes, HA titres decreased with six, remained unaltered with eleven, and increased with two strains. With guinea-pig cells, the HA titres were unaltered with five and increased with sixteen strains.



LIEUT-COL. J.W. CORNWALL, C.I.E., M.A., M.D., D.P.H., I.M.S.
Director (1907–'26)



LIEUT-COL. T. H. GLOSTER, M.B., D.P.H., I.M.S.
Director (1926–'28)



LIEUT-COL. K. R. K. IYENGAR, C.I.E., O.B.E., M.D., D.P.H., I.M.S.
Director (1928–'38 & 1941–'46)



COL. H. W. MULLIGAN, C.M.G., M.D., D.SC
I.M.S.
Director (1938–'41)

Comments

It was found that all the Asian strains agglutinated fowl, guinea-pig, monkey and horse erythrocytes. While all the strains isolated during 1957–60 agglutinated sheep red cells, only some of them isolated during 1961–64 agglutinated them in a good titre. The isolates obtained during 1965 and 1966 did not agglutinate sheep cells. Similarly, the strains isolated during 1957–60 did not agglutinate calf erythrocytes, while some of those isolated during 1961–64 agglutinated them in varying titres. The isolates obtained during 1965 and 1966, however, did not agglutinate calf red cells.

All the type B isolates, except one A1 strain isolated in 1951, agglutinated fowl, guinea-pig, monkey, calf and horse erythrocytes.

It was found that the A1 strains isolated during the period 1950–56 varied in their sensitivity to normal sheep and calf serum inhibitors. The absence of inhibitors in fowl sera against these strains was striking. The Asian strains were found to be susceptible to inhibitors present in sheep, calf, rabbit, monkey, fowl, guinea-pig and horse sera. They were generally heat-stable and were destroyed by periodate.

It was found that except for normal horse serum all the animal sera tested contained inhibitors in low titres against most of the type B strains. They were not destroyed by periodate.

Heating at 56°C. for 30 minutes was found to reduce the HA titres of a majority of the Asian and type B strains.

Ether treatment increased the HA titres of some of the Asian strains. With the other A2 and type B strains it was observed that though there was no increase in fowl cell titre, guinea-pig cell titres increased considerably.

(3) INFLUENZA VIRUS

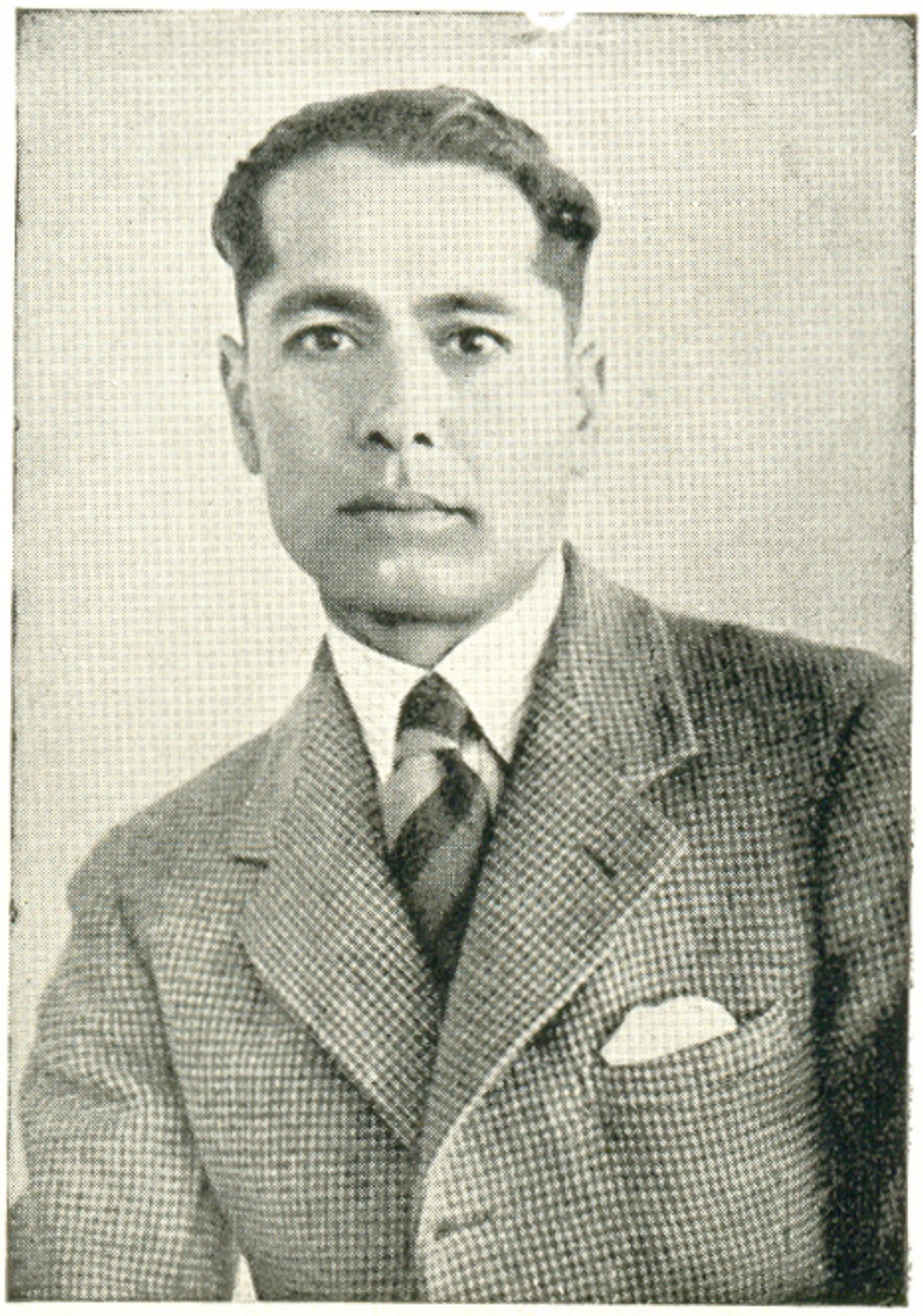
Attempts to increase the yield of influenza virus from stored infected allantoic fluids

Virus infected allantoic fluids which were stored at -20°C. for some time and then thawed often showed a fine precipitate or coarse deposit, the amount being dependent to some extent on the period of storage and the frequency of freezing and thawing. The presence of precipitate seemed to be associated with a fall in the haemagglutination titre of the supernatant fluid. On investigation these precipitates were found to contain considerable amount of virus. Sometimes the virus content of the precipitate exceeded that of the supernatant, thus accounting for the irregular results on titration.

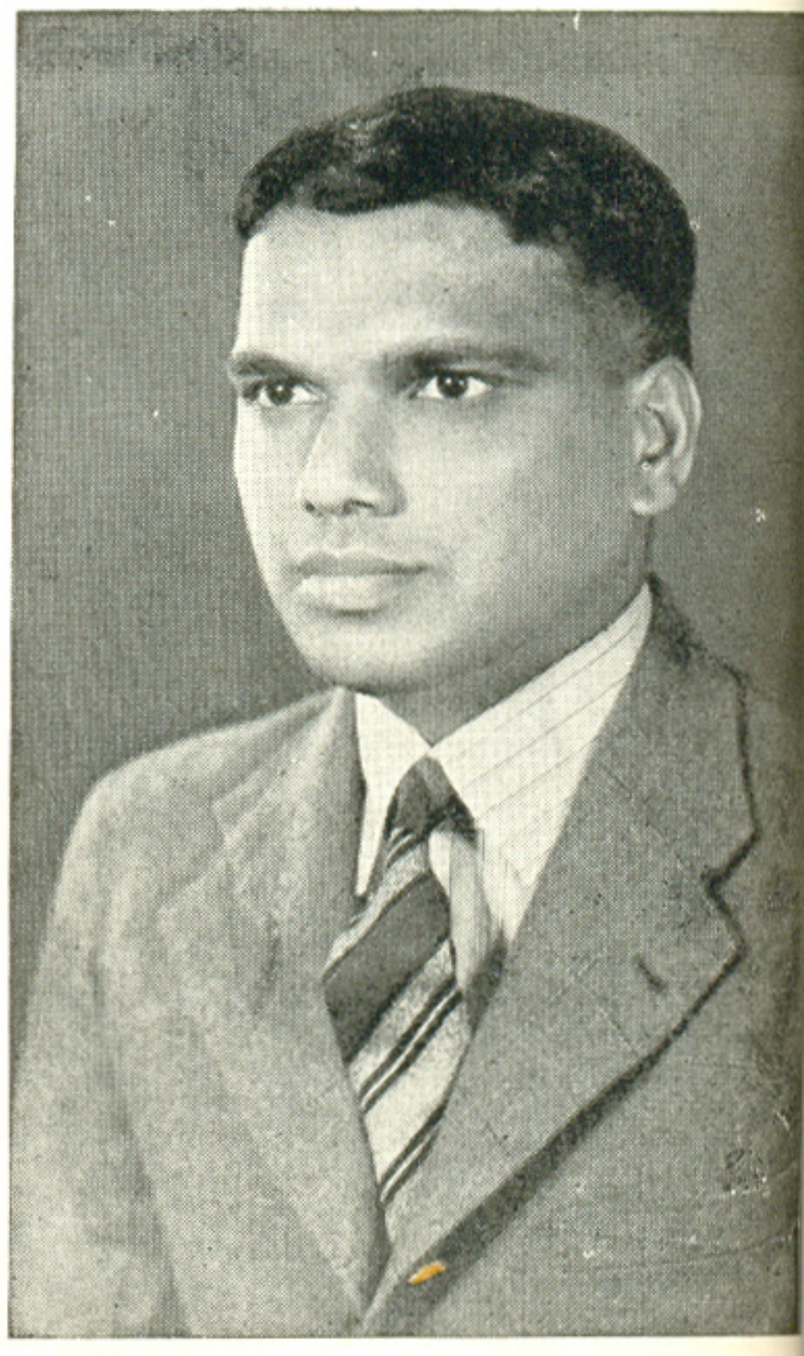
Studies were undertaken to recover the virus present in such fine precipitates and coarse deposits. It was found possible to recover all the virus by using appropriate concentrations of sodium chloride.

It was found that the haemagglutinin and virus content of the deposits was considerable, and that treatment with 1.25M NaCl was selective and superior to the other methods tried for recovering the virus present in the deposit. The results indicated that the virus recovery was better by treating the deposit separately with 1.25M NaCl and adding it to the supernatant than by treating the pool with NaCl in a final concentration of 1.25M NaCl. The total yield obtained by treatment of the deposit with 1.25M NaCl was 50%–100% higher with all the strains tested.

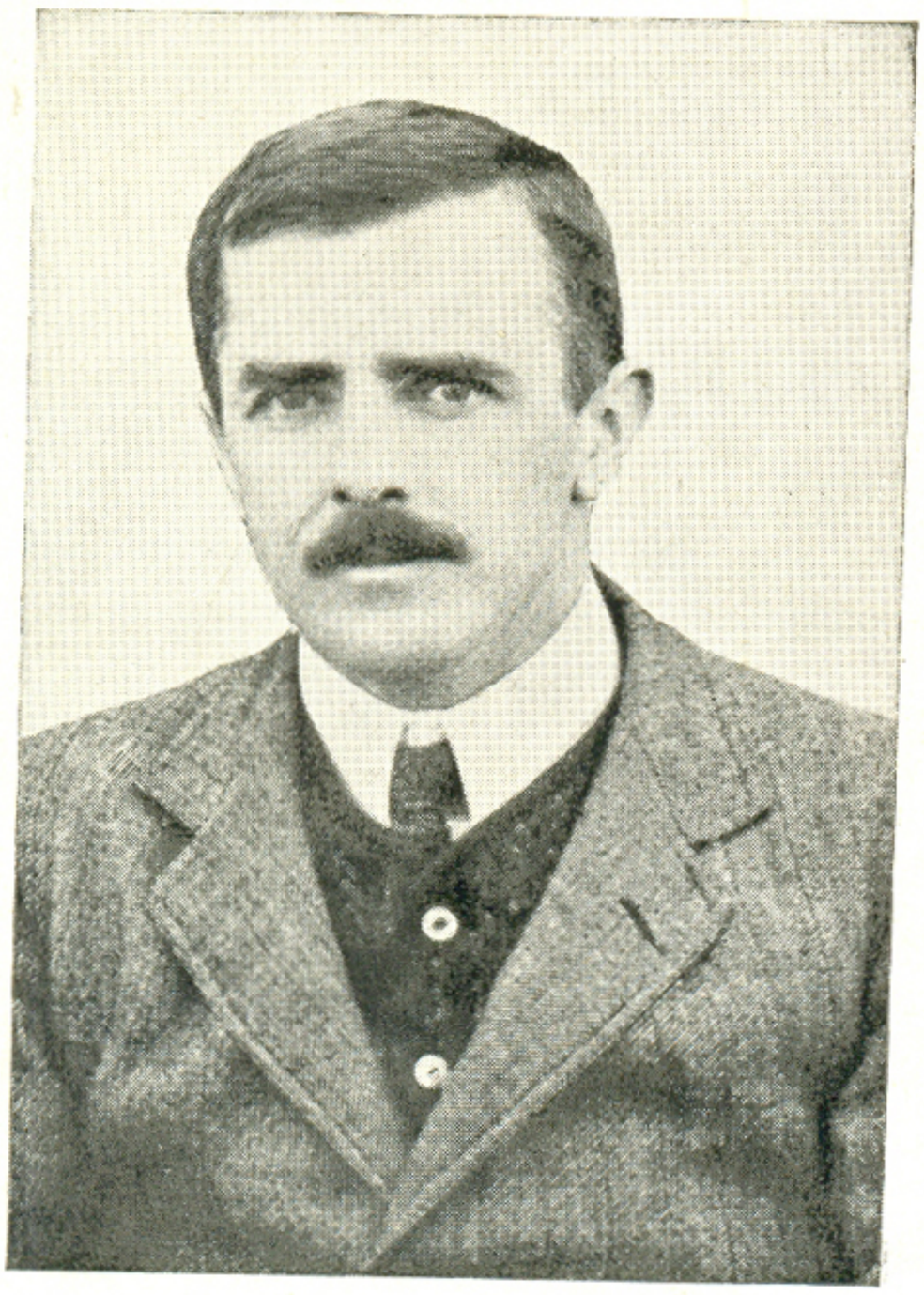
Our studies showed that irrespective of the method employed there was a loss in virus recovery, the degree of which depended on the strain of virus and the



LIEUT-COL. M.L. AHUJA, M.D., D.P.H., I.M.S.
Director (1946–'47)



DR. N. VEERARAGHAVAN, M.B., B.S.,
Director 1947–



LIEUT-COL. A.G. MCKENDRICK, M.B.,
CH.B., D.SC., F.R.C.P.E., F.R.S.E., I.M.S.
Acting Director (1911)



MAJOR J.A. CRUICKSHANK, M.B., I.M.
Acting Director (1912–'15)

method of purification employed. But, with NaCl treatment and subsequent purification by any one of the methods recommended, this loss would be made good. This should prove useful in large-scale production of influenza vaccine when the material has to be stored for some time.

Concentration and purification of influenza virus

There are several methods which could be used for the concentration and purification of influenza virus. But, since Stanley's paper in 1944 comparing the four methods—(a) high-speed centrifugation, (b) adsorption on and elution from adult fowl red cells, (c) freezing and thawing, and (d) cell adsorption and elution of the virus using embryonic red cells—there has been no report on the relative merits of the newer techniques. It seemed desirable to assess the efficacy of the newer methods in order to determine which of them would be most suitable for the production of influenza vaccine on a large scale. With this object in view the methods employing (a) high speed centrifugation, (b) red cell adsorption and elution, (c) methanol precipitation and (d) adsorption on aluminium phosphate gel and elution were studied. These were compared with a modification of the method using zinc hydroxide described by Newton & Bevis (1959) for the purification of animal viruses such as vesicular stomatitis and vesicular exanthema of swine.

The methods chosen were those which could be used in the large-scale production of influenza vaccine. In certain cases they were modified to suit this purpose. The aim was to recover by simple methods a relatively pure antigen without much loss in the haemagglutinin and virus contents during processing.

The zinc hydroxide method employed did not give the degree of purification required and was, therefore, considered to be of not much value. The red cell adsorption method gave very good results with A-prime as well as the Asian strains which had undergone several egg passages. But, with low egg passage Asian strains the haemagglutinin and virus yields were poor. Apart from this, our experience during the 1957 pandemic was that in spite of all care there was a certain degree of lysis of red cells during processing, resulting in a coloured product. This fact, together with the large quantities of red cells required, rendered this method not quite suitable for large-scale production. Two cycles of aluminium phosphate treatment gave the purest product. Ultracentrifugation with or without methanol treatment was only slightly inferior. When methanol precipitation method described by Cox *et al.* was employed, strict adherence to conditions regarding temperature etc., was essential. The aluminium phosphate method had the advantage that it was relatively simple and could be carried out with the minimum facilities.

Cultivation of influenza virus *in vitro*

Studies were undertaken at the Coonoor Influenza Centre in 1959 to evolve a method for the cultivation of influenza virus *in vitro* which would be suitable for growing the virus in large quantities and which would prove as sensitive as the fowl egg for puposes of titration of the virus and its antibodies. Attempts were made to grow the virus in monkey kidney cell cultures but the titres of haemagglutinins and virus obtained were poor. The technique of 'allantois-on-shell' developed by Fulton & Armitage (1951) and modified by Fazekas de St Groth & White (1958) were then tried but the haemagglutinin titres obtained were low.

Eaton *et al.* (1960) reported that with minced chorioallantoic membrane (CAM) tissue cultures using the sodium-pyruvate glucose medium described by them, the

virus titres were about one log value less than the egg titres. Our studies with the low egg passage Asian virus indicated that the titres in tissue cultures with the above medium were about 2 log values less. Systematic studies were undertaken to improve the medium so that the results with CAM tissue cultures were as good as those obtained in the egg.

Early in the investigations it was observed that low haemagglutination titres were associated with clumping of the CAM fragments. Attempts were made to prevent the clumping by coating the bottom of the roller tubes with agar and by the addition of small sterile beads. The results with both the methods were unsatisfactory. Glass wool was tried next and gave excellent results. The fragments of membrane adhered to the glass wool, and in bottles they formed almost a sheet. After an hour in the roller drum all the pieces of membrane were found adhered to the glass wool, leaving the medium clear. Microscopic studies indicated that the fragments of membrane attached to the glass wool showed a certain degree of fibroblastic and epithelial cell proliferation. The success of the method evolved was in a large measure due to use of glass wool.

A large number of compounds singly and in different combinations were tested for their growth promoting properties. These consisted of acids and their salts; amides, amines and amino alcohols; amino acids, carbohydrates including sugars, alcohols and glycosides; nucleic acids; polypeptides; purines and pyramidines; trace elements; growth factors and vitamins. It was found that a medium containing sodium pyruvate, glycine, sodium molybdate, folic acid and glycerol (Medium V), and another containing sodium pyruvate, glycine, glycerol, pimelic acid and putrescine gave very good results. In view of the possibility of putrescine being toxic to human beings the former medium was selected and the method of cultivation was standardized.

The method developed for the cultivation of influenza virus is simple and elegant. Medium V is easy to prepare and gave good results with the different strains of virus used.

The CAM tissue culture method using medium V was comparable to the egg in its sensitivity for purposes of titration of different strains of influenza virus and their neutralizing antibodies.

It was economical in as much as about 25 culture tubes can be put up with CAM obtained from one egg. The saving in the number of eggs required, in incubator space, and in the labour involved in handling a large number of eggs before and after inoculation was obvious. For instance, in a small roller drum it was possible to produce about 1750 ml. of virus, which would otherwise mean, inoculation, incubation and collection of allantoic fluid from about 500 eggs. Further, the variations due to differing susceptibilities of eggs could be avoided by employing this method.

The greatest advantage of this method was the possibility of growing different strains of influenza virus in large quantities in a short time. This should prove useful in the large-scale production of influenza vaccine.

It was found that the method could be used for the production of good complement fixing antigens for diagnostic purpose.

An interesting observation was that the recent Asian virus isolates could be cultivated readily, immediately after amniotic isolation. But primary isolation of Asian virus from throat gargles was not possible. Also, the mouse adapted Asian strains could be readily cultivated by this method.

Cultivation of pure influenza virus

Elimination of tissue nitrogen

Studies on the purity of the culture virus obtained by using medium V and the glass wool technique showed that its nitrogen content varied from 0.1832 to 0.2832 mg. per ml. while that of the infected allantoic fluid ranged between 0.6561 and 0.7131 mg. per ml. The nitrogen content of the virus purified by ultracentrifugation and made up to the original volume of the allantoic fluid with medium V varied from 0.1196 to 0.1416 mg. per ml., the nitrogen content of the medium being 0.04 mg. per ml. Since the nitrogen content of the culture virus was higher than that of the ultracentrifuged material experiments were undertaken to enhance its purity.

Preliminary investigations showed that the higher nitrogen content of the culture virus was due partly to the inadequacy of the method used for washing the CAM and partly to the tissue nitrogen from the CAM liberated into the medium during rolling. It was found possible to eliminate most of the tissue nitrogen by rolling the CAM on glass wool with medium V for 3 hours at 37°C., removing the medium, washing the CAM with two changes of Hanks' balanced salt solution without glucose (BSS) and then adding fresh medium V followed by the virus. By this procedure it was possible to obtain water clear cultures of influenza virus, whose purity as judged by tissue culture infective doses ($TCID_{50}$) per mg. of nitrogen was as good as the material purified by ultracentrifugation.

This procedure, however, was considered rather uneconomical in view of the large quantities of medium V that had to be used for the preliminary rolling of CAM on glass wool. Experiments undertaken to determine the constituent of the medium V responsible for the elimination of nitrogen indicated that it was glycerol. Controlled investigations showed that preliminary rolling of CAM with BSS containing 0.4% glycerol (Analar) for three hours at 37°C. gave consistently good results, although the final virus yields tended to be slightly lower. It was found that washing with two changes of BSS could be dispensed with provided the pH of the medium V added was adjusted to 7.3 instead of 7.1. Another improvement found beneficial was to roll the glass wool in the culture bottles with sterile, double-distilled water containing penicillin and streptomycin for one hour at 37°C. prior to the addition of glycerinated BSS. This removed the traces of alkalinity which tended to persist in the glass wool in spite of careful acid treatment and washing.

Improvement of virus titres of cultures

Improvement of the medium. It was obvious that the above method, which was more economical, should prove very useful if the virus titres of the cultures could be stepped up. Attempts were therefore made to improve the medium.

(i) It was found that the addition of L–glutamic acid and L-aspartic acid in a concentration of 10 mg. and 5 mg. respectively per 100 ml. of the medium V gave consistently good results. The improved medium was designated as **medium V1.**

The HA and $TCID_{50}$ per ml. titres obtained with medium V1 were generally greater than those obtained with medium V. The high $TCID_{50}$/HA values indicated that in each case complete virus was being produced. The results were similar when the strains were grown in 350 ml. volumes.

The $TCID_{50}$ per mg. of nitrogen values showed that the culture viruses obtained with medium V1 were purer than those obtained with medium V and as good as those purified by ultracentrifugation.

The culture using medium V1 was also found to be more sensitive than that employing medium V for purposes of titration of different strains and their antibodies.

The pure virus obtained by this method should prove useful for live virus immunization against influenza.

(ii) Attempts were made to improve the medium V1 with a view to increase the concentration of influenza virus obtained in CAM cultures. It was found that better titres were obtained with a medium containing only one-fifth the concentration of sodium molybdate and folic acid used in medium V1. This improved medium was designated as medium V2.

(iii) Attempts are continuously being made to improve the medium V2. The effect of adding several compounds have been investigated and are presented in the Scientific Reports for the years 1963 – 66. Of the various compounds tested, only sodium succinate and *i*-inositol added to the medium V2 appeared to have some beneficial effect on the growth of Asian and type B influenza viruses in CAM tissue cultures *in vitro*.

Age and concentration of CAM

(i) Investigations were undertaken to determine the influence of age of CAM on the final virus titre. It was found that a higher titre of virus was obtained when CAM of 11-day-old embryos was used.

(ii) The final concentration of 50% washed CAM used for cultures was 0.07 ml. per ml. The possibility of stepping up the virus yields by increasing the concentration of CAM added to the culture was studied. It was found that there was no advantage in increasing the concentration of CAM.

(iii) Experiments were undertaken to determine whether the titres of influenza virus in CAM cultures could be stepped up by adding fresh minced CAM and, if so, the optimum period at which the addition should be made. It was found that the titres could be stepped up by the addition of extra CAM. The optimum period for the addition of CAM was 48 hours from the commencement of incubation of the culture.

Cultivation of influenza virus in duck eggs

In embryonated eggs

In view of the difficulty of obtaining fowl eggs in large numbers during an emergency, experiments were undertaken to determine whether duck eggs could be used for the cultivation of influenza virus.

It was found that duck eggs could be used for the cultivation of the Asian and B type viruses. Twelve- to 14-day-old eggs gave better results than 10-day-old eggs. The haemagglutination titres obtained were generally higher if the allantoic fluid was harvested after incubation at 37°C. for 48 to 72 hours. The haemagglutination titres obtained with duck eggs were much lower than those obtained with fowl eggs but the infectivity titres were high. They were, however, slightly lower than those obtained with fowl eggs.

Based on these results studies were undertaken to determine whether embryonated duck eggs could be used for the primary isolation of influenza virus from throat gargles. Fifteen-day-old duck eggs were used for amniotic inoculation and the results compared with those obtained when fowl eggs were employed.

In the throat gargles tested it was found possible to isolate the strains by amniotic

inoculation of duck as well as chick embryos.

In duck CAM tissue culture

In view of the finding that the infectivity titres of allantoic fluids of infected duck eggs were high, experiments were undertaken to cultivate influenza virus strains in duck CAM cultures *in vitro* using the glass wool technique and medium V2 and compare the titres with those obtained when fowl CAM was used.

It was found that haemagglutination titres of the cultures using duck CAM were lower than those where chick embryo CAM was employed. Good virus titres were obtained in cultures with all the strains when duck CAM was used. They were however slightly lower than those obtained using chick CAM.

Growth cycle of influenza virus cultivated *in vitro*

The growth cycle of some strains of influenza virus cultivated *in vitro* in CAM tissue cultures using the glass wool technique and medium V or VI was studied. It was found that with the improved method of cultivation and the use of medium VI, the results obtained were different from those reported with the old method and medium V. The important differences were the following.

There was no demonstrable fall in the virus titre during the first 2 to 3 hours. On the other hand, there was a steady rise in the virus titre upto the end of 4 hours, the rise being 2 to 3 log values.

From 4 to 18 hours (the interval varying with each strain from 8 to 14 hours), the virus titre remained stationery.

From 12 to 24 hours (the interval varying with each strain from 3 to 9 hours), there was a second rise in virus titre.

Again from 18 to 36 hours (the interval varying with each strain from 6 to 15 hours) the virus titre remained stationery.

From 24 to 60 hours (the interval with each strain being 24 hours), there was a third rise in virus titre.

From 48 to 96 hours, depending on the strain, the virus titre remained almost stationery.

RNA from influenza viruses

Several reports have appeared about the isolation of infectious RNA from different viruses using the hot and cold phenol methods. An attempt was made to isolate the RNA from influenza virus preparations by the different methods and test its infectivity. The cold phenol method described by Holland *et al.* for the isolation of infectious RNA from poliovirus infected monkey kidney culture fluids was adopted for isolating the fraction from infected allantoic fluids and red cell purified preparations of influenza virus. The hot phenol method was the same as that used by Wecker for the isolation of RNA from Western Equine Encephalitis virus. The method described by Ada and Perry using sodium chloride extraction and subsequent purification of the RNA with alcohol was also tried. The results based on three separate experiments for each method were as follows :

The RNA fractions obtained by the different methods were not infective when inoculated by the amniotic or allantoic route.

The fractions did not agglutinate fowl red cells.

They did not fix complement in complement fixation tests.

These observations were similar to those reported by Ada *et al.* (1959).

Mutation and recombination

It was found that some of the marker characteristics for the strains employed by previous workers in the field, e. g., capacity to agglutinate fowl or mammalian cells, character of haemagglutination and average level of haemagglutination in 44-hour egg fluids, were found unsatisfactory when using freshly isolated strains, because they changed so rapidly just with further egg passages, without any experimental manipulation. As the objective of the study was to work with strains as close as possible to the original isolation and then find out whether the changes induced, if any, were permanent on subsequent passages, the marker characteristics selected had to be such as were not likely to be affected by further passages alone.

After a detailed study of the effect of egg and mouse passages by themselves on various characteristics of the virus strains, it was decided to select the strains on the basis of two of them, viz., the serological behaviour or antigenic pattern on the one hand and sensitivity or otherwise to normal inhibitors,particularly of fowl sera, on the other.

Investigations on the above lines were carried out on 10 strains. It was found that there were certain intrinsic difficulties in drawing conclusions from the experimental results. Thus when PR8 and Lee viruses were inoculated together in the same eggs, the resulting harvest showed that much of the material reacted with both A and B sera. Hirst and Gotlieb explain the phenomenon as due to the ability of the virus genetic core to accept overlays or coats of specific virus material from distantly related agents like NDV.

Effect of salts on the stability of influenza virus

Studies were undertaken to determine the effect of different salts in one molar concentration on influenza viruses. Recently isolated Asian and type B viruses cultivated in CAM tissue cultures were used. It was found that only $MgSO_4$ conferred some protection on Asian and type B viruses against thermal inactivation.

Antiviral properties of iodine against influenza virus

Interest in the study of the antiviral properties of iodine arose from the findings of Menon and Shaw (1957) that colloidal iodine prevented the spread of influenza. In view of the encouraging results the antiviral properties of iodine against influenza virus were investigated.

The action of a solution of iodine in potassium iodide on the haemagglutination property of the PR8 strains of influenza virus was studied. It was found that 20 mg. of free iodine completely destroyed the haemagglutination activity.

The action of aqueous solution of iodine in potassium iodide, collosol iodine and Betadine on the infectivity of different strains of influenza virus were investigated. The results, which were similar with the different strains of virus used, showed that the dilutions of aqueous solution of iodine in potassium iodide, collosol iodine and Betadine required for destroying the infectivity of the virus completely was 1:16000, 1:500 and 1:30000 respectively. Thus Betadine appeared to

be the best while iodine in potassium iodide was better than collosol iodine.

Different dilutions of the iodine preparations were given into the allantoic cavity of embryonated eggs 4 hours before inoculating allantoically 0.25 ml. of a 1 in 100 dilution of the virus. Controls were run by inoculating 0.25 ml. of distilled water in place of iodine. The results showed that in all the cases there was no protection against infection.

Action of formaldehyde on influenza virus

Formaldehyde produced varying degree of fall in the haemagglutination titre of strains of influenza virus used for the preparation of vaccine. The effect was not consistant or regular. It was found that with the Asian strains the purified material showed a greater diminution in haemagglutination titre than the unprocessed infective allantoic fluid. The concentration of formaldehyde employed was found to decide the degree of fall in haemagglutination titre. Thus, in a concentration of 0.04 mg./ml., it reduced the titre from 128 to 64, while at 0.4 mg. ml. the titre was less than 16.

However, the effect was not common on all strains. Thus no fall in haemagglutination titre was observed with A/Sweden/51 and B/Coonoor/4/51 when treated with formaldehyde in the above concentrations.

Action of some organic compounds on influenza virus

A series of organic compounds were tested to determine whether they were capable of preventing the multiplication of influenza virus. The choice of the compounds depended on the presence of certain chemical linkages, which are known to inhibit the growth of some micro-organisms or which form the basis of some biological phenomena.

Of the compounds tested, 3-hydrazinoquinoline dihydrochloride inhibited haemagglutinin formation and greatly suppressed the mulitplication of influenza virus in CAM cultures *in vitro*.

(4) INFLUENZA VACCINE

Preparation

During the 1957 pandemic a representative strain of Asian virus isolated in Malaya and labelled PAR was used for vaccine production. It was found to be unsatisfactory in many respects for the purpose. It gave consistently low haemagglutination titres in the early passage levels, was of poor antigenic value and gave very erratic results in the course of processing by different methods. The methods tried included adsorption of the virus on embryonic as well as adult chick cells, adult guinea-pig and human red cells, and on other adsorbents like calcium phosphate, aluminium phosphate and aluminium resinate. After trials on the effectiveness of the methods as well as the quality of the processed material from the point of view of yield, antigenicity and other factors, the methanol precipitation method of Cox *et al.* (1947) capable of giving a highly purified concentrated virus suspension from infected allantoic fluids was selected. The aluminium phosphate method was also used for the preparation of vaccine.

Evaluation of potency

In rabbits. The potency of methanol precipitated vaccine prepared with the PAR strain was tested in rabbits. It was found that the results with complement fixation test were better than those obtained with the haemagglutination-inhibition test. The vaccine administered intradermally appeared to give as good results as when it was given subcutaneously or intramuscularly. It was

found that the rabbit was a suitable animal for testing the potency of influenza vaccine.

In human volunteers. With a view to assess the antigenic value of influenza vaccine prepared at the Institute some trials were undertaken among human volunteers These included studies with different batches and types of vaccine.

It was found that the antibody response evoked by PAR strain against itself and a homologous strain like K VI as well as A-prime strains like A/Ned/56 and Liverpool was very poor. The Asian strains K VI and CN, on the other hand, appeared to produce an appreciable level of antibody against both the A-prime strains.

Effect of storage

Under the emergency production scheme of influenza virus vaccine in 1957–58, the Institute produced 55,000 doses of vaccine out of which 25,535 doses were supplied to various State Governments and Administrations. About 29,465 doses remained in stock and all of them became time-expired by January 1959. The vaccine was prepared by the methanol process. Random samples from each batch were taken and titrated for haemagglutination against fowl cells at the end of one and two years storage at 4°C. after preparation.

Of the samples tested at the end of one year only 43% retained the original titre. At the end of two years the percentage was only 34%.

The aluminium phosphate vaccine showed a slight advantage over the plain saline vaccine. It was found that 50% of the ampoules retained the full titre at the end of one year.

(5) LYOPHILIZATION OF INFLUENZA VIRUS

The effect of freeze-drying a strain of Asian virus grown in CAM cultures *in vitro* after the addition of glucose, sucrose, gelatin, polvinylpyrrolidone, dextran and peptone, either singly or in combination was studied. It was found that results similar to those obtained with sucrose and gelatin could be had with dextran alone, sucrose and dextran, polyvinylpyrrolidone and peptone, and sucrose, gelatin and polyvinylpyrrolidone, in the concentrations employed. The loss in titre after lyophilization was minimal when 10% sucrose and 1% peptone were added before freeze-drying.

(6) HISTOLOGICAL AND HISTOCHEMICAL CHANGES IN LUNGS OF INFECTED MICE

The histological changes in the lungs of mice infected intranasally with the Asian strain of influenza virus were found to be progressive. These changes which were most marked at 96 hours included: marked dilatation of the bronchioles with round cell cuffing; change of columnar to cuboidal epithelium; marked piling up of epithelial cells; presence of inflammatory cells and desquamated epithelium in the lumen of bronchioles; areas of atelectasis and emphysema; presence of pinkish acellular material in some distended alveoli; marked focal infiltration of interstitial spaces with round cells and neutrophiles; and marked congestion and dilatation of blood vessels with round cell cuffing.

The histochemical studies showed that there was progressive increase in the RNA content of the bronchiolar epithelial cells, septal cells and endothelial cells of blood vessels, commencing 24 hours after infection and reaching to a maximum at 72 hours. While the RNA content increased there was a steady decrease in the DNA content. Also, there was a progressive increase in the alkaline phosphatase content of the basal epithelial cells of the bronchioles. There was no demonstrable change in the acid phosphatase content.

(7) THE 1957 PANDEMIC OF INFLUENZA IN INDIA

A detailed account of the studies by the Coonoor Centre on the 1957 pandemic of influenza in India has been presented by Menon (1959). Some of the important findings are briefly summarized below.

Spread of the pandemic

The first intimation that the influenza outbreak in South-East Asian countries such as Japan and Malaya was about to spread to India was received at the Government of India Influenza Centre at Coonoor on 11 May 1957. It was decided to keep a special watch on the arrival of infected cases at Calcutta and Madras and to isolate the virus from such cases. Information was received on 15 May that the s. s. *Rajula*, which had left Singapore on 9 May with 1622 passengers and about 200 crew members had been directed to proceed to Madras instead of to its first port of call in India, Negapatam, in view of an outbreak of influenza on board affecting 254 persons in seven days. On the ship's arrival at Madras on the morning of 16 May it was found that there were 44 active cases of influenza on board, four of them showing temperatures above 103°F. (39.4°C.). The steamer was placed in quarantine at sea and was boarded by a medical team which examined all on board and gave the necessary treatment. A laboratory team from Coonoor collected throat washings from the patients. These specimens were collected on 16 and 17 May and sent to Coonoor with adequate safeguards for preservation and safety in transit. In the laboratory at Coonoor, eggs were inoculated amniotically and the first isolation of the virus from the cases from the steamer was made on 22 May. The strain was sent to the World Influenza Centre in London and identified there as A/Asia/57 virus.

Four of the nurses who boarded the steamer on 16 May came down with fever on 18 May, i.e., 48 hours after exposure to infection. If this is taken as the first date of the epidemic in India, it can be stated that the pandemic was noticed in North China in January, Shanghai in February, Canton in March, Hong Kong in April, Singapore early in May and Madras in mid-May, or the 20th week of the year. The first cases in Bombay were reported on 21 May or in week 21, while Calcutta reported a few cases in the same week and over 1000 cases in week 22 with the first two deaths on 1 June. Whether the ports of Madras, Bombay and Calcutta were affected independently of each other or whether Madras was the first port to be affected and Bombay and Calcutta received infected cases from that city cannot be decided easily on the information available; the likelihood was that they were seeded independently but within a few days of each other by infected cases arriving by ship or aircraft. The southern States of Madras, Mysore, Kerala and Andhra appeared to have received massive infection through the hundreds of passengers coming from Singapore by the two steamers s. s. *Rajula*, which discharged 1622 passengers on 21 May, and s. s. *State of Madras*. which discharged about 1065 passengers on 28 May. Medical examination of those on board the latter steamer on arrival at Madras revealed 49 active cases, besides 35 persons convalescing after attacks during the voyage. Another factor tending to confuse the issue was the report that there were several cases of influenza in the city of Madras before 15 May. It was not clear at that time whether they represented stray infections with the Asian virus or were due to type A or B virus strains current earlier in the country.

In each area, the pattern was one of sweeping spread through the most crowded capital and other cities, followed by a relatively slow spread across villages and other towns. Passengers from the s. s. *Rajula*, allowed to disembark on 21 May at Madras, arrived at Madurai and provided the starting-point of the outbreak in that city in

week 21. The heavy influx of visitors from Madras city to Ootacamund for the annual flower show on 18 May was probably responsible for the sharp outbreak among the staff and students of the Lovedale School, commencing on 21 May and infecting 256 out of 533 children within five weeks. In the 22nd week, cases appeared for the first time in several widely separated areas, with a frequent history of arrival of infected persons or of contacts from infected towns. Thus 45 midwives returned from Madras to Coimbatore and six of them, along with eight nurses in contact with them, provided the first admissions of influenza to the Coimbatore Hospital. In Thandaraiputhur, Tiruchi district, a compounder returning from Madras developed influenza on 27 May and his contacts provided the next four cases. In Hyderabad, Andhra State, the primary focus was provided by a patient admitted to hospital on 26 May who had returned from Singapore in the s. s. *Rajula*. Bangalore and Mysore also each received one passenger from the s. s. *Rajula* on 27 May, and in the former city, three sick persons arriving by train from Madras on 31 May were removed to the infectious diseases hospital. Other towns and cities affected in the 22nd week were Erode and Vellore in Madras State, Belgaum and Shimoga in Mysore, Dibrugarh in Assam, and New Delhi, the capital. The source of infection was not clear in these areas.

The maximum spread of the epidemic appeared to take place in the 23rd week (2–8 June). Over 60 passengers from the s. s. *Rajula* had arrived in various parts of the Palghat district of Kerala State and on 4 June, 12 persons in four families were down with influenza. Cochin port received its earliest cases by the s. s. *Indian Shipper* in that week, and had its first fatal case in the next week in a 60-year-old member of the crew of the s. s *City of Chelmsford*. Trivandrum, the capital of the State, Trichur, Kozhikode and most of the bigger towns were severely affected in the 23rd week. In the same period, Mysore city and Kolar in Mysore State; Bezwada, Nellore, Gudur and other towns in Andhra; Poona, Nagpur, Ahmedabad and sholapur in Bombay; Raipur and Sambalpur in Orissa; Lucknow and Agra in Uttar Pradesh; and Ludhiana and Ambala in the Punjab were among the numerous towns affected all over India. The spread of the epidemic from the 24th week onwards was one in depth and intensity in the areas already affected. Thus New Delhi, which had a total of 80,573 cases in June with 15 deaths, had 1,000 cases in the first three days of June, 2,796 on 6 June, 3,830 on 7 June, 4,718 on 8 June, with a peak of 6,460 on 11 June, falling to 3,057 on 16 June and 1,761 on 22 June. The main wave of influenza would thus appear to have swept across the country within a period of 12 weeks ending 10 August 1957. The cases occurring subsequent to that date in all the affected areas represented the permanent infiltration of a new virus into the population, as distinct from its early massive pandemic attack.

Attack-rates

By age-group. An interesting feature was that the maximum incidence percentage for June was in the 6–10- year group and for July in the 0–5-year group. The possibility existed that this phenomenon was due to the school–going children becoming infected early in the epidemic and the pre-school children acquiring their infection later on from the older ones. All the data were in agreement as to the gradually diminishing incidence in the higher age-groups.

The age-group 31–35 years seemed to be the dividing line between the high and low incidence. The possibility existed that individuals born well after the 1918 pandemic—say in 1923, by which time the pandemic strain might have disappeared—were affected to a significantly greater degree during 1957 than those alive in 1918 and the succeeding five years.

By State. If the incidence of the disease was worked out from the figures in relation to the population of the various States according to the 1951 census figures, it was seen to vary from 0.4% in Rajasthan, Uttar Pradesh and Bihar, 0.6% in New Delhi, 0.7% in Andhra Pradesh, 0.8% in the Punjab, 0.9% in Madhya Pradesh, 1.0% in Orissa, 1.1% in West Bengal and Manipur, 1.3% in Tripura, 1.4% in Himachal Pradesh and 1.9% in Madras to the relatively high incidence of 2.1% in Assam and Kerala, 2.2% in Mysore and Andaman and Nicobar and 2.8% in Bombay. The figures reported, however, were likely to be much lower than the actual number of cases, while the 1951 census figures for population were also lower than actual ones for 1957.

By occupational group. The only occupational group that showed a distinctly higher than average incidence of the disease in many cities and towns was that of the medical and nursing personnel. In a large teaching hospital, for instance, cases occurred in 147 nurses (43%), 30 house surgeons (25%), 17% medical students (21%) and 33 other staff (6%). Patients in many towns and villages experienced great difficulty owing to the large proportion of medical persons who fell ill.

Mortality rates

For the period of 38 weeks from 19 May 1957 to 8 February 1958, there were 44,51,785 cases of influenza with 1,098 deaths in the whole of India. With a population of 360 million according to the 1951 census, this gave an attack-rate of 12,366 and a mortality rate of 3 per million of population. The case-fatality rate for the whole of India worked out to 242 deaths for every million cases. It has been pointed out earlier that the attack-rates varied from a low of 0.4% or 4,000 per million population in Rajasthan, Uttar Pradesh and Bihar to a high of 2.8% or 28,000 per million in Bombay. There were some unusual features in the distribution of mortality in adjacent States. The case-fatality rates brought out particularly clearly the differences in behaviour of the disease in different areas.

Despite this anomaly, it was perhaps worth noting that Bengal had the lowest mortality rate (8.5 per 1,000) in the 1918 pandemic but the highest (1,539.5 per million) during 1957; indeed West Bengal, with 7.2% of the total population of the country accounted for only 6.4% of all influenza cases but for 40.5% of all influenza mortality. No convincing explanation of the differences shown by the various States with regard to mortality was available.

Second attacks

The only instance of two outbreaks both proved to be due to Asian virus by isolation of the strains and by serological investigations was in a regimental centre. In June 1957 an influenza outbreak in that centre affected over 330 persons out of 3,000 at risk and in November there was another outbreak with over 112 cases in seven days. On each occasion, the Asian virus was isolated at Coonoor from two of the throat washings.

Clinical features

Nausea and vomiting were present in an unusually high percentage of cases in 1957 in most areas in India. While they did not pose a serious threat to life in the majority of cases, in a few patients vomiting gradually increased in frequency and led to deepening stupor and death. Bradycardia was a constant feature. Jaundice appeared in a very small number of patients. Epistaxis was reported in about 10% of cases, followed in a small number of cases by haemetemesis, melaena and death. These features suggested a possibility that the 1957 virus had a specially damaging effect on the liver.

Reports were received from many areas of the presence of a dysenteric condition simultaneously with or immediately following the main outbreak of influenza. No laboratory studies were made on the aetiology of the dysentery, but the reports came from widely scattered areas in Kerala, Madras and Uttar Pradesh as well as outside India. It was diagnosed in some areas as gastric influenza or influenzal dysentery.

Another feature of the pandemic was the frequency of signs and symptoms related to the nervous system. Clear-cut evidence in support of the production of encephalitis or meningitis by the virus directly was not available; but signs and symptoms included stiffness of the neck, stupor, delirium, complete disorientation, mental depression and boisterous and violent behaviour requiring physical control of the patient.

The frequency of involvement of the lungs during influenza was rather difficult to decide on a purely clinical basis. It was likely to be high in cases admitted to hospital. In a small series of 25 patients subjected to radiological investigations at Lawley Hospital, Coonoor, the lung picture showed some abnormality in 14 cases and pronounced changes in six of these. As the patients were selected for investigation on account of the severity of disease, the findings were not applicable to the ordinary run of cases.

Another important finding was the evidence of myocarditis in cases in which electrocardiograms were taken. Though its incidence was higher in the group of severe cases, it was demonstrated in the mild and moderate groups also. The danger of going back to work too soon after an attack of influenza was obvious from these findings.

Organisms isolated and pathological observations

The fatal cases at Coonoor were too few for statistical study. The first fatality at Lawley Hospital was in a young girl aged 12 years admitted on 12 June 1957 with a history of fever for one day only, but in a highly toxic state, with delirium and persistent vomiting; she died the same day. Her throat swab yielded *Strept. haemolyticus* as well as *Strept, non-haemolyticus* (*Enterococcus*) on culture and A/Asia/57virus on egg ioculation. *Diplococcus pneumoniae* was obtained on culture from a very serious case whose recovery was unexpected. Coagulase-positive *Staph. aureus* was found in several cases of varying degrees of severity. H. *influenzae* was seen only once, in a case with severe broncho-pneumonia. Other organisms isolated from about 30 cases of influenza studied bacteriologically included, in addition to the bacteria mentioned above, *B. proteus*, *B. lactis aerogenes*, coliform and diphtheroid organisms and a particular Gram-negative organism, not fermenting any of the sugars, that could be labelled *B. faecalis alkaligenes* or *H. bronchisepticus*. It had the unusual property of haemagglutinating fowl as well as guinea-pig cells to high titre when present in egg fluids or even in suspensions taken from culture tubes. It was a particularly troublesome contaminating organism, being insensitive to sulfonamide compounds and all the antibiotics tested against it. During the pandemic wave, no studies could be undertaken on the sensitivity of the bacteria to antibiotics. But early in 1958, of 25 strains of *Staph. aureus*, 8 were found to be resistant to penicillin in concentrations over 10 units per ml. All the 8 strains were found resistant to streptomycin also in concentrations of 20 ug. and above per ml. Both penicillin-sensitive and penicillin-resistant strains were found to be destroyed equally well by iodine in a dilution of 1 in 20,000. Out of the 25 strains, 12 were isolated from cases of influenza and two of these were resistant to penicillin in concentrations as high as 3,125 units per ml.

Post-mortem notes kindly made available by the medical officers concerned covered four cases only. Generalized congestive changes were evident in the

brain, in the mucosa of the stomach, and in two cases in the spleen and kidneys. Patchy congestion in the lungs was present in all four, with pleural adhesions on both sides in two cases and four ounces of blood-stained fluid in the left pleural cavity in one. The liver was congested in one and showed greyish-yellow patches in two others.

The cause of death in the majority of patients seemed to be related to the development of pneumonitis, frequently with haemorrhagic changes. In two cases where severe vomiting supervened after the patient became afebrile 48 hours after onset of illness, the blood urea was raised to 45 mg. and 60 mg. respectively the day before death. It was not clear to what extent the changes in the liver, brain and other organs were due to the severe anoxaemia resulting from extensive pneumonitis. Histopathological examination of the liver sections and liver biopsy specimens showed degenerative and fatty changes quite different from those in infective hepatitis and compatible with the results of continued lack of oxygen.

Virus isolation

It was found that the newly isolated strains differed considerably from the previous A and A1 strains, not only antigenically but also in biological characteristics. They grew with difficulty in the early egg-passages, gave low haemagglutination titres with both fowl and guinea-pig cells and did not show the characteristic 0 phase. Previous studies at Coonoor had shown that normal fowl sera did not generally contain non-specific inhibitors against the A1 strains, although against a few strains low titres of the order of 1:8 or 1:16 were seen. In contrast, they were inhibited by normal rabbit sera in dilutions 1:256 and even higher. Antisera prepared in fowls were, as usual, used for testing the Asian strains in 1957. Very erratic results were obtained that led to further studies on their possible causation. It was then found that some of the 1957 strains were totally insensitive to the non-specific inhibitors in the sera of all animals tested, unlike the A1 strains; but other Asian strains were highly sensitive not only to the inhibitors in normal human, guinea-pig and rabbit sera, like the A1 ones, but also to those in normal fowl sera, unlike the A1. The totally insensitive Asian strains resembled the HVJ or Sendai virus in this respect.

Another unusual characteristic of some of the Asian strains was their reaction to ether treatment. Some of the Asian strains gave increased haemagglutination titres with fowl cells. After treatment with equal parts of ether, at 0°C. and room temperature as well as at 37°C., the haemagglutination titres with fowl cells were 4–16 times greater than the pre-treatment titres in the case of the PAR strain sent from Singapore and the Kolar VI strain isolated from Mysore. The guinea-pig cells titres showed even higher rates of increase. On further egg-passages, PAR showed a threefold and Kolar VI a sixfold rise in titre after treatment. Many of the Asian strains as well as all the well-adapted A and A1 strains showed, on the contrary, the usual fall in titre with fowl cells observed by Henle.

The strains were tested for their sensitivity to the lethal action of iodine observed with other A and A1 viruses. They were found to be destroyed by iodine in the same dilutions as were effective earlier.

Serological studies

Paired sera from patients, deactivated at 56°C., were tested against the PAR strain in haemagglutination–inhibition tests and found to give few positive results. Clinically typical cases as well as some others from whom the Asian virus was isolated sometimes showed no significant rise in antibody titre. The effect of ether in increasing the haemagglutinating titres of some of the Asian strains against fowl cells has already been mentioned.

The PAR strain that was totally insensitive to non-specific inhibitors was found to become sensitive to inhibitors and also to specific antibodies in greater degree after ether treatment. The Kolar VI strain, already sensitive to inhibitor, became more so on ether treatment. If we can imagine the Asian virus donning, in the final stages of its production, a non-reactive, protective, incomplete surface-coating, it is easy to explain its high infectivity, low haemagglutinating capacity, poor antigenicity, low reactivity to specific antibodies, insensitivity to inhibitors and erratic behaviour in elution. Ether apparently removes the coating partially or fully and thus causes an increase in haemagglutinating capacity, reactivity to antibodies and sensitivity to inhibitor. By removal of the protective coat, the infectivity of the virus is lost because it is more open to the destructive action of its environmental conditions. As the strain moves through the population, the "armour" gets worn out or stripped in varying degree and we get a series of strains with the same antigenic core but differing widely in biological characteristics. Finally the armour is completely removed and the virus, devoid of its protection, is unable to resist the hostile influence in nature and is effaced. The localized outbreaks and periodic epidemics may be caused by strains in the partially protected phase while a pandemic is initiated by a fully "armoured" virus.

Preparation of Asian influenza vaccine

The unusually rapid and extensive spread of influenza in Asia in the early months of 1957 indicated the possibility of its being due to a new strain or subtype. It was realized that vaccines prepared from older and mouse-adapted strains might therefore be ineffective, and if vaccine was to be prepared, the aetiological strains had to be obtained. A representative strain isolated in Malaya and labelled PAR was flown out to India, well packed in a CO_2-ice flask, and inoculated into eggs on 26 May at Coonoor. It was successfully established in eggs and the first consignment of material from it was sent to the Armed Forces Medical College, Poona, by 30 May for vaccine production there. Later on, it was distributed to other vaccine production centres and laboratories in India.

A target production of 100,000 doses of vaccine was decided on, out of which the Coonoor Centre had manufactured 55,000 doses by 31 January 1958.

Since influenza had spread all over India by the first week of June, within three weeks of its introduction into the country, and since the first massive wave of infection had spent itself by 10 August, the vaccine could hardly receive a fair field trial, as the earliest batches were not ready until July.

Other prophylactic and therapeutic measures

Experiments carried out in India before 1957 had shown that iodine was effective in the laboratory in destroying different strains of influenza virus, various preparations such as Mandl's paint, Lugol's solution and tincture of iodine being used. Laboratory experiments with the new Asian strains gave identical results.

Research was in progress on the details of the action of iodine when the advent of the 1957 pandemic precipitated trials of its prophylactic and therapeutic value in human beings. In view of the rapid spread of the pandemic and of the urgent necessity to take protective measures, it was not possible always to insist on the use of control groups, or consequently, on as accurate an assessment of the results of field trials as would otherwise have been desirable.

Early in the pandemic, however, controlled trials were carried out; these have been reported elsewhere by Menon & Shaw (1957). Among the uncontrolled trials worthy of mention was one in a military hospital where 189 cases were reported in the first three days of an outbreak in June. Starting on the morning of the

fourth day, the throats of all those at risk were painted twice daily with Mandl's paint. There were 144 further cases that day and a steady drop to 18, 6, 5, 1, 2 and 1 cases on subsequent days. Similar results were obtained in a further outbreak in November. Mention may also be made of use of Mandl's paint among 183 persons on a tea estate, of whom only five (2.8%) developed influenza, as against 689 cases in 4,647 untreated persons (14%) on the same estate.

The use of iodine in the treatment of influenza gave the best results in severe cases, to whom 1–3 doses of 10 ml. of colloidal iodine were generally administered intravenously. In several such cases, treatment was followed by rapid drop in temperature and the disappearance of toxaemia. In mild cases, however, it was not possible to show statistically that the results of iodine treatment were superior to those obtained in control groups given placebo injections or antibiotics. The simultaneous use of antibiotics and iodine did not apparently interfere with the action of either.

REFERENCES

Sci. Rep. Pasteur Institute, Coonoor. 1951–66

Cox H.R. Van Der Scheer, J., Aiston, S. and Bohnel E. (1947) *J. Immunol.*, **56**, p. 149.

Eaton, M.D., Jewell, M. and Scala, A.R. (1960) *Virology*, **10**, p. 112.

Fazekas De St Groth, S. and White, D.O. (1958) *J. Hyg.*, **56**, p. 151.

Fulton, F. and Armitage, P. ((1951) *J. Hyg.*, **49**, p. 207.

Menon, I.G.K. (1959) *Bull. Wld Hlth. Org.*, **20**, p. 199.

Menon, I.G.K. and Shaw, C.W. (1957) *Antiseptic*, **54**, p. 692.

Newton, N. and Bevis, R.E. (1959) *Virology*, **8**, p. 344.

Veeraraghavan, N. (1961) *Bull. Wld Hlth. Org.*, **24**. p. 679.

Idem. (1962) *Bull. Wld Hlth. Org.*, **27**, p. 808.

Veeraraghavan, N. and Kirtikar, M.W. (1961) *Bull. Wld Hlth. Org.*, **24**, p. 684.

Veeraraghavan, N. and Sreevalsan, T. *Bull. Wld Hlth. Org.*, **24**, p. 695.

Idem. (1961) *Bull. Wld Hlth. Org.*, **24**, p. 703.

Veeraraghavan, N., Kirtikar, M.W. and Sreevalsan, T. (1961) *Bull. Wld Hlth. Org.*, **24**, p. 711.

Veeraraghavan, N., Natarajan, S. and Sreevalsan, T. (1962) *Ind. Jour. Med. Res.*, **51**, p. 1.

3. OTHER RESPIRATORY VIRUSES

(I) ADENOVIRUSES

(i) Virus isolations

The wide use of *in vitro* cultures of cells has brought to light many new viruses. One such important group affecting mainly the respiratory system of man is the adenoviruses. As they are not pathogenic to common laboratory animals and the chick embryo, cultures of appropriate tissue cells are essential for their isolation and identification. Clinically, infections with this group of viruses lead to pharyngo-conjunctival fever, ocular symptoms like kerato-conjunctivitis, lymphadenitis of regional lymph nodes, non-streptococcal pharyngitis, febrile catarrh or primary atypical pneumonia.

Since 1958, systematic attempts are being made to study the incidence of adenovirus infections in the Nilgiris. The throat swabs from sick children are regularly collected from the paediatric ward of the local hospitals. Also, throat gargles are collected daily from patients with respiratory illness attending the hospitals at Coonoor, Wellington and Ootacamund. In addition, every outbreak of respiratory illness occurring among the army personnel and in the various residential schools has been investigated. The results of these studies are presented in Table 7.

A total of 111 strains of adenoviruses was isolated from 4,988 specimens of throat swabs and gargled specimens. Of these, 60 belonged to type 21 virus. There were 8 strains of type 2, 6 of type 4, 4 each of the types 1 and 7, 3 each of the types 3 and 5, and 2 each of the types 6 and 24. The isolations from other sources included: 1 strain of type 21 virus from

TABLE 7

Year	Total No. of specimens tested	Adenovirus Types											
		1	2	3	4	5	6	7	15	21	24	Undetermined	Total
						THROAT SWABS/GARGLES							
1958	92	...	...	...	...	...	...	1	...	...	...	...	1
1959	409	...	3	...	...	...	...	1	...	...	...	1	5
1960	468	1	2	2	...	...	...	...	...	...	...	2	7
1961	477	1	...	...	1	1	...	...	...	2	1	1	7
1962	1,293	1	...	...	...	...	2	...	...	23	...	1	27
1963	755	...	1	...	...	...	...	...	...	15	...	...	16
1964	559	...	...	1	2	...	...	1	...	3	...	...	7
1965	390	1	1	...	...	...	...	...	...	5	1	9	17
1966	545	...	1	...	3	2	...	1	...	12	...	5	24
						SPUTUM							
1962	3	...	...	...	...	...	..	...	...	1	...	...	1
1964	24	...	...	...	...		...	...	...	...	...	...	...
						RECTAL SWABS/FAECES							
1961	42	...	...	...	...	...	...	...	...	1	...	...	1
1962	16	...	...	...	...	...	...	...	...	1	...	...	1
1963	34	...	...	...	...	...	...	...	...	...	...	..	...
1964	61	...	...	...	...	...	...	...	...	6	...	...	6
1965	26	...	...	...	...	...	...	...	...	...	...	2	2
1966	14	...	...	...	...	..	...	...	...	...	...	6	6
						CONJUNCTIVAL SCRAPINGS							
1961	123	...	...	1	...	...	...	...	1	...	...	...	2
1962	43	...	...	...	...	...	...	...	...	...	...	2	2
1963	3	...	..	...	...	...	...	...	...	...	...	...	...
1964	22	...	...	...	...	...	...	...	...	...	...	...	...
1966	22	...	...	...	...	2	...	...	...	...	...	3	5
						TONSILS AND ADENOIDS							
1961	6	3	1	...	...	1	...	...	...	...	...	...	5

27 sputa; 7 strains of type 21 from 193 rectal swabs or faeces; 2 strains of type 5 and one strain each of the types 3 and 15 from 213 conjunctival scrapings; and 1 strain each of the types 1, 2 and 5 from tonsils and adenoids.

(ii) Investigations of outbreaks among recruits

Several outbreaks of respiratory illness occurring among children in residential schools and among army recruits were investigated during the period 1958–66. The results of investigations of the outbreaks in army recruits is briefly summarized below.

From October 1961 to May 1964, there were three outbreaks of respiratory infections amongst the army personnel in Wellington, Nilgiris, situated at a height of 5,800 ft. above mean sea level. During this period, about 1,500 persons were admitted to the wards of the Military Hospital, with symptoms of acute upper or lower respiratory tract infection or those resembling influenza. During the outbreaks acute and convalescent sera and throat swabs were collected from as many patients as possible.

First outbreak. This lasted from November 1961 to March 1962. During these 5 months, of the 168 cases hospitalized for respiratory infection, 117 were in the 3 months, November to January. Clinically, 84 patients were suffering from pharyngitis, 12 from bronchitis, and 72 from pneumonitis; there were two deaths. Six strains of adenovirus type 21 were isolated from 121 throat swabs. Paired sera were available from 4 patients who yielded adenovirus; all the four gave significant rise in titre of neutralizing antibody for type 21. In 8 patients from whom adenovirus was not isolated, paired sera gave evidence of infection with adenovirus type 21 with significant rise in both complement fixing and neutralizing antibody. In the bacteriological study of 91 throat swabs from the same group, 17 strains of *H. influenzae* and 7 strains of *Staph. aureus* were isolated besides some commensals.

Second outbreak. This lasted from November 1962 to May 1963. Out of 518 throat swabs, 34 strains of adenovirus type 21 were obtained. It was found that hospital admissions were unusually high during the 3 months from December to February and that there were 9 patients who gave evidence of double infection with adenovirus type 21 and influenza virus A2.

Third outbreak. The number of specimens collected was small when compared to the previous years as the period of maximum incidence of cases occurring in November and December 1963, was missed. From 62 throat swabs collected between January and May 1964, only 3 strains of adenovirus were obtained. None was isolated from 24 specimens of sputum, whereas 21 specimens of faeces yielded 6 strains of adenovirus type 21.

Forty-nine pairs of sera collected were subjected to complement fixation tests with adenovirus, influenza A2, *Mycoplasma pneumoniae and Parainfluenza* type 3 antigens to find out the role of other agents. While 33% of specimens showed significant titres for adenovirus in both acute and convalescent samples, 35% recorded a significant rise in titre. Only 15 pairs were tested for neutralizing antibody against type 21; of these 9 (60%) gave significant rise in titre indicating a specific infection with the virus. Only about 4% showed any significant rise in titre in complement fixing antibodies for *Mycoplasma pneumoniae* or *Parainfluenza* type 3, indicating the dominant role played by adenovirus type 21.

Bacteriological investigation of 28 throat swabs yielded five strains of *H. influenzae*, four of *Strept. pyogenes* and one of *Staph. aureus*.

(iii) Epidemiological studies among recruits

In 1962, extensive epidemiological studies were carried out on healthy recruits soon after their arrival and after 4 to 6 weeks of training. Within a week after their arrival at the station, a throat swab for virus isolation and blood for serological tests were collected from each recruit. This was repeated 4 to 6 weeks later.

The first survey was carried out between 2 February 1962 and 6 April 1962 when specimens were collected from 121 recruits. During the second survey on October 1962 specimens where collected from 218 recruits.

The results of these investigations were interesting. None of the 502 throat swabs examined yielded adenovirus. Complement fixation studies on sera of these recruits revealed that 60% to 64% had CF antibodies in a signficant titre (1 in 16 and above) on arrival, probably due to prior latent infection. There was a seroconversion of 29% during the period of February to April, and 50% during October to November. Specific neutralizing antibodies developed as a result of infection with the particular type. Titration of the neutralizing antibody for type 21 showed that 33% (58 out of 70) were devoid of type 21 neutralizing antibody on arrival. Fifty-three per cent remained so at the end of 4 to 6 weeks of training and 23% showed a four-fold or greater rise in titre probably due to subclinical infections.

The studies indicate the necessity for continued studies of adenovirus infection in recruits to the Armed Forces in different areas of the country.

(iv) Incidence of adenoviruses during the pandemic of Asian influenza

Investigations were carried out to study the occurrence of respiratory viruses of the adeno group during the pandemic of Asian influenza in 1957. A large number of specimens were collected during the main epidemic in 1957 and a smaller number from localized outbreaks caused by the same virus in 1958.

Out of the 84 specimens examined, adenovirus type 7 was isolated from one case. These results indicated that there was no simultaneous infection with adenoviruses during the pandemic of Asian influenza.

(v) Serological studies

Since the isolation of adenoviruses from throat swabs of children and adults with respiratory infections did not yield many positive results, it was considered worthwhile to investigate the presence of complement fixing antibodies in the sera received at the Institute for other serological examinations. These included (a) sera from healthy blood donors and of persons whose specimens where sent for serological test for syphilis, (b) sera of febrile patients sent mainly for agglutination reactions against enteric group of organisms or for their isolation, and (c) paired sera from patients suffering from influenza and other respiratory infections.

During 1959–66, 3,932 sera have been examined, Of these, 1,360 were from febrile cases and 2,572 from afebrile cases. The sera from 29% of febrile patients and 30% of afebrile cases showed the presence of CF antibodies against adenoviruses in a dilution of 1 in 16 and above.

The finding indicates that adenovirus infections are quite common in the community.

Persistence of CF antibodies against adenoviruses. Out of 11 persons studied, 8 maintained the titre over a period 11 to 15 months. In one, the antibodies persisted for 7 months and in another for over

3 months. Only in one case there was a complete loss in titre.

CF tests using different types of adenovirus as antigens. Complement fixation tests using adenovirus types 4, 7 and 21 as antigens were carried out simultaneously on 44 specimens of sera from 16 patients suffering from adenovirus infection. The readings were identical in 23 sera. In the rest there was only one tube difference.

Preparation of adenovirus antisera. Prototype strains were obtained from the American Type Culture Collection at Washington. Subcultures of all the types were made in primary amnion cells. Rabbits were immunized with these cultures by giving an intravenous injection of 10 ml. followed a week later by an intraperitioneal injection of 10 ml. The animals were bled a week after intraperitoneal injection.

The combined intravenous and intradermal method also gave good results in rabbits.

An attempt was made to produce antisera against types 3 and 7 adenoviruses in fowls. The results were not satisfactory. The sera gave only a CF titre of 64.

(2) HAEMADSORPTION VIRUSES

(i) Isolation

From throat swabs. All specimens of throat swabs collected for various examinations are routinely examined for the presence of haemadsorption viruses in monkey kidney cell cultures. All the 1,307 throat swabs examined yielded negative results.

From monkey kidney cell cultures. Primary monkey kidney cell cultures prepared from the kidneys of individual monkeys were tested for the presence of haemadsorption viruses. It was not possible to demonstrate them in 96 batches of uninfected cultures examined so far.

(ii) An outbreak of parainfluenza virus infection in Madras city

An outbreak of respiratory illness in Madras city during July 1964 was investigated.

The cases belonged to the age group 12 to 40 years and were predominently males. The patients complained of pain all over the body, headache, fever and cough. The duration of fever ranged from 1 to 2 days. The patients were not acutely ill and most of them were treated as out-patients. Physical examination did not reveal any positive findings except inflammation of the pharynx.

Specimens of throat gargles were collected from 32 fresh cases. Specimens of blood could be obtained from only 2 cases.

All the throat gargles were inoculated into amniotic cavities of 13–days–old chick embryos but influenza virus was not isolated from any of the specimens. An interesting observation was that in some cases the amniotic fluid from the eggs inoculated with the throat gargles gave positive haemagglutination with guinea-pig red cells but not with fowl cells. These materials when passed into monkey kidney, human amnion or HeLa cells showed cytopathic effects with giant cell formation and the presence of cytoplasmic inclusion bodies. There was haemadsorption with guinea-pig red cells indicating that these belonged to the group of parainfluenza viruses. Also, some of the throat gargles when inoculated directly into human amnion or monkey kidney cells yielded agents with similar properties. A total of 19 strains were isolated.

Neutralization tests indicated that the strains belonged to *Parainfluenza*, group 3.

(3) OTHER RESPIRATORY VIRAL AGENTS

(i) Mycoplasma pneumoniae

In a recent study among army recruits, despite the use of special PPLO media, none of the specimens of sputum yielded *M. pneumoniae*. Serological studies, however, showed a significant rise in titre for *M. pneumoniae* in three patients.

(ii) Respiratory syncitia virus

In an investigation among army patients, out of 42 specimens of sera tested for complement fixing antibodies against respiratory syncitial virus, 8 gave a titre of 16 or more.

REFERENCES

Sci, Rep. Pasteur Institute, Coonoor. 1957–66.

KURIAN, P. V., MUKUNDAN, P. and KARPAGAGANAPATHY, P. R. (1962) *Lancet*, 2, p. 355.

KURIAN, P. V., ROSHAN LAL and PANDIT, V. (1966) *Ind. Jour. Med. Res.*, **54**, p. 812.

ROSHAN LAL and KURIAN, P.V. (1959) *Armed Forces Med. J.*, **20**, p. 61.

4. INTESTINAL VIRUSES

(1) ENCEPHALITIS

Investigation of epidemics

DELHI

Delhi had an epidemic of encephalitis among children during 1957. Specimens of throat swabs, faeces and CSF collected from children were examined at the Institute.

Virus isolation

It was found that a larger number of viruses could be isolated from faecal samples concentrated by ultracentrifugation. This procedure was therefore regularly adopted.

Thirteen viruses were isolated from 43 samples of faeces. These were: poliovirus type 2, 2; Coxsackie A9, 5; and ECHO, 2. One strain of virus was isolated from the CSF.

Serological studies

The two ECHO viruses isolated were found to be aetiologically related to the patients' illness as the convalescent samples neutralized the homologous virus to a titre of log 1.5 to 1.9 $TCID_{50}$, whereas acute sera did not show the presence of neutralizing antibody in a dilution of 1 in 10 and above.

The paired serum samples available from 3 of the cases from whom Coxsackie A9 virus was isolated did not show neutralizing antibody in 1 in 10 dilution against 1.25 to 2.5 log $TCID_{50}$ of homologous virus.

NAGPUR

Nagpur and surrounding areas had a severe epidemic of encephalitis among children during 1958. The epidemic was investigated by a team sent from the Institute.

The illness was of acute onset associated with pyrexia, frequency of bowel evacuation, vomiting, varying degrees of disturbance of consciousness and evidence of cerebral and meningeal irritation with convulsions, etc. A large proportion of cases occurred among children below 5 years of age. The case fatality rate was high in the early phase of the epidemic. The interval between the onset of illness, time of seeking medical aid and fatal termination was very short in most of these cases. The terminal picture in fatal cases was peripheral circulatory failure, pulmonary oedema or hyperpyrexia in association with cerebral

manifestations which did not show localizing signs.

Laboratory studies

Virus isolations

A total of 47 strains of enteroviruses were isolated from 24 patients, 32 from faeces and 15 from throat swabs. Of these, 20 were re-isolations in HeLa, KB and human amnion cells. The 27 strains isolated included: poliovirus type 1, 1: poliovirus type 3, 2; Coxsackie A9, 3; Coxsackie B3, 3; ECHO 11, 3; ECHO (untypable), 1; and unidentified, 2.

Serological studies

Coxsackie B3 virus isolated from both faeces and throat swab of a patient appeared to be aetiologically related to the patient's illness as shown by a rise in antibody content in the convalescent serum. A strain of the same virus was isolated from the throat swab of another patient. Paired sera of this patient did not neutralize the homologous virus.

Three strains of Coxsackie A9 virus isolated from the throat swabs and faeces of three patients did not neutralize the homologous or the standard Coxsackie A9 strain.

An ECHO virus isolated from faeces was not neutralized by the paired sera of a patient.

OTHER AREAS

In addition to the major epidemics small outbreaks of encephalitis occurring among children in different areas were investigated.

Madras. One strain of Coxsackie A9 virus was isolated from the faeces of a case of encephalitis in Madras. The specimen was collected on the sixth day of illnesss. The serum sample obtained from the patient on the 17 day neutralized the standard Coxsackie A9 strain. The two other strains of Coxsackie viruses isolated could not be typed. No viruses were isolated from the CSF.

Mysore. One strain of Coxsackie B5 virus was isolated from the faeces of a case of encephalitis. No viruses were isolated from the C.S.F.

(2) POLIOMYELITIS

Investigation of epidemics

ANDHRA PRADESH

Poliomyelitis occurred in an epidemic form during 1960–61 in the State of Andhra Pradesh. According to the public health authorities the cases occurred over an area of 12,300 square miles comprising the districts of Krishna, Guntur and West Godavari. Cases tended to concentrate in areas where special facilities for treatment were available viz., Vijayawada and Guntur, irrespective of the place of residence of the patients. In the two municipal towns of Guntur and Vijayawada all cases were spotted by a survey conducted with the help of the permanent public health staff.

Incidence in different areas. Out of 160 cases studied, 42 occurred in Guntur municipality and 40 in Vijayawada municipality. The remainder occurred in other portions of the affected area.

Period of occurrence. There was a progressive increase in the number of cases from November 1960 onwards. In Vijayawada municipality the number of cases decreased during March 1961 indicating that the epidemic was subsiding there while in other areas the increased incidence during the same period indicated that the epidemic was still active.

Age incidence. The youngest patient was 6 months old and the oldest 34 years. In general the disease was not met with in subjects under 6 months nor

in those aged over 11 years. Children aged 2 to 3 years appeared to be the most susceptible as shown by 50% of the cases occurring in that age group. It was also found that in all the 3 areas 87% of the cases occurred in children below 6 years of age.

Clinical manifestations. The clinical manifestations correlated with laboratory diagnosis in 150 cases which occurred during October 1960 to March 1961. A short febrile illness preceded the paralysis in most cases.

Out of the 150 cases 78% showed spinal manifestations. In 8% of cases the presenting symptom was facial paralysis with or without spinal manifestations. In none of these were vital cranial nerves involved during the course of the illness. The course and nature of the illness justified the classification as bulbar poliomyelitis in 15 cases. Six cases presented symptoms of encephalitis.

The overall mortality was 9.3% (14 out of 150). The fatalities were confined to the bulbar and encephalitic types of illness and the mortality in both the groups was 66.7%.

Infection of household contacts. Clinical history of 215 household contacts associated with 54 cases was obtained. Two cases were reported in 4 of the 54 families. In three of these only, the index case occurred during the epidemic. The interval between the cases in the families was 10 to 40 days and the index case was always the older sibling. In another family a pyrexial illness occurred in all the four household contacts at the same time. Poliovirus was demonstrated in only one contact from whom specimen was available for examination.

Virus excretion was detected in 3 out of the 12 families studied. Five contacts, 4 children and 1 adult, excreted poliovirus type 1. Virus was isolated from the faeces of contacts only when the virus was detected in the throat swab and/or faeces of the index case.

Laboratory studies

The results of laboratory studies carried out on the specimens collected from cases and their household contacts as were available, without any selection, can be summarized as follows.

(*i*) *In patients*

Virus isolations. Thirty-nine strains of type 1 poliovirus were isolated from 24 of the 47 patients examined. The viruses were isolated from the faeces of 18 cases, from the faeces and throat swabs of 3 cases and from the throat swab only of the remaining cases, who had bulbar poliomyelitis and from whom faecal samples could not be collected. The poliovirus isolation in 71% of the cases was from specimens collected within 8 days of the onset of paralysis. Although virus isolation was possible from faeces collected from 9 to 24 day of illness, it was found that consecutive samples collected beyond the 8 day from cases were not always positive. Three non-polio viruses were isolated one from the throat swab and two from the faeces of three patients.

Serology. Serological studies were possible in only 11 out of 23 cases from which poliovirus could not be isolated. Among these, the diagnosis of poliovirus type 1 infection was possible in 9 out of 11 cases. The positive serum samples were collected within 8 days of onset in 4 cases and later on in others.

In 14 cases from whom type 1 poliovirus was isolated, serological diagnosis was positive in 9 (64%). The positives were 67% and 60% respectively in the sera collected before and after the 8 day of illness.

Out of 3 patients from whom non-polio viruses were isolated, 1 showed type 1 antibody in high titre.

Based on virus isolation and serology the diagnosis of type 1 poliovirus infection

was confirmed in 35 out of 49 cases studied.

(*ii*) *In contacts*

Virus isolations. Six strains of type 1 poliovirus were isolated from faeces of contacts belonging to 4 families. The index case had virus in the throat and faeces in 3 families and in the faeces of only one in another. Household contacts showed virus in faeces only when the index case was excreting virus and the duration of illness was 7 days or less (5 out of 6 cases). The virus isolation rate in contacts was 1 out of 8 (12.5%) and 5 out of 11 (45.5%) respectively for adults and children.

Two non-polio viruses, one each of Coxsackie B5 and B2, were isolated from the faeces and throat swab respectively of 2 contacts. The former specimen was obtained while the index case in the family was excreting the poliovirus.

Serology. Serological studies were carried out in 8 contacts (6 children and 2 adults) from whom virus isolation could not be obtained. Four of the children showed diagnostic titre of type 1 and another to type 3 polio antibody.

Polio antibodies were determined in the sera of 4 children excreting type 1 virus and 3 of these showed diagnostic titre for type 1 antibody and the remaining to type 3 antibody.

Based on virus isolation and serology a diagnosis of polio type 1 infection could be established in 10 out of the 19 contacts studied.

(*iii*) *In the environment*

Several samples were collected on swabs from different areas in the Vijayawada municipality for virus isolation. These were pooled into 8 lots, extracted in Hanks' BSS and used for virus isolation after ultracentrifugation. No viruses were isolated.

PALGHAT

An epidemic of poliomyelitis was reported in the Palghat Taluk in the Kerala State during August 1964.

Fifty-seven cases were reported from the taluk. Of these, 34 were considered to be poliomyelitis based on the presence of residual paralysis. There were 4 deaths in cases diagnosed as bulbar or spinal form of poliomyelitis due to respiratory involvement.

Out of the 57 cases reported, 56 occurred among children less than 8 years old. Of these, 7 (12.3%) were in children less than 1 year, and 40 (70%) among children aged 1 to 4 years.

Laboratory studies

Virus isolations

From patients. Type 1 poliovirus was isolated from 13 out of 24 patients from whom specimens were collected for virus isolation within 27 days of onset of illness. One strain each of Coxsackie B1, and type 1 poliovirus were also isolated from the faeces and throat swabs of 2 patients in whom type 1 poliovirus excretion was intermittent.

From family contacts. Seven strains of type 1 poliovirus were isolated from the faeces of 5 contacts belonging to 3 families. An interesting finding was that the virus was excreted from family contacts 1 to 2 weeks after the death of the index case in 2 families and 10 weeks after the onset of illness in the index case in the third family, while the patient was not excreting the virus.

The isolation of 26 strains of type 1 poliovirus from 13 patients and 5 contacts showed that type 1 poliovirus infection was prevalent in the area.

COIMBATORE

An outbreak of poliomyelitis occurred in Coimbatore and surrounding areas from April to August 1964.

Out of 53 cases reported 49 were considered clinically as cases of poliomyelitis. Out of the 49 cases 48 occurred among children less than 9 years. Of these, 6 (12.2%) were in children less than 1 year and 37 (75%) among children aged 1 to 4 years.

Fever was present in nearly all the cases during or immediately prior to the onset of paralysis. In 34 cases the paralysis was severe as it involved more than one limb. Further, the residual paralysis was present in 21 out of 26 cases examined during convalescence.

Two deaths occurred in the 49 cases. One was in a boy aged 3 years, who died on the 7 day of bulbar poliomyelitis. The other was in a boy aged 7 years who died on the 35 day of respiratory paralysis.

Virus isolations

From patients. Poliovirus type 1 was isolated from 3 of the 5 patients who had paralysis and from whom samples were collected within 27 days of onset of paralysis. No virus was isolated from the 11 specimens collected from 9 patients after this period.

From family contacts. Four samples were obtained from 2 members in each of 2 families. Type 1 poliovirus was isolated from 1 of the 2 contacts aged 8 years, while the index case in the family was also excreting the virus 9 days after the onset of paralysis.

The isolation of poliovirus type 1 from 3 patients and 1 contact showed that the outbreak was due to poliovirus type 1 infection.

DHALAVOIPURAM

The prevalence of cases of poliomyelitis in Dhalavoipuram in Ramnad district from November 1965 was reported to the Institute in February 1966.

Over 50 cases suspected to be poliomyelitis were examined. Though all of them gave a history of fever of short duration, signs and symptoms suggestive of polio infection were present only in 10 cases. A survey in the area revealed another 20 cases of paralytic polio with onset of illness before 1965.

Only children less than 4 years of age developed the disease. In 83.3% of cases the age was below 3 years. All the cases except one appeared to have been infected at Dhalavoipuram.

The population at Dhalavoipuram is about 8,000 and paralytic polio has occurred there since 1951. From 1959, cases were met with every year, the number of cases during 1965–66 being four. The incidence of paralytic polio was high in the area compared with that in the other areas studied so far.

Laboratory studies

Virus isolation

In patients. Type 1 poliovirus was isolated from the 2 faecal samples collected 9 and 10 days after the onset of paralysis from a patient aged 11 months.

In contacts. One strain of type 1 poliovirus was isolated from the faecal sample of a family contact aged 10 months: The index case in the family was aged 2.5 years and no virus was isolated from the faeces of the patient collected 9 days after the onset of illness.

Basic data on poliomyelitis

The data on the incidence of poliomyelitis in the country are meagre. In

connection with the programme of the Institute to produce and try out live polio vaccine, it was considered essential to collect basic data on (i) the incidence of poliomyelitis, (ii) the types of virus prevalent, (iii) the age at which the children came in contact with the virus as judged by the appearance of antibodies, and (iv) the other viruses prevalent among them, which were likely to interfere with live virus vaccination. Coimbatore city was chosen for this study.

Since October 1960, rectal and throat swabs and blood are being collected from children, new-born to 12 years of age. The subjects are those attending paediatric section of the Government Headquarters Hospital, Coimbatore, either as in-patients or out-patients. Standard methods were employed for the collection, transport, storage and examination of the specimens.

Virus isolations

From rectal swabs. The examination of rectal swabs from 1,811 children have been completed. The virus isolations obtained in the different age groups are given in Table 8.

It was found that out of 66 viruses isolated, 22 belonged to the polio group, 25 to the Coxsackie group, 17 to the ECHO group. Two viruses are yet to be identified.

From throat swabs. Out of 1,844 throat swabs examined, 26 viruses were isolated. Of these, 13 belonged to the polio group, 7 to the Coxsackie group, and 6 to the ECHO group. The virus isolations obtained in different age groups are given in Table 9.

Serological studies

The sera of 1,486 children were examined for the presence of neutralizing antibodies against the different types of polioviruses.

The results are presented in Table 10.

Summary

Ninety-two strains of viruses were isolated from 83 children examined giving an overall isolation rate of 4.5%. Virus strains were isolated from the rectal swabs of 57 and throat swabs of 17 children. In 9 children, viruses were recovered from both the rectal and throat swabs. Among these, in 5 instances the same viruses (i.e., polio type 2, Coxsackie A9 and untypable, ECHO) were isolated from both the rectal and throat swabs. In the remaining children, 3 strains of ECHO viruses and 1 strain of Coxsackie A9 virus were isolated from the rectal swabs, while 2 strains of polio type 2 virus and one strain each of Coxsackie B5 and A9 virus were isolated from the throat swabs.

The polio group of viruses formed 38.0% (35 out of 92) of the total virus isolates. Further, these viruses were present in 37.3% (31 out of 83) of children who showed evidence of infection with enteric viruses. The isolation rate for poliovirus types 1, 2 and 3 was 20.7%, 12.0% and 5.4%, while the infection rate was 22.9%, 12.0% and 6.0% respectively. The polioviruses contituted 50.0% and 33.0% of the isolates from the throat and rectal swabs respectively.

Type 1 poliovirus was isolated from the rectal and/or throat swabs of children of all age groups below 8 years of age. This virus was also isolated from the throat swab of a child aged 11 years in whom the clinical diagnosis was not paralytic poliomyelitis. Type 2 poliovirus was not isolated from children aged 5 years and above. The isolation rate for type 3 poliovirus was the lowest though serological data confirmed the presence of infection with this virus in the area studied.

The other intestinal viruses, which can be isolated in tissue culture were met with upto the age of 7 years. These

TABLE 8

Age group	No. of rectal swabs examined	Virus isolations		Nature of virus										
				Poliomyelits type			Coxsackie					ECHO		Not yet identified
		Total No.	Percentage	1	2	3	A9	B2	B5	Untypable	To be typed	Untypable	To be typed	
New-born	175	...	...	...	...	...	...	...	...	...	...	...	...	...
6 months	57	2	3.5	...	...	...	1	...	...	...	...	1	...	...
<1 year	117	9	7.7	2	2	...	...	...	1	2	...	2	...	...
*1 year	245	18	7.3	3	2	2	3	1	...	3	...	4	...	...
2 years	259	17	6.6	4	1	...	2	...	...	5	...	5	...	...
3 ,,	209	7	3.2	...	1	1	...	...	...	2	...	2	...	1
4 ,,	184	8	4.3	1	1	1	...	...	...	3	...	1	...	1
5 ,,	204	2	1.0	...	...	...	1	...	...	...	...	1	...	...
6 ,,	138	1	0.7	...	...	...	...	...	...	...	...	1	...	...
7 ,,	66	2	3.0	1	...	...	...	...	...	1	...	...	...	...
8 ,,	48	...	...	...	...	...	...	...	...	...	...	...	...	...
9 ,,	47	...	...	...	...	...	...	...	...	...	...	...	...	...
10 ,,	45	...	...	...	...	...	...	...	...	...	...	...	...	...
11 ,,	10	...	...	...	...	...	...	...	...	...	...	...	...	...
12 ,,	7	...	...	...	...	...	...	...	...	...	...	...	...	...
Total	1,811	66	3.6	11	7	4	7	1	1	16	...	17	...	2

* Denotes age one year to less than two years.

TABLE 9

Age group	No. of throat swabs examined	Virus isolations		Nature of virus										Not yet identified
				Poliomyelitis type			Coxsackie				ECHO			
		Total No.	Percentage	1	2	3	A9	B5	Untypable	To be typed	Un-typable	To be typed		
New-born	173	1	0.6	...	...	...	...	...	...	...	1	...		...
6 months	63	2	3.2	1	...	...	...	...	...	...	1	...		...
<1 year	120	2	1.6	...	2	...	...	...	...	...	...	...		...
*1 year	249	8	3.2	...	2	1	1	...	1	...	3	...		...
2 years	263	2	0.8	1	...	...	1	...	...	...	...	...		...
3 ,,	205	6	3.0	2	...	...	3	...	...	...	1	...		...
4 ,,	183	...	...	...	...	...	...	...	...	...	...	...		...
5 ,,	205	3	1.5	2	...	...	...	...	1	...	...	...		...
6 ,,	137	1	0.7	1	...	...	...	...	...	...	...	...		...
7 ,,	70	...	...	...	...	...	...	...	...	...	...	...		...
8 ,,	53	...	...	...	...	...	...	...	...	...	...	...		...
9 ,,	48	...	...	...	...	...	...	...	...	...	...	...		...
10 ,,	46	...	...	...	...	...	...	...	...	...	...	...		...
11 ,,	15	1	6.6	1	...	...	...	...	...	...	...	...		...
12 ,,	14	...	...	...	...	...	...	...	...	...	...	...		...
Total	1,844	26	1.4	8	4	1	5	...	2	...	6	...		...

* Denotes age one year to less than two years.

TABLE 10

Age group	No. of sera examined	Percentage of sera showing antibody** to poliomyelitis virus		
		Type 1	Type 2	Type 3
New-born	155	91.6	89.7	83.2
Upto 6 months	41	31.7	48.7	14.6
<1 year	78	11.5	18.1	12.8
*1 year	183	18.6	29.0	26.1
2 years	198	47.5	50.5	47.0
3 ,,	169	64.5	65.7	54.4
4 ,,	157	75.1	75.1	67.5
5 ,,	168	86.9	80.4	72.0
6 ,,	120	85.0	90.2	81.7
7 ,,	59	96.6	93.2	83.0
8 ,,	45	97.7	98.7	97.7
9 ,,	43	95.4	93.1	86.4
10 ,,	47	93.6	93.6	93.6
11 ,,	14	92.9	100.0	92.6
12 ,,	9	88.9	88.9	77.8
Total	1,486			

**Antibody present in 1:8 dilution of serum.

*Denotes age one year to less than two years.

viruses formed 50% or less of the total virus isolates in the three age groups, 1, 6 and 7 years; while in seven others they constituted more than 50% of the total isolates.

The virus isolations indicate that the susceptible age to infection with types 1, 2 and 3 polioviruses may be upto 7, 5 and 5 years respectively. It is possible that the data for type 3 poliovirus, which is least prevalent in the area, is not quite reliable.

In sera from new-born children, antibodies to poliovirus types 1, 2 and 3 were present in 91.6% 89.7% and 83.2% respectively. Children aged 6 months to less than 1 year were most susceptible to infection with these viruses. By two years of age, nearly 50% of the children showed serological evidence of infection with all three types of poliovirus. Beyond this age the infection rate was lower to all the polioviruses in all the age groups studied. Over 90% of children showed antibody to poliovirus types 1, 2 and 3 at the age of 7, 6 and 8 years respectively.

The susceptible age to infection with poliovirus type 1 is upto 7 years as shown by virus isolation from rectal swab and serology. Serological data indicate that the susceptible age to infection with poliovirus types 2 and 3 is upto 6 and 8 years respectively, even though these viruses were isolated only from children 5 years of age.

Preparation of live oral poliomyelitis vaccine

In recent years poliomyelitis in an epidemic form is being reported in increasing frequency in different parts of India. This has resulted in a demand for a reliable prophylacti against the disease in the country. The Government of India decided that live poliomyelitis vaccine (Sabin) would be the most suitable for the purpose, and on the advice of Dr. A.B. Sabin selected the Pasteur Institute, Coonoor, for the preparation of the vaccine for the whole country.

The imported as well as some of the indigenously available items of equipment required for this were provided by the USAID Mission, New Delhi, while other locally available items of equipment, staff and other expenses are met by the Ministry of Health, New Delhi.

Strains of attenuated poliovirus were kindly made available by Dr. Sabin.

The seed viruses for all the three types of poliovirus were prepared at the Institute and tested. Through the good offices of the WHO, the Medical Research Council Laboratories, London, kindly retested the batches and carried out the neurovirulence tests in monkeys. They confirmed the suitability of the seed viruses in June 1967. Since then the production of vaccine has commenced.

The first batches of vaccine prepared at the Institute are being retested at the Institute of Poliomyelitis and Encephalitis, Moscow, and the Haffkine Institute, Bombay, before release.

(3) LABORATORY PROCEDURES

Attempts are continuously being made to improve the various laboratory procedures used in connection with the work on enteroviruses. Some of the techniques which have been standardized are: (i) metabolic inhibition test for the assessment of polio antibody in sera, (ii) plaque formation of polio viruses in monolayers of monkey kidney cell cultures, and (iii) a micro-method for viral complement fixation tests using antigens prepared from infected tissue culture fluids.

(4) PREPARATION OF ANTISERA

The Institute prepares its requirements of antisera against the various enteroviruses. During the past 10 years

methods have been standardized for the preparation of antisera against polio, Coxsackie and ECHO group of viruses in monkeys, rabbits and sheep.

REFERENCES

Sci. Rep. Pasteur Institute, Coonoor. 1957–66.

5. VACCINIA

(1) STUDIES ON THE CULTIVATION OF VACCINIA VIRUS

Cultivation in sheep kidney cell cultures

Several investigators have reported the cultivation of vaccinia virus in a variety of primary mammalian and avian cell tissue cultures and established mammalian cell lines. Studies were undertaken to find a suitable cell population that could be prepared with relative ease and in an economical manner for the assay of vaccinia virus and its antibodies and investigate its usefulness for the preparation of a vaccine against smallpox. Cell cultures prepared from chicken embryo kidney, heart, liver and whole chicken embryo were found to support the multiplication of the virus. The cells showed marked cytopathic changes and were destroyed within 72 hours. The preparation of cell cultures from chicken embryo tissue in quantity and their maintenance after establishment in monolayers were, however, considered not quite suited for large scale cultivation of the virus. Sheep kidney cells have been found useful for the cultivation of many viruses but there was no report of the cultivation of vaccinia virus in these cultures. In view of the relative ease of obtaining sheep kidneys from the slaughter house and their value in virus studies, attempts were made to determine their usefulness for the cultivation of vaccinia virus. Kidneys obtained immediately after slaughter were found useful for the purpose.

The medium used for the cultivation of sheep kidney monolayers was Hanks' balanced salt solution, 80%; sheep serum, 20%; lactalbumin hydrolysate, 0.5%; yeast extract, 0.1%, and antibiotics at a concentration of penicillin 200 units, and streptomycin, 200 ug. per ml. The medium was replaced on the third day. After good monolayer formation, usually 6 to 9 days after the preparation of the cultures, the medium was replaced with a maintenance medium consisting of Earle's balanced salt solution 80% with 10% sheep serum, the concentration of other substances being the same as in the growth medium. This medium was used for virus cultivation.

The inoculation of vaccinia virus in sheep kidney cell cultures was followed in 48 hours by cytopathic effects, which progressed to complete cell necrosis by the seventh day. Growth cycle studies revealed a steady and rapid multiplication of the virus, the highest concentration obtained in some cultures being $10^{8.5}$ $TCID_{50}$ per ml. on the fourth day following inoculation. The virus was found to multiply equally well in serum-free medium. It was found that the virus could induce plaques in sheep kidney monolayers similar to those induced by the virus in chicken embryo and monkey kidney cell cultures, and that this property could be utilised for the assay of virus and its antibodies. The sheep kidney cultures appeared to offer a better medium for the titration of the virus than embryonated chicken eggs. Also, they were more sensitive than the rabbit skin for titration purposes.

Vaccinia virus grown in sheep kidney cultures could be freeze-dried without significant loss in virus concentration, and the freeze-dried material was found to produce a satisfactory antigenic response.

The ease and economy with which primary sheep kidney cultures could be prepared, the high concentration of virus obtained in these cultures even in the absence of serum, and the fact that culture virus could be freeze-dried without suffering a significant loss in titre or anti-

genicity suggested the value of such cultures for the production of a vaccine against smallpox.

Improvement of the method of preparation of sheep kidney cell cultures

The preparation of cell cultures from the kidneys of freshly slaughtered young sheep was according to a method, which was a modification of that described by Youngner. Later, the simplified method of Bodian for the dispersion of kidney cells with trypsin was employed. Subsequent work showed that with overnight trypsinization of kidney cells and the use of lactalbumin hydrolysate medium, the growth of cells was not consistent. While occasionally the results were good, in general the cell growth was poor and the formation of cells sheets was delayed. Attempts were, therefore, made to improve the method of cultivation of sheep kidney cells.

(*i*) *Period of trypsinization.* According to the method used earlier, sheep kidney cells were subjected to continuous trypsinization for 16 to 18 hours at 4°C., the concentration of trypsin used being 0.2%. Experiments were undertaken to determine whether a reduction of the period of trypsinization would increase the viability of the cells. It was found that good monolayers were obtained when the cells were collected after 1 to 4 hours trypsinization. The viability of the cells collected upto 4 hours was very high compared with those collected after 16 hours trypsinization.

Recent studies indicated that the period of trypsinization could be further reduced to 1.5 hours. When Hanks' basal salt solution was used for the preparation of trypsin (0.2%) instead of GKN, the viability of the cells was found to be better. The pH of the trypsin solution was adjusted to 7.6.

(*ii*) *Improvement of the growth medium.* The growth medium originally employed consisted of Hanks' BSS, 80%; lactalbumin hydrolysate, 0.5%; yeast extract, 0.1%; and sheep serum, 20% with antibiotics. Attempts were made to (a) improve the above medium, (b) evolve a medium without lactalbumin hydrolysate and yeast extract, and (c) reduce the concentration of serum.

Several media were tried with this object in view. Of these, two media have given consistently good results and have been designated as medium V1 and medium V2. Medium V1 is essentially Eagle's medium with 7.5% sheep serum. Medium V2 contains lactalbumin hydrolysate, arginine, histidine, glycine and yeast extract with 15% sheep serum. Of these, medium V1 is superior as the cell sheets are regularly formed within 5 to 6 days.

Attempts to increase the titre of vaccinia virus in sheep kidney cultures

Although good titres were obtained with lactalbumin hydrolysate medium described earlier, they were not consistently high. Studies were, therefore, undertaken to improve the titres obtained in sheep kidney cultures.

(*i*) *Improvement of the medium.* Several media were tried. Of these, medium V3, which is a synthetic medium without serum, gave consistently good results. Employing this medium titres of $10^{7.0}$ to $10^{8.0}$ $TCID_{50}$ per ml. could be obtained in cultures with several of the vaccinia virus strains tried. Similar results were obtained when the virus was grown in 40 ml. quantities in Roux bottles.

Attempts are continuously being made to improve the medium V3 by the addition of several compounds. Over 50 compounds have been tried so far, singly or in various combinations. Of these, phosphotidyl-L-serine and protamine sulphate appeared to have some beneficial effect.

(*ii*) *Effect of adding fresh sheep kidney cells to cultures.* Investigations were undertaken to determine whether the virus titres could be stepped up by the addition of fresh primary sheep kidney cells at varying intervals. The results indicated that slightly higher virus titres could be obtained in cultures when extra cells were added to the infected sheep kidney monolayers at 0, 24, 48 and 72 hours after infection.

Cultivation in CAM and chick embryo tissue

Attempts were made to grow the Lister Institute strain of vaccinia virus in bottles containing glass wool according to the technique used for the cultivation of influenza virus. Minced CAM from 12-day-old chick embryos or minced 8-day-old chick embryo tissue with medium V3 was employed. Specimens were collected at different hours and titrated in sheep kidney cultures. It was found that the virus titres obtained with CAM and chick embryo tissues were similar and ranged from $10^{5.3}$ to $10^{5.5}$ $TCID_{50}$ per ml. However, they were lower than those obtained with sheep kidney cell cultures.

Titration of vaccinia virus in different cell cultures and in the egg

Barne's strain of vaccinia virus was adapted to the chorioallantoic membrane (CAM) of eggs and sheep kidney cells (SK). The CAM and SK adapted viruses were titrated simulaneously in human amnion, HeLa, rabbit kidney, sheep kidney and chick embryo cell cultures and in the egg. With CAM adapted viruses the highest titre was obtained in chick embryo tissue cultures ($10^{8.1}$ $TCID_{50}$). The titres obtained with human amnion, rabbit kidney and sheep kidney cells were similar ($10^{7.5}$ $TCID_{50}$). The titre in eggs was $10^{6.7}$ EID_{50} while that in HeLa cell cultures was only $10^{5.5}$ $TCID_{50}$ per ml.

With the SK adapted virus the titres were generally lower. The titres per ml. in the different systems were: chick embryo cells, $10^{6.7}$; human amnion cells, $10^{5.7}$; sheep kidney and rabbit kidney cells, $10^{5.5}$ and HeLa cells, $10^{4.7}$ $TCID^{50}$. The titre in the egg was very low, $10^{3.9}$ EID_{50} per ml.

(2) Lyophilization of vaccinia virus

Studies were undertaken to determine the best suspending medium for freeze-drying of vaccinia virus grown in tissue culture. A batch of the Lister Institute strain of virus grown in sheep kidney cultures employing medium V3 was used for the purpose. The effect of using solutions of sucrose, gelatin, polyvinyl–pyrrolidone (PVP), dextran, and peptone, 1% and 5%, either singly or in different combinations was investigated.

It was found that there was no fall in virus titre during lyophilization when peptone 5% alone, gelatin and peptone 5%, PVP alone or PVP and dextran were used as the suspending medium. But, there was a slight fall in the titre when these were added to the virus pool. This initial fall was less and the final titre of the lyophilized material good when sucrose and dextran, gelatin and peptone (1%), PVP and peptone (5%), or sucrose, gelatin and peptone (5%), were used as the suspending media.

The ampoules stored at 4°C. were retitrated in sheep kidney cultures at the end of 14 and 27 months. It was found that the fall in virus titre was minimal with sucrose and peptone (5%), gelatin and peptone (5%), PVP and peptone (5%) and peptone (5%).

REFERENCES

Sci. Rep. Pasteur Institute, Coonoor. 1960–66.

SUBRAMANYAM, P., DIVAKARAN, S. and VINOD RAJ, P. (1961) *Bull. Wld Hlth Org.*, **25**, 33.

6. TRACHOMA INCLUSION CONJUNCTIVITIS AGENTS

(1) Isolation from clinical cases

The viral aetiology of follicular conjunctivitis is under investigation at the Institute since 1960. In addition to the specimens received from the ICMR Trachoma Research Unit, Aligarh, and the Government Opthalmic Hospital, Madras, conjunctival scrapings collected from children from other areas were examined. The results of these investigations are summarized below.

COONOOR

With the assistance of a specialist in ophthalmology, 25 children between 4 and 12 years of age, studying in two primary schools were selected for study. The clinical classification of the cases were: trachoma: stage 1, 14; stage 2, 6; doubtful, 4; and bilateral blepharitis, 1. In most of these cases the upper tarsal conjunctiva and upper fornix were covered with follicles of varying sizes. The lower palpebral conjunctiva and fornix contained fewer follicles. The children did not complain of any symptoms, probably because there was no secondary infection.

No inclusion bodies were found in all the 25 smears examined.

None of the 25 specimens yielded a viral agent in human amnion cell cultures after 2 serial passages.

By yolk sac inoculation, 3 strains of trachoma virus were isolated. Particles resembling elementary bodies were detected in smears from yolk sacs during the first three passages. These increased in number during the subsequent passages.

Two of the strains isolated were from cases diagnosed as trachoma, stage 1, and the third was from the case of bilateral blepharitis.

KOTAGIRI

The conjunctival scrapings from an adult having trachomatous follicles and early pannus were examined. Elementary particles were found during the first passage of the material in chick embryo yolk sac. The strain was successfully passaged thrice in chick embryo yolk sacs.

BANGALORE

Conjunctival scrapings and smears collected from cases of trachoma attending the Minto Eye Hospital, Bangalore, were kindly forwarded by Dr. Puttanna.

In 1964, seventeen conjunctival scrapings from 12 patients were received in tryptose phosphate broth containing 20 mg. per ml. of streptomycin. All these were inoculated into the yolk sac of 7-day-old chick embryos. Specimens, which were free from contamination, were passed 4 times before being declared negative. From seven of these specimens a yolk sac transmissible agent was demonstrable, which morphologically resembled trachoma virus. One strain was isolated from a case diagnosed as trachoma, stage 1, two strains from two cases of trachoma, stage 2, and one strain from a case of trachoma, stage 3. One strain was isolated from a case showing follicles in upper palpebral conjunctiva and another from a case of corneal ulcer of the left eye.

In 1965, conjunctival scrapings from 13 patients were received and 2 strains of trachoma virus were isolated. Of these, one strain was from a case showing follicles in the upper eyelids without pannus and the other from a case with follicles in the upper eye lid with pannus.

(2) Maintenance of strains

The strains of trachoma virus isolated were maintained by serial passage in yolk sacs of 6 to 8-day-old chick embryos. It

was found that the infected yolk sacs could be stored at –20°C.

(3) Growth in chick embryos

Some of the strains of trachoma virus were adapted to grow in the yolk sac of duck eggs. It was found that 10- to 11-day-old embryos were best suited for the growth of the virus.

(4) Adaptation to allantoic and amniotic cavities of chick embryo

Allantoic passage. An attempt was made to adapt a strain of trachoma virus grown in yolk sac to the allantoic cavity of 9-day-old chick embryos. The strain was passaged in the allantoic cavity 5 times but no virus was demonstrable in any of the passages.

Alternate amniotic and yolk sac passages. Experiments were undertaken to determine whether trachoma virus could be adapted to the amniotic cavity of 11-day-old chick embryos by alternate amniotic and yolk sac passages. It was not possible to demonstrate the virus in smears of the yolk sac, and amniotic and allantoic fluids during any of the four passages.

(5) Adaptation to the mouse lung

Experiments were carried out to adapt the agent to the mouse lung by nasal installation. Seven-day-old mice were used after anaesthetising them with ether-chloroform mixture. None of the mice showed any evidence of illness. Gross lesions were not found during post-mortem examination of the mice and the impression smears from the lungs did not reveal any inclusion bodies. Another experiment in which one strain was serially passaged twice in the lungs of 3-weeks-old mice ended in failure.

(6) Adaptation to tissue cultures

Attempts were made to adapt 5 strains of trachoma virus isolated in the yolk sac of chick embryos to various cell cultures. Infected yolk sac material was used to initiate serial passage of the strains in HeLa, FL and primary human amnion cell cultures. It was not possible to adapt any of the virus strains to the above cell cultures.

(7) Acridine orange staining of yolk sac smears

During serial passage of trachoma virus strains in yolk sac it was noted that normal yolk material in yolk sac smears was also stained by Giemsa and Machiavello stains and gave rise to some confusion in the recognition of true viral particles. Therefore, the fluorochrome, acridine orange, was tried as it specifically stains deoxyribonucleic acid green and ribonucleic acid red. The smears were examined under ultra-violet light.

Infectivity experiments showed that when both acridine orange and Giemsa strains were positive, or when acridine orange stain alone was positive, the specimens contained the virus. This was confirmed by the observation that it was possible to demonstrate a transmissible agent in yolk sacs when acridine orange staining was positive, but not consistently when Giemsa stain alone was positive.

REFERENCES

Sci. Rep. Pasteur Institute, Coonoor. 1960–66.

7. HERPES SIMPLEX

(i) Isolations

The throat swabs being collected regularly for the isolation of respiratory viruses, as well as swabs from suspicious skin lesions were examined regularly for the presence of herpes simplex virus. The number of examinations done and the strains of virus isolated are given in Table 11.

TABLE 11

Year	Herpes virus isolations from			
	Throat swabs		Skin lesions	
	No. examined.	No. of strains isolated	No. examined	No. of strains isolated
1960	442	4	...	...
1961	479	7	1	1
1962	1,293	12	1	1
1963	755	2	3	...
1964	559	2	...	...
1965	390	...	...	...
1966	375	...	...	...

It was found that the isolations of the virus from children were greater than those from adults. The age distribution of the sick children from whom 16 strains of herpes virus were isolated was as follows : 1 yr., 3; 2 yrs., 5; 3 yrs., 1; 5 yrs., 2; 6 yrs., 1; 7 yrs., 3; and 9 yrs., 1.

(2) Properties of virus

Virus isolation

Primary human amnion cell cultures were used for the isolation of the virus and its subpassage. The maintenance medium consisted of Earle's BSS, 75%; tryptose phosphate broth, 20%; deactivated sheep serum 5%, and antibiotics.

Unlike adenoviruses, which take about 7 days or more to show cytopathic effect, herpes virus usually showed characteristic changes within 24 to 48 hours. Tissue culture fluid from a positive tube had to be passaged repeatedly in other tubes to increase the virus titre.

Behaviour in other cell cultures

The morphological changes in HeLa cells were similar to those observed in human amnion cells. Only they occurred a little slower. Both the monkey and sheep kidney cells became rounded and shrunken in focal patches within 48 hours. This finding suggested that the changes caused by herpes in sheep kidney cells might be useful in differentiating it from enteric viruses. Rabbit kidney cells appeared to be more sensitive than monkey and sheep kidney cells to herpes virus. They were completely destroyed within 48 hours and the titres obtained were also higher.

Behaviour in eggs

Six strains were passaged on the chorio-allantoic membrane of 13-day-old eggs. Fine, pin-point, opaque lesions were found along the course of the blood vessels of the membrane. With some strains, thickening and gelatinous oedema of the membrane, was a prominent feature.

Behaviour in animals

Mice. New-born, 3-week-old and adult mice were inoculated with the strains of herpes virus. New-born mice received 0.2. ml. intraperitoneally and the adults 0.5 ml. intraperitoneally, or 0.03 ml. intracerebrally. The mice died

in 7 to 10 days and herpes virus was isolated from their brains.

Since adenoviruses do not kill mice, inoculation of these animals might be a simple method of distinguishing between adenoviruses and herpes virus. It was also found that while there was a marked fall in the titre of herpes virus in tissue culture fluids, a fall to the same extent was not encountered in the brains of mice on storage.

Rabbits. The strains were given intracerebrally in 0.5 ml. amounts into rabbits. They died in 7 to 10 days and herpes virus was reisolated from their brains in amnion cell cultures and in mice.

Plaque studies

Using bottles of primary human amnion cells as host cells and 10% suspension of the brain of an infected rabbit as the inoculum, plaques not more than 3 mm. in diameter were obtained. Herpes virus was recovered from the plaques.

Neutralizaiion

The strains were neutralized by specific antiserum for herpes.

(3) Cultivation in chorioallantoic membrane of duck eggs

Four strains of herpes virus were inoculated in 0.2 ml. amounts on the CAM of 14–day–old duck eggs. The eggs were incubated at 36°C. After incubation for 72 hours the embryos were found to be alive. The eggs were chilled and opened. It was found that the plaques were slightly bigger than those usually obtained on the CAM of chick embryos. They were also more discrete and opaque. One fresh strain produced vesicular lesions with ballooning instead of the usual plaques. The titre of this strain was 100–fold greater than that of a strain which produced plaques.

These findings suggest that it might be advantageous to use duck's eggs instead of hen's eggs for isolation and propagation of herpes virus.

(4) Preservation of herpes virus

When herpes virus was stored as tissue culture fluid at –20°C. in a deep-freeze cabinet, it was found that the titre of the virus decreased markedly after some weeks. It was found possible to store the virus for longer periods by the following methods: (a) Tissue culture fluid was mixed with an equal quantity of sterile skimmed milk and stored frozen at –20°C. (b) Intracerebral injection of tissue culture fluid killed young mice in about 5 days. The brain was harvested aseptically when the animals were moribund and emulsified in Hanks' BSS with sterile glass powder. The suspension was then mixed with an equal quantity of sterile glycerine or sterile skimmed milk and stored frozen.

REFERENCES

Sci. Rep. Pasteur Institute, Coonoor. 1961–66.

8. B VIRUSES (*HERPES VIRUS SIMIAE*)

(i) Preservation of the virus

This is one of the human pathogenic viruses latent in monkey renal tissue. It is not a stable virus. The stability of the virus stored at 4°C. and –40°C. was investigated.

The virus maintained its titre well in one of the five pools tested for a period of 8 months when stored at 4°C. In four others the fall in titre was less than 1 log up to 3 months' storage, beyond which it increased progressively.

In two pools titrated during one month's storage at –40°C. the fall in titre was less than 1 log. Subsequently, there

was a sudden fall in titre in all the pools. This titre was maintained without significant change in all the pools during further storage.

The virus titre was lower at all periods of storage at –40°C. during the period of study.

(2) Presence of antibodies to B virus in normal animal sera

The presence of antibodies to B virus in the sera of normal calves and monkeys was investigated.

B virus antibody was not demonstrable in the sera of normal calves tested.

It was found that sera from only 3 monkeys caught during 1963 neutralized the virus upto a dilution of 1 in 4. Serological evidence of infection was not present in the monkeys caught after 1963. The virus was not demonstrable in any of the cultures prepared from the renal tissue of monkeys used in the study.

REFERENCE

Sci. Rep. Pasteur Institute, Coonoor. 1965–66.

9. MEASLES VIRUS

Throat swabs were collected from three children who had measles rash of less than 24 hours' duration. Primary human amnion cell cultures were used for growing the virus. The material was inoculated in 0.3 ml. amounts into tubes followed by 0.7 ml. of the maintenance medium. Early degenerative changes were noticed on the fourth day and definite cytopathic changes by the tenth day. Subpassages were made in amnion cells using 0.4 ml. of the culture fluid as inoculum.

Cytological studies were carried out on coverslips with cell sheets infected by this agent. The changes appearing in the stained preparations varied. In primary human amnion cells mitotic figures disappeared from the cell sheets after infection. Multinucleated giant cells appeared early in some preparations. In others, cytoplasmic inclusion body and large vacuoles in the cytoplasm pushing the nucleus to an eccentric position appeared early. Intranuclear inclusions were fewer in the early stages. Coverslips prepared with sheets of KB cells demonstrated the inclusion bodies better.

REFERENCES

Sci. Rep. Pasteur Institute, Coonoor. 1958.

10. SV_{40} VIRUS

Isolation from normal monkeys

Infection with SV_{40} virus is usually latent in monkeys. Virus is present in the renal tissue of infected animals whose sera also show antibodies to the virus. The virus can be isolated from the renal cells or medium obtained from the *in vitro* cultures of these cells. Experiments were undertaken to study the presence of SV_{40} virus infection in the animals used for the preparation of primary monkey kidney cell cultures at the Institute. The monkey was considered to be infected with the virus if the characteristic cytopathic effect was produced in cultures of BSc-1 cell line which were inoculated with the medium collected from the primary monkey cell cultures prepared from its renal tissue.

The virus was isolated from 12 out of 16 (75%) of cultures prepared from monkeys caught during 1963. All the specimens collected from these cultures between 3 and 30 days after setting up showed the virus, indicating that the virus if present in the renal tissue of the monkey is readily isolated in BSc-1 cell cultures.

A virus was isolated from the specimen collected on the third day from one of

the cultures set up from a monkey caught during 1964, while the specimen collected on the seventh day from the same culture did not yield the virus.

It is interesting to note that SV_{40} virus has not been demonstrated in monkeys caught after 1964.

Sci. Rep. Pasteur Institute, Coonoor. 1965, 1966.

11. TISSUE CULTURE METHODS

The important feature of viruses is their ability to multiply in living cells. In recent years tissue culture has been used extensively for the study of viruses.

It is well known that monolayer cultures of monkey renal epithelial cells have a wide viral spectrum. They are the cells of choice for the isolation of enteric group of viruses as they are more resistant to the faecal toxins than other epithelial cell cultures. Sheep and rabbit kidney cell cultures are suited for the isolation and cultivation of certain groups of viruses. Human amnion cells are found to be very valuable for the isolation of many viruses, some workers claiming them to be superior to HeLa and fibroblastic cells. Some of the wild viruses occasionally encountered in monkey kidney cells are notably absent in human amnion cells. The easy availability of human placenta renders it possible for even small laboratories to prepare these cells.

All the above cells are grown at the Institute in large quantities in connection with the various lines of work on viruses in progress. Attempts are continuously being made to improve the technique of growing these cells. Some of the important observations in respect of the different cell cultures are summarized below.

Monkey kidney cell cultures

It was found that better results were obtained when following anaesthesia the monkey was exsanguinated before removal of the kidneys.

The cells were obtained either from the cortical tissue or from decapsulated kidneys following treatment with 0.25% trypsin solution with the aid of a magnetic stirrer.

When cortical tissue alone was used any of the following methods for releasing cells were found to be equally good as judged by cell yield and percentage of successful cultures: (i) Allowing 1 to 2 litres of trypsin solution to flow through the flask in which the tissue was suspended in 250 ml. of the same solution, in 6 to 8 hours, (ii) treatment with 200 ml. of fresh solution for 15 min. each time, 10 to 12 times, and (iii) extraction up to 4 times at intervals of 4 to 8 hours with 300 ml. of fresh solution every time for a period of 16 to 18 hours at 4°C.

With decapsulated kidneys alternate extraction with 0.25% trypsin and growth medium containing 2% inactivated sheep serum gave larger cell and culture yields and was regularly employed.

Growth medium containing Hanks' balanced salt solution (HBSS), 0.5% lactalbumin hydrolysate and 5% deactivated sheep serum without yeast extract gave good yields of primary monkey renal cell cultures.

It was found that demineralised water could be substituted for double distilled water in making the medium.

The concentration of cells used was 5×10^5 and 2.5×10^5 cells per ml. respectively for distribution into tubes and bottles.

Rabbit kidney cell cultures

Rabbits about 4 weeks old were used. They were exsanguinated before the kidneys were removed. Two methods were employed for setting up cultures.

In the first method, minced, washed cortical kidney tissue was trypsinized in 0.2% trypsin solution for 18 hours at 4°C. The resulting cell suspension was filtered through two layers of gauze into centrifuge tubes. The cells obtained after centrifugation at 1,000 r.p.m. for 10 min. were washed three times in growth medium consisting of HBSS, 0.5% lactalbumin hydrolysate, 0.1% yeast extract and 10% sheep serum with antibiotics. The cells were suspended in sufficient growth medium to give a concentration of 3 x 10^5 cells per ml. and distributed into tubes and bottles.

In the second method decapsulated kidneys were used. The cells were extracted from the tissue by alternately stirring it over a magnetic stirrer with 150 ml. each of growth medium consisting of 2% calf serum at pH 7.2. The cells were recovered in 4 to 6 extractions, each of 30 min. duration. They were dispersed in growth medium containing HBSS, 0.5% lactalbumin hydrolysate, 0.1% yeast extract and 10% calf serum, concentration adjusted to 4 x 10^5 to 6 x 10^5 cells per ml. and distributed in tubes and bottles.

Sheep kidney cell cultures

The cortical tissue from kidneys of freshly slaughtered young sheep was used. Trypsin (0.2%) was prepared in HBSS and the pH adjusted to 7.6.

It was found that medium V1 which was essentially Eagle's medium with 7.5% sheep serum gave the best results as the growth medium. Medium V2 containing lactalbumin hydrolysate, yeast extract, arginine, histidine, and glycine with 15% sheep serum also gave good results.

Human amnion cell cultures

The cells from human placenta were recovered by 3 or 4 trypsinizations with 150 ml. of 0.25% trypsin in phosphate buffered saline. The pooled trypsin extract was filtered through double layers of sterile gauze, centrifuged at 1,000 r.p.m. for 10 min., repeatedly washed and resuspended in about 70 ml. of growth medium consisting of HBSS, 0.5% lactalbumin hydrolysate, 0.1% yeast extract and 20% pooled human serum. The concentration of cells were adjusted to 3 x 10^5 to 5 x 10^5 cells per ml. and distributed.

In the preparation of human amnion cells, it was found that the yield of viable cells could be increased by overnight trypsinization at 4°C. of pieces of membrane remaining after the usual four extractions.

Papain was tried in place of trypsin in the preparation of human amnion cells, and the results were found to be encouraging. This should prove useful when good quality trypsin is difficult to obtain.

Chick embryo tissue cell cultures

The growth medium for chick embryo cell cultures consisted of HBSS, 0.5% lactalbumin hyrolysate, 0.1% yeast extract, and 2% deactivated sheep serum. The concentration of cells used for setting up cultures was 4 x 10^5 cells per ml.

Cell cultures from tonsils

Cell cultures from human tonsils were prepared with a view to isolate adenoviruses from surgically removed tonsils.

Cultures of established cell lines

The standard cell lines HeLa, KB, MS, FL (human amnion), HEp 2 and BSc-1 are being maintained.

It was found that in processing stable cell lines sent by air from distant places, bottles of cells which were allowed to rest in the incubator overnight after replacing the nutrient medium fared better than those which were trypsinized and distributed soon after receipt in the laboratory.

HeLa cells. HeLa cells maintained better when transferred with 0.25% trypsin instead of with 0.05% versene.

Two types of growth media were used with good results. They were: (i) Eagle's basal medium with 0.5% lactalbumin hydrolysate, 0.1% yeast extract and 20% human or calf serum. The maintenance medium used for these cultures consisted of Eagle's basal medium 75%, Tryptose broth, Difco, 20% and sheep serum 5%; (ii) Hanks' BSS containing 0.5% lactalbumin hydrolysate, 0.1% yeast extract with 25% human serum. The maintenance medium used was Earle's BSS containing 0.5% lactalbumin hydrolysate, 0.1% yeast extract and 10%rabbit serum.

HeLa cells could be stored frozen at –56°C. and recovered after 6 weeks in a medium consisting of Earle's BSS 80%, calf serum 20%, lactalbumin hydrolysate 0.5%, yeast extract, 0.1% with 10% Analar glycerine. There was no difference in the viability of cells when they were rapidly frozen or frozen in stages by keeping at 4°C ,– 20°C. and –56°C. It was found that the critical factor in the success of the method was the concentration of cells used. If it happened to be less than 10^6 cells per ml. the results were negative.

FL (human amnion) cells. It was found that 0.02% versene was better than 0.25% trypsin solution for the transfer of FL cells.

The cell line propagated well when double distilled water or demineralised water was used in the growth medium consisting of Earle's BSS containing 0.5% lactalbumin hydrolysate, 0.1% yeast extract and 20% calf serum with antibiotics.

The FL cells in a concentration of 1×10^6 or 2×10^6 cells per ml. could be stored frozen at –40°C. or –70°C. and successfully recovered for periods upto 22 weeks, when 10% glycerine was added to the growth medium containing 20% calf serum.

Establishment of stable cell lines

Attempts are continuously being made to establish cell lines from human tissues as well as those of the monkey, guinea-pig, mouse, chick and the duck. The cells were grown by trypsinization and plasma clot methods.

From a still-born baby a kidney cell line was successfully established. It was found that all types of adenoviruses, herpes simplex and the three types of polioviruses, produced cytopathic effects as in HeLa cells. No cytopathic effect was noted with Coxsackie A9 and influenza viruses.

All attempts to establish stable cell lines from the brains of human embryos, as well as those of the monkey, guinea-pig, mouse, chick and the duck were unsuccessful.

REFERENCES

Sci. Rep. Pasteur Institute, Coonoor. 1957–66

KURIAN P.K. and MUKUNDAN, P. (1960) *J.I.M.A.*, 35,p. 405.

12. CHOLERA

(1) STUDIES ON THE CULTIVATION AND ISOLATION OF *V. CHOLERAE*

Studies have been in progress, continuously at the Institute to improve the medium used for the cultivation and isolation of *V. cholerae.* The results of these investigations have been presented regularly in the Scientific Reports of the Institute. Some of the important observations are summarized below.

(*i*) *Influence of pH*

It is a general assumption that *V. cholerae* require alkaline conditions for multiplication and many specific media employ a pH range from 8.0 to 9.5.

Experiments were undertaken to determine the effect of lower pH on the growth of *V. cholerae* under standard conditions. It was found that with several strains tested the growth was the same in peptone water or broth in the pH range 7.0 to 8.2.

(ii) Evaluation of different liquid media

Twenty-four liquid media recommended for the cultivation of *V. cholerae* were compared under standard conditions. Both *V. cholerae* and NAG vibrios were used.

It was found that *V. cholerae* grew more profusely in complex organic media than in chemically defined media and that glucose was better for growth than organic acids. When a strain with simple nutritional requirements was used, the growth in synthetic media reached a maximum in 24 hours and than ceased. In some cases the organisms were lysed. On the other hand, in complex media increase in growth was observed even after 72 hours. The different enrichment media employing high pH, high salt concentration, addition of bile salts, potassium tellurite, boric acid etc., were found actually to reduce the growth of many strains and even inhibit some. A larger inoculum was necessary for obtaining growth in chemically defined media whereas in complex media growth was the same irrespective of the amount of inoculum used.

In experiments with NAG vibrios it was found that in all complex organic media the organisms grew profusely compared to Inaba and Ogawa strains. Their growth was not satisfactory in some of the chemically defined media.

(iii) Effect of some compounds on the growth in liquid media

The effect of the addition of some compounds on the growth of vibrios in peptone water was studied.

It was found that the growth of NAG vibrios was markedly enhanced by sodium fumarate, lactate, maleate and pyruvate, adenosine and thymidine.

Sodium fumarate, lactate and maleate caused 3 to 4-fold increase in the growth of water vibrios, while guanine, N–methyl taurine, sodium pyruvate, sodium ribonucleate and taurine caused a significant growth promotion. Protamine sulphate inhibited growth.

With El Tor vibrios, sodium lactate and fumarate caused a slight enhancement while the remaining compounds resulted in a slight suppression of growth. Protamine sulphate was found to be inhibitory.

In the case of true cholera vibrios, sodium fumarate, lactate, maleate and succinate did not inhibit the growth while the remaining compounds were slightly inhibitory. Protamine sulphate suppressed growth.

(iv) Effect of some compounds used in selective solid media

We have found that the simplest medium, i.e., nutrient agar, is excellent for isolation of *V. cholerae*. The use of several compounds have been reported for the selective isolation of *V. cholerae* and other pathogens. Studies were undertaken to determine the effect of incorporating these compounds into nutrient agar at neutral pH on the growth of *V. cholerae*.

The inhibitory concentrations of rosolic acid, cetavlon and sodium lauryl sulphate on *V. cholerae* were found to be 0.005, 0.002 and 2% respectively.

(v) Attempts to prepare a medium specific for V. cholerae

(a) The following media were tried: (i) peptone water with 0.5% ethanolamine and (ii) nutrient agar after addition singly or in combinations of the following: (a) bile salts, (b) boric acid, (c) borax,

(d) potassium chromate, (e) potassium dichromate, (f) sodium carbonate, (g) starch, (h) phenolphthalein and (i) thymolphthalein. All the compounds were found to be either toxic or non-specific.

(*b*) *Marmite–starch medium.* The medium has the following composition: marmite, 1%; sodium chloride, 0.5%; starch, 3%; saturated solution of phenolphthalein in 50% alcohol, 0.1 ml. Sodium hydroxide solution (2N) is added till the medium turns pink.

Strains of organisms were inoculated and incubated at 37°C. After 18 hours incubation the medium became colourless in the case of vibrios alone. But, there was no difference between Inaba, Ogawa, NAG and water vibrios.

Twenty different Gram–negative strains belonging to different genera were tried and in no case was the medium bleached even after 96 hours incubation. With only one strain of paracolon bacillus, a faint decolorisation was observed.

The experiments were repeated with several vibrio strains. The results indicated that this may be used as a highly specific diagnostic biochemical reaction for the vibrio genus.

The principles involved in the test are: (i) marmite is a good source for the multiplication of the vibrios; (ii) starch is degraded under highly alkaline conditions only by vibrios and not by other organisms; and (iii) the breakdown products of starch, probably acids, lower the pH and turn the phenolphthalein indicator colourless.

The experiments were repeated using thymolphthalein as an indicator instead of phenolphthalein. The pH was greater than 9.6 and the bleaching of the blue colour was observed only in the case of vibrios. However, a heavy inoculum had to be used.

The value of the marmite-starch medium was investigated in the field. About one ml. of the stool was added to 5 ml. of the medium. Many of the stools bacteriologically positive for *V. cholerae*, decolorised the medium after 8 hours incubation at room temperature. But, some false positive and negative results were obtained. The cause for this was investigated.

The decolorisation of the medium is due to change in the acid-base indicator system. In some cases it was found that the colour was bleached by the acidity of the stool itself. When the stools were highly alkaline, the pH of the medium became very alkaline so that the vibrios could not bleach it. This might be due to their inability to degrade the starch in the medium at high alkaline pH. It was found that these difficulties could be overcome by (a) adjusting the pH of the acid stools, and (b) adding smaller inocula.

(c) *Formic acid-agar medium.* Baars (1940) found that El Tor ferments glucose more energetically than the true cholera vibrio and that formic acid was oxidized to carbon-dioxide better by El Tor than by cholera vibrios. Experiments were undertaken to see whether this observation could be used in developing a medium for differentiation of types.

After repeated experiments employing a varying range of pH from 6.5 to 8.5 and using different concentrations to 1.0% formic acid in the range 0.1%, it was found that 0.3% formic acid was the optimum concentration and a pH of 6.8 was the best. The medium was prepared by adding 0.3 ml. of 89% formic acid to molten nutrient agar, followed by 4 mg. of bromothymol blue and sodium hydroxide solution (1N) till a green colour was obtained. This was then poured into plates.

The inoculated plates were examined between 24 to 36 hours after incubation at 37°C. The colonies of Inaba and

Ogawa were coloured yellow or yellowish green, those of water vibrios green or greenish blue and NAG colonies blue or deep blue. The NAG strains were found to grow well even at a concentration of 0.6% formic acid. Identical results were obtained with 50 cholera vibrios, 10 NAG strains and 6 water vibrios.

It should be emphasized that the medium is not specific for vibrios. Actually several other organisms grow in it. But, once a strain of vibrio is isolated, the medium appeared to be useful in determining its serotype.

Attempts were made to improve the formic acid-agar medium. The effect of using a mixed indicator, BDH '6676' instead of bromothymol blue in the medium was investigated. The medium was prepared at pH 6.6 with 0.5% formic acid. In this medium *V. cholerae* appeared as grey colonies and NAG vibrios as blue black colonies. With NAG vibrios the whole medium turned blue black or violet. El Tor was inhibited.

(*d*) *Benzoic acid-agar medium.* Since in the aliphatic series, formic and propionic acids behaved similarly in the nutrient agar medium containing, bromothymol blue, the studies were extended to the aromatic series, using benzoic acid. With this medium it was found that the growth and multiplication of Inaba and Ogawa strains were considerably retarded, while those of El Tor, NAG and water vibrios were not. In 40 hours all the Inaba and Ogawa strains produced yellowish green transparent colonies, whereas El Tor, NAG and water vibrios produced bluish green colonies. The colonies of El Tor vibrios were less opaque than those of NAG and water vibrios.

(2) BIOCHEMICAL STUDIES

(*i*) *Nitrosoindole reaction*

The classical cholera red reaction described by Bujwid and elaborated by Gallut still presents many anamolies. The colour is due to the formation of nitrosoindole but the optimal concentrations of nitrous acid and indole necessary for this reaction to occur are not known. It has been found that when the nitrite concentration is in slight excess, the nitrosoindole reaction fails to occur in cholera cultures.

In a study employing 103 vibrio strains, results of nitrosoindole formation were not reproducible using 72 hours and 120 hours old peptone water cultures. It was observed that peptone water cultures which yielded a positive colour after 3 days failed to do so after 5 days and *vice versa*. Often, the same strain showed divergent results. Therefore, the nitrosation of indole was studied using pure indole and Analar sodium nitrite from which nitrous acid was liberated by sulphuric acid. The results showed that in the nitrosation of indole 5 or 6 different products were formed depending upon the different proportions of the ingredients and the redox conditions. In cholera cultures it is also possible that apart from the concentrations and redox conditions, inhibition of tryptophanase and/or nitrate-reductase may be responsible for the marked variation in the cholera red reaction.

(*ii*) *Phenylpyruvic acid production*

The capacities of vibrios to produce phenylpyruvic acid from phenylalanine was tested using Inaba, Ogawa, NAG and water vibrio strains. The results were negative after 48 hours. No green coloration was produced with ferrous ammonium sulphate.

(*iii*) *Haemolysin production*

The use of haemolysis as a criterion for differentiating non-cholera vibrios from cholera vibrios has received considerable attention. The behaviour of typical cholera vibrio producing laking round colonies on blood agar plates, though the culture filtrates contained no haemolysin

is explained on the basis of the high alkaline pH, usually employed in cholera cultures. This was found to be no longer true when cultures mixed with phosphate buffers of different pH were tested for haemolysis using sheep cells. Using 10 NAG vibrios, which were all known to be pathogenic, positive results for haemolysin production were obtained in 5 cases, negative results in 4 and a doubtful reaction in 1 case. A NAG strain after 30 serial passages in the guinea-pig by the oral route produced more haemolysis.

(*iv*) *Cytochrome containing respiratory systems in V. cholerae*

The presence of a cytochrome containing system that serves as a terminal electron carrier during aerobic respiration in *V. cholerae* was demonstrated by the modification of the benzidine test for iron-porphyrin compounds. The procedure employed was essentially the same as that followed by Diebel and Evans.

Using 28 Inaba and 21 Ogawa strains of *V. cholerae*, 21 non-agglutinable vibrios and 8 water vibrios, it was found that all cultures gave a positive test. The intensity of coloration was not uniform for all the cultures and a strain to strain variation was noticed even among the same serotype. These findings are in accord with those reported by Saxena *et al.* from a study of the catalase activity of the vibrios.

(*v*) *Diamine oxidase activity of liver*

The observation that pentamethylenediamine (PMD) is present in the stools of cholera patients and in the accumulated intestinal fluid in experimental infection in guinea-pigs suggested the possibility that the natural detoxifying mechanism may be inhibited or absent in these cases. The invariable presence of PMD in the intestinal fluid and the beneficial effect of semicarbazide, an inhibitor of diamine oxidase (DAO), in producing infection, suggested the possibility that the DAO activity may be altered or inhibited in cholera infection. This enzyme is known to be distributed in the wall of the gut, liver, kidney and serum. With a view to determine the role of DAO activity in cholera, experiments were undertaken to study the activity of the enzyme in the livers of normal guinea-pigs and those infected with *V. cholerae*. It was found that there was an individual variation in the enzyme activity of the livers of different guinea-pigs. In addition, there was a general decrease in the DAO activity of livers of cholera infected animals compared with that of normal controls.

(*vi*) *Vibriolytic activity of glycine, thymol and other compounds*

Lysis of vibrio cells by thymol was noted very early in the 20th century and was quoted by Buchanan and Fulmer (1930). Gordon and Gordon (1943) found that glycine produced involution forms up to 0.5%, inhibition up to 1.5% and no growth at 2%concentration, when added to nutrient agar medium. Saxena *et al.* (1955) noticed lysis of vibrio cells with amino acids and glycine had the maximum activity. It was, therefore, considered desirable to try glycine derivatives, products related to glycine, and a combination of glycine and thymol for their vibriolytic power.

The optimum, condition for the lysis by glycine was at pH 8.0 and at a concentration of 1M. But, under the conditions employed the percentage of lysis was only 10.3%. All substituted derivatives were less effective than glycine while methyleneaminoacetonitrile, an intermediate in the manufacture of glycine, and ammonium acetate, an ultimate reduction product of glycine, were more effective.

The effective concentration of thymol was very low (about 3 mg.%) as the solubility is only 0.1 gm. per 100 ml. It was found that even at a concentration of 0.5 mg. per ml. it was many times more active than glycine. The combination of thymol and glycine was additive. Spermine phosphate was able to afford a

certain degree of protection to the vibrio cells against lysis by thymol, though it did not prevent it completely. But, it afforded no protection against lysis by glycine.

The observation that thymol, which is of low toxicity, possesses marked vibriolytic property and that this activity could be enhanced by combination with certain compounds, may prove useful in the clinical treatment of cholera.

(vii) Vibriolytic action of ethylenediaminetetraacetic acid

It has been reported that ethylenediaminetetraacetic acid (EDTA) causes lysis of *V. succinogenes.* Experiments were undertaken to determine whether EDTA caused a lysis of *V. cholerae* also.

It was found that the lysis caused by EDTA was practically negligible during the first 2 hours, but a 20 hour incubation caused lysis of the vibrios when the concentration was up to 0.5 mg. per ml. Above this concentration there was a decrease in the lysis as the concentration of EDTA increased. At 5 mg. per ml. concentration there was practically no lysis suggesting that EDTA at this concentration had no lytic effect.

(viii) Inhibition of V. cholerae by plant products

The usefulness of some common Indian medicinal plants in inhibiting the growth of *V. cholerae* was tested. These included: (a) *Piper betle*—Linn (Piperceae); (b) *Mentha aquatica*—Linn (Labiatae); (c) *Majorana hortensis*—Moench (Labiatae); (d) *Michelia champaka* (Nilagirica)—Linn (Magnoliaceae) and (e) *Oscimum sanctum*—Linn (Labiatae).

Saline extracts of the above five plants (25%, w/v) as well as their steam distilled products were tested for their inhibitory activity on a strain of Ogawa by the standard cup and the filter paper disc methods on nutrient agar plates. Plants a, b, and c inhibited growth while d and e had no effect.

(3) ANTIGENIC AND SEROLOGICAL STUDIES

Extensive studies have been carried out on the antigenic and serological properties of *V. cholerae* and NAG vibrios. Some of the observations are summarized below.

(i) V. cholerae

(a) Attempts were made to grow a strain of Ogawa in association with the following organisms: *B. faecalis alkaligenes*, paracolon bacillus, *E. coli*, *B. proteus*, *S. typhi*, *Sh. flexneri*, *Sh. sonnei*, *Staph. aureus* (coagulase positive), *Strépt. haemolyticus*, enterococcus and *Sarcinae.*

The Ogawa strain was grown with each of the above organisms in nutrient broth at 37°C. Serial transfers were made daily for a week. Isolations were made at the end of the period and the organisms identified. It was found that the Ogawa strain studied suppressed the growth of *S. typhi*, *Sh. flexneri*, *Sh. sonnei* and enterococus. *E. coli*, paracolon bacillus and *B. proteus* suppressed the growth of the Ogawa strain. It was found to grow in association with *B. faecalis alkaligenes* and *Staph. aureus. Sarcinae* could not be isolated after a week and streptococcus was present only in small numbers.

(b) In a study of cholera agglutination reaction, the Ogawa specific antiserum was mixed with an equal quantity of other enteric group antisera and their agglutination titres determined against the Ogawa strain using formalin killed 18 hours broth culture as antigen. It was found that the other enteric group antisera, though by themselves did not agglutinate the Ogawa strain, did enhance the titre of the Ogawa antiserum.

(ii) NAG vibrios

(a) Thirty-three serial passages of a NAG vibrio through guinea-pigs by the oral route and 6 passages of the strain in the guinea-pig ileum by the ligation method did not result in the strain becoming agglutinable although its virulence to the guinea-pig had considerably increased.

(b) Repeated serial passage in alkaline peptone water by daily transfer of the surface scum for 60 days resulted in the strain becoming auto-agglutinable. But, this property was lost when the strain was transferred to nutrient agar medium.

(c) Antisera were raised in rabbits against formalin killed agar slant saline washings of a strain of NAG and a strain of Inaba. Sera showing a titre of 1 in 5,000 to 1 in 10,000 were grown in nutrient agar containing 1% of their homologous antiserum and in broth containing 5% antiserum. After 15 serial passages in broth the NAG strain showed agglutination against the NAG antiserum at a higher dilution. On the other hand the Inaba strain grown in 5% Inaba antiserum broth showed a slight loss of activity.

The phagocytic indices for the NAG and Inaba strains both before and after serial passage in antiserum agar were tested using the phagocytes of the guinea-pig peritoneal fluid collected 18 hours after injection of Locke's solution containing 0.1% glucose. The results indicated that the strains after serial passage in antiserum agar seemed to show greater resistance to phagocytes.

(d) A considerable amount of work has been reported on the phenomenon of haemolysis by cholera and El Tor vibrios. But, very little work has been done on vibrios which do not agglutinate with cholera antiserum. Taking the pathogenic NAG vibrios as a class, there is a wide variation among the strains. Experiments were undertaken to study the haemolytic behaviour of NAG vibrios.

It was found that the haemolysins from the NAG strains differed in their ability to lyse red cells of different species.

Goyal (1931) found that the sensitivity of red blood cells of animals and birds were in the order, guinea-pig, goat, sheep, rabbit, pigeon, monkey, horse and man, guinea-pig cells being the most sensitive and human cells the least. The same order of sensitivity was found in this study also, except that chicken cells were the most refractory.

Many purines, pyramidines, calcium, magnesium and ammonium salts, organic acids, sodium azide, fluoride and glycerophosphate inhibited or retarded haemolytic action.

These studies indicated that haemolysis was associated with some metabolites formed in the cultures and the availability of some specific substances released from the red cells.

(iii) Agglutination studies with dioxan

It has been reported by Grant that dioxan exerts an inhibitory effect on precipitin reactions between protein antigens and rabbit antibodies and also between pneumococcal polysaccharide antigen and horse antiserum. While extending this finding to the cholera and typhoid agglutination reactions some interesting observations were made.

When dioxan was added in concentrations varying from 5% to 32% a thick precipitate was formed at low dilutions of the serum and an agglutination at high dilutions. With increasing concentrations of dioxan, positive results were obtained at the highest dilution of serum.

The precipitation or agglutination occurred only after 2 to 4 hours at 56°C. and not in the cold. Treatment of the serum alone or antigen alone with dioxan did not produce any change. These results appeared to support the concept that the antigen-antibody reaction depend-

ed on attractions between oppositely charged groups and the effect of dioxan might be due to the lowering of the dielectric constant of the solution.

When agglutination tests were done with (i) normal guinea-pig serum and *V. cholerae*, (ii) Widal negative serum and *S. typhi* H, and (iii) rabies immune guinea-pig serum and *V. cholerae* using 20% dioxan, it was found that agglutination occurred in some cases at and below 1 in 1000 dilution of serum. Thus the specificity of the antigen-antibody reaction seemed to be lost.

(4) EXPERIMENTAL INFECTIONS OF ANIMALS WITH *V. CHOLERAE* AND TREATMENT

Several important problems connected with the epidemiology and pathogenesis of cholera have remained unsolved for long because of the lack of a suitable experimental animal in which the enteric infection, simulating the human disease could be regularly produced. Freter (1955) described a method for the production of an enteric infection in the guinea-pig following intra-gastric administration of *V. cholerae*. He found that the animals could be regularly infected by the oral route provided that (a) their normal intestinal flora had been reduced by starvation and inhibited by streptomycin administration, and (b) a streptomycin-fast strain of *V. cholerae* was used. The experimental infection was fatal and limited to the bowel.

During the past 10 years a considerable amount of work has been done to improve the method and also utilise it for solving some of the problems in connection with the epidemiology and pathogenesis of the disease. The results of these investigations which have been presented regularly in the Scientific Reports of the Institute are briefly summarized below.

Experimental cholera infection in guinea-pigs

First an attempt was made to study the bacterial flora of the intestines of guinea-pigs. It was found that coliforms, enterococci, *B. faecalis alkaligenes*, and *B. pyocyaneous* formed the normal flora and their numbers were considerably reduced after starving the animals for four days.

Different strains of *V. cholerae* varied in their sensitivity to streptomycin *in vitro*. It was found possible to induce cholera infection in guinea-pigs by a preliminary starvation of the animal, alkalinisation of the gastric contents and inhibition of the normal flora of the intestines by streptomycin. On an average the large intestines contained 30–40 ml. of fluid rich in vibrios.

Experiments showed that it was possible to produce an infection simulating the human disease in guinea-pigs with 0.001 ml. of a culture compared with the 15 ml. used by Freter. The infection was localised to the intestines and *V. cholerae* were not recoverable from the heart blood, liver and spleen.

The cholera vibrios could be passaged serially from guinea-pig to guinea-pig orally. It was found that while apparently there was no alteration in the biochemical characteristics of the vibrios during passage, the intestinal lesions tended to become haemorrhagic in animals fed with organisms which had undergone several passages.

After standardization of the technique the behaviour of strains of *V. cholerae* freshly isolated from epidemics was investigated. It was found that with fresh epidemic strains an enteric infection similar to that described by Freter could be produced in guinea-pigs by feeding them with vibrios which were not rendered streptomycin resistant. There was no need to alkalinise the gastric contents with calcium carbonate nor inhibit the intestinal flora with streptomycin. Only starvation seemed to be adequate.

In view of the above findings experiments were undertaken to determine

whether it was possible to produce a fatal enteric infection in guinea-pigs fed with non-agglutinable (NAG) vibrios isolated during an epidemic. It was found that animals fed with NAG vibrios also became sick after variable intervals of time and died. On post-mortem they showed severe congestion of the gastro-intestinal tract with ballooning of the intestines and collection of varying amounts of fluid. The appearances were similar to those obtained when animals were fed with *V. cholerae*. Vibrios were isolated from the intestinal contents. They were non-agglutinable and did not show any change in their biochemical properties.

The NAG vibrios could be passaged in guinea-pigs by the oral route. Serial passage appeared to enhance the virulence of the organism. The vibrios during passage maintained their characteristics such as non-agglutinability and other biochemical properties.

(*i*) *Experiments with ligated loops*

In 1953, De and Chatterjee showed that cultures of *V. cholerae* injected into ligated loops of small intestines of rabbits resulted in the distension of the loops with fluid and desquamation of the lining epithelium with haemorrhagic necrosis of the wall. They also demonstrated the enterotoxicity of the cholera filtrate by the ligated loops. Employing the ligated loop of guinea-pigs instead of rabbits the effect of injecting different compounds was tested.

In animals in whom the ligated loop was injected with vibrios, large quantities of straw-coloured or haemorrhagic fluid accumulated in the stomach and foregut. The isolated loops were turgid and presented varied appearances.

Growing broth cultures were found to be more toxic than suspensions of the vibrios in saline with regard to the appearance of haemorrhagic patches in the isolated loop and the digestion of the intestinal wall. Similariy, lactic acid produced more severe lesions than sodium lactate. In the former case distension was more marked. Sodium nitrite produced marked haemorrhage in the isolated loop. Ammonium butyrate caused a very severe haemorrhagic lesion in the loop with comparatively less congestion in the stomach and the ileum.

Cadaverine (PMD) proved to be more toxic than putrescine. The lesions produced with cadaverine were more severe than those produced by growing broth cultures of vibrios. The haemorrhagic appearances extended to the anus and a very large quantity of haemorrhagic fluid with a peculiar odour was present in the ligated loop.

(*ii*) *Influence of some compounds*

Investigations were undertaken to see whether the lesions in guinea-pigs fed pathogenic NAG vibrio suspensions could be enhanced by the administration of certain compounds like ammonium butyrate, sodium lactate, sodium nitrite and filtrates of heat killed vibrios. Also, feeding experiments were carried out with sodium bicarbonate, culture media, glucose etc., with a view to (i) maintain the alkalinity of the stomach and intestines, (ii) aid the multiplication of the vibrios *in vivo*, and (iii) enhance the toxin production *in vivo*.

Maintenance of alkalinity by feeding with sodium bicarbonate always resulted in early deaths and gastro-intestinal manifestations, like congestion, collection of fluid, ballooning of the intestines and digestion of the stomach wall, were marked. Subsequent feeding with peptone water and a synthetic medium described by Veeraraghavan for the cultivation of *V. cholerae* to aid in the multiplication of vibrios *in vivo*, and feeding with broth containing glucose and sodium bicarbonate to increase the toxin production did not appear to have any enhancing effect. The administration of ammonium butyrate seemed to produce marked thirst but did

not enhance the toxic symptoms when fed after vibrio suspension. Sodium lactate produced flatulence of the stomach and in some cases distension of the foregut. The administration of sodium nitrite after infection caused vasodilatation and transudation of fluid into the gut resulting in dehydration. Animals fed after infection with the filtrate prepared from heat-killed meat infusion broth cultures did not show any remarkable lesions.

(*iii*) *Influence of bile salts*

It has been reported that mucin-like enhancement of microbial virulence for mice is produced by bile salts and other surfactants. Since it is known that mucin enhances the virulence of *V. cholerae*, it was considered desirable to study the effect of bile salts in experimental cholera infection. In these experiments one ml. of culture and one ml. of a mixture of 2% solutions of sodium desoxycholate and sodium glycotaurocholate were injected into the small intestines after opening the abdomen. The results indicated that bile salts appeared to enhance the virulence of *V. cholerae* in experimental infections in guinea-pigs. Also, the isolation of the organism from the contents of the gall bladder of infected animals suggested that bile did not have a lethal action on *V. cholerae*.

(*iv*) *Influence of sodium malonate*

Sodium malonate is known to be a general enzyme inhibitor, A reduction in the survival time of mice infected with *S. typhimurium* and given sodium malonate has been reported. In studies on the disease Ekiri in Japan, Yamagami was able to produce a decrease in the natural resistance of mice and guinea-pigs to the infection by the administration of sodium malonate. In view of these findings, experiments were undertaken to determine whether sodium malonate would prove useful in producing experimental infections in guinea-pigs with *V. cholerae*.

Different routes of infection and administration of malonate were employed. They were: (i) vibrios orally and malonate intraperitoneally; (ii) vibrios into the small intestines and malonate intraperitoneally; (iii) vibrios and malonate into the intestines, and (iv) vibrios and malonate into isolated intestinal loops.

It was found that *V. cholerae* given along with sodium malonate intraperitoneally appeared to enhance the susceptibility of guinea-pigs to experimental infection. Simultaneous injection of sodium malonate into the intestines or into the ligated loop did not give consistent results.

(*v*) *Effect of carbon tetrachloride*

Carbon tetrachloride is a well studied hepato-toxin. Apart from inducing fatty changes in the liver, it depresses the complement and properdin levels of animals. Formal *et al.* (1958) reported that carbon tetrachloride could replace four days' starvation in causing liver injury and utilised this method for producing shigella infection in animals.

The starvation of guinea-pigs for successful infection with *V. cholerae* frequently resulted in death, without symptoms, of a certain number of animals either during starvation or soon after infection. So, the administration of carbon tetrachloride was tried in experimental cholera infection in guinea-pigs in place of starvation. The results indicated that the administration of carbon tetrachloride cannot replace starvation in the experimental cholera infection of guinea-pigs.

(*vi*) *Entero-toxicity of V. cholerae grown in different media*

Though there is no definite evidence that *V. cholerae* produces an exotoxin, the entero-toxicity of vibrio-free culture filtrates has been reported. Studies on the pathogenesis of cholera also indicate

the possibility of the production of an enzyme or an antimetabolite or some toxic substance in the intestines during or before the onset of the symptoms.

Experiments were undertaken to determine the entero-toxicity of *V. cholerae* grown in different media. For this purpose, a strain of Ogawa was grown in the following media: (i) synthetic medium (Veeraraghavan); aerobic; 48 hours; (ii) the above medium, anaerobic; 48 hours; (iii) peptone water with 1% glucose and 1% sodium bicarbonate; aerobic; 18 hours; (iv) nutrient broth with 1% glucose and 1% sodium bicarbonate; aerobic; 24 hours; and (v) Robertson's cooked meat medium; anaerobic; 24 hours. Each culture was filtered through a Seitz pad, tested for sterility and the filtrate given the corresponding number. They were used fresh.

Guinea-pigs were starved for two days. Without the administration of alkali to neutralise the gastric contents the animals were fed with two doses of 5 ml. of each filtrate on the first day and with one dose of 2.5 ml. on the following day.

Two animals given filtrate (i) escaped infection, while both the guinea-pigs given filtrate (ii) succumbed. They showed marked congestion and accumulation of haemorrhagic fluid in the stomach and the proximal part of the intestines. One of the animals given filtrate (iii) showed a gelatinous appearance of the intestines and accumulation of straw-coloured fluid. The animals fed with filtrates (iv) and (v) escaped infection.

The experiments were repeated using the ligated loop technique. The animals were sacrificed after 24 hours. Post-mortem examination revealed varying degrees of congestion and fluid accumulation. The filtrates (ii) and (iii) produced more congestion, haemorrhage and accumulation of straw-coloured and haemorrhagic fluids in the loop, intestines and stomach than a control agar slant suspension of the organism in broth. Further, animals given the filtrate (iii) presented a gelatinous appearance of the intestines with digestion of the stomach wall. It was interesting to note that while the growth was very scanty in medium (ii) the filtrate proved to be the most toxic.

The findings suggest that the vibrio cells, either by their lysis or during their growth in the intestines liberate some toxic substances which may help their enzymic activity, provide suitable conditions for their rapid multiplication, and also bring about a change in the permeability of the intestinal wall. Anaerobic conditions and a medium containing glucose appear to favour the release of this toxic substance.

(*vii*) *Value of thymol in the treatment of experimental cholera infection*

The lytic action of thymol on *V. cholerae* has been confirmed by us. In view of this finding, experiments were undertaken to test the action of thymol *in vivo* in guinea-pigs.

It was found that there was an absence of intense congestion and accumulation of fluid in the intestinal loops of guinea-pigs infected with *V. cholerae* when thymol was given. Also, the intestinal lesions in animals which succumbed to the infection in spite of treatment with thymol were generally mild.

These findings together with the observation that thymol was of low toxicity and significant vibriolytic activity would suggest its use as a remedy for the treatment of *V. cholerae*.

(*viii*) *Value of copper sulphate in the treatment of experimental cholera infection*

Bose and Charaborty (1944) reported a bactericidal action of metallic copper on *V. cholerae*. They found that in distilled water containing copper turnings, the

vibrios could not be recovered later than 30 min., while in the controls it survived for 48 hours.

Our recent studies also showed that copper ions in very small concentrations caused a 100% inhibition of the production of PMD *in vitro* by *V. cholerae* from extracts of monkey intestines. Based on these observations investigations were undertaken to determine the value of copper sulphate in experimental cholera infection in guinea-pigs. Both the ligated loop and intra-intestinal methods were used.

The studies indicated that the administration of copper sulphate appeared to reduce the severity of the lesions in experimental cholera infection in guinea-pigs even when the challenges were severe.

(5) PATHOGENESIS

Role of pentamethylenediamine (Cadaverine)

(i) In experimental cholera infection

Breiger (1889) suspected the presence of pentamethylenediamine (PMD) in cholera stools. Following this, several workers detected PMD in the body fluids of cholera victims and in cultures of *V. cholerae*. It was thought that the peculiar odour emanating from cholera patients and the semen-like odour of cholera stools might be due to PMD.

In experimental cholera infections in guinea-pigs it was observed that the fluid which accumulated in the intestines had an odour resembling that produced when *V. cholerae* were incubated in large amounts. This fluid on alkalinization and distillation yielded a fraction which produced mild swelling and congestion in ligated intestinal loops of guinea-pigs and rabbits. It was also found that oral administration of PMD to animals already fed with *V. cholerae* produced an enhancement of the lesions.

It was, therefore, considered desirable to study the production of PMD in experimental animal infection and its probable role in the pathogenesis of cholera. In view of the localization of cholera infection to the intestines, experiments were undertaken to study the appearance of PMD *in vivo* during cholera infection and its formation *in vitro* from intestinal extracts and other sources.

The difference in the amount of PMD obtained by the action of *V. cholerae* on different intestinal extracts and some proteins suggested that the nature and the specificity of the substrate was an important factor. The influence of time and pH upon PMD production and the absence of PMD when vibrio cell proteins were precipitated by trichloroacetic acid soon after addition to the intestinal extract indicated an enzymatic reaction. The best results were obtained after 18 hours incubation and the amount of PMD produced continued to increase even after this period.

When *V. cholerae* were grown in chemically defined media, PMD was not found but appeared when protein matter was added as a growth factor. PMD was absent in heat killed saline suspensions and in sonic lysates of organisms. These results suggested that it was neither a metabolic product of vibrio growth nor was it released by the disintegration of virbio cells. The formation of PMD from intestinal extracts by water clear sonic lysates of vibrios and the long interval required for its appearance also supported this view. The production of PMD from gastric mucin and its absence when intestinal extracts were prepared after completely scraping the mucosa, suggested the possibility that some of the enzyme systems of *V. cholerae*, such as mucinase, produced a suitable substrate for PMD production by acting upon intestinal mucosa.

Experiments done with different types of vibrios showed that there was a difference in the production of PMD by pathogenic and non-pathogenic types. It was interesting to note that pyridoxal

LIBRARY
HAFFKINE INSTITUTE
BOMBAY-400 012.

phosphate was a cofactor for water, NAG and some El Tor vibrios, but not for Inaba, Ogawa and certain other El Tor types. PMD was also produced by other micro-organisms capable of producing diarrhoea. All the strains of *V. cholerae* tested produced PMD *in vitro* and *in vivo* experiments. Further, they were found to tolerate the alkalinity of PMD without marked inhibition of growth. These observations suggested the possibility that PMD itself might be responsible for producing diarrhoea.

The symptoms produced by PMD in animals resembled those produced by *V. cholerae* in several respects. PMD was absent in stools when cholera infection failed in animals, but was invariably found when the symptoms were very severe.

The maximum amount of chloride in the fluid from control loops was 248 mg.% with little or no protein. On the other hand, the chloride and protein content of the PMD infected loops were high and resembled those where *V. cholerae* had been given.

The histological changes observed in the intestines of animals given PMD resembled those encountered in fatal cholera infections.

Experiments to determine the rate of flow as well as those when Evan's blue was given intravenously suggested that the permeability of the intestines wall was altered by PMD.

It is known that intestinal epithelium is impervious to macromolecules of the size of toxins. Even if PMD did not exert any toxic effect by itself, it might alter the absorptive capacity of the intestinal wall in such a way that it was rendered pervious to the toxic fractions liberated by *V. cholerae*.

Further, by altering the permeability of the intestinal wall PMD might start a disease process. PMD is known to bring about renal abnormalities, disturbances in the electorolytic fluid balance, functions of ACTH, etc., in the Japanese Ekiri disease.

All these findings suggest a probable role for PMD in cholera infections.

It has been found that there was a general decrease in diamine oxidase activity of livers of guinea-pigs showing evidences of cholera infection compared with that of normal controls The presence of diamine oxidase is known in several micro-organisms and the occurrence of these organisms in the intestines might be responsible for the oxidation of PMD. The consistent results obtained when the ligated loops to be injected with PMD were selected in the duodenum or jejunum, which were relatively free of organisms, and the beneficial effect of semicarbazide, an inhibitor of diamine oxidase enzyme system, supported this view.

It would appear that *V. cholerae* act on the intestinal mucous membrane and produce PMD. It is possible that if the natural defence mechanisms are successful PMD may be detoxified and recovery occurs. On the other hand, if PMD is present in larger amounts for longer duration, the defence mechanisms may fail leading to a chain of disease processes.

(ii) *Occurrence of PMD in cholera stools*

There was an epidemic of cholera in Madurai city during January to March 1965. About 1,440 persons were attacked of whom about 50 died.

Workers from the Institute reached Madurai only during the last stages of the epidemic. The main purpose of the team was to investigate the presence of PMD in the stools and vomits of cholera cases.

One vomit and 24 stools collected were tested for the presence of PMD.

It was found that in 10 specimens positive for *V. cholerae*, PMD was present in varying amounts. The concentrations were high in acute cases and in severe cases with marked dehydration. In 6 specimens, also positive for *V. cholerae*, PMD was not detected. PMD was present in 4 specimens from which *V. cholerae* could not be isolated. It was possible that in these cases the excretion of the vibrios had ceased. Another explanation was that the PMD was liberated by other micro-organisms.

It should be emphasized that the work suffered from certain drawbacks. The specimens were collected during the last stages of the epidemic when it was possible that the virulence of the organism had declined. Also, in some instances the samples were collected after the acute stages of the infection had passed off and the patient was convalescing, i.e., when the natural defence mechanism was operating. Apart from these, the estimation of PMD could not be carried out immediately as the spectrophotometer was out of order.

REFERENCES

Sci. Rep. Pasteur Institute, Coonoor. 1956 – 66.

VEERARAGHAVAN, N., KALYANARAMAN, V. and VISALAKSHI, V. K. (1966) *Ind. Jour Med. Res.*, 54, p. 117.

13. ENTERIC FEVERS

(1) Phage typing of enteric organisms

Vi phage typing of *S. typhi* has proved to be an epidemiological tool of great precision. In countries where typhoid fever is rare, determination of phage types has aided public health workers to trace the source of infection and also establish the chain of spread.

Since December 1956, the phage typing of all the strains of enteric pathogens isolated at the Institute has been carried out with the co-operation of the Phage Typing Centre at the Seth G.S. Medical College, Bombay, and the ICMR Phage Typing Centre at the Lady Hardinge Medical College, New Delhi.

The distribution of the phage types among 644 strains of *S. typhi* isolated at the Institute from December 1956 to August 1966, is given in Table 12.

Among the 607 strains typed it was found that 500 (82.3%) belonged to type A, 66 (10.9%) to type E_1, 11 (1.8%) to type 0, and 10 (1.7%) to type D_6. Thus, less than 18% belonged to the 11 phage types other than type A encountered in this study.

(2) Evaluation of a rapid slide agglutination test

A comparative study of Dreyer's modification of Widal Reaction and the rapid slide agglutination test of Werbin and Kasher using bacterial suspension of *S. gallinarium* stained with 0.25% solution of 2, 3, 5–tripheny1 tetrazolium in physiological saline has been in progress at the Institute since 1961.

Out of 1,615 sera received for Widal test there was agreement between the two tests in 1,040 (64.4%). Among 214 sera in which *S. typhi* was isolated the results were the same in the case of 145 (67.7%) sera. The agreement between the tests in 43 sera where *S. paratyphi* A was isolated in clot culture was in 26 (60.5%) cases.

REFERENCE

Sci. Rep. Pasteur Institute, Coonoor. 1956–66.

TABLE 12

Source	Phage types of 644 strains of *S. typhi* isolated in Coonoor, South India.														
	A	C_4	C_5	D_1	D_6	E_1	E_2	E_4	J_1	O	T	28	Degraded strains	Untypable strains	Number of strains typed
Nilgiri District															
Coonoor	186	—	—	5	5	22	—	1	—	2	1	2	9	1	234
Ootacamund	157	—	1	—	4	22	2	1	—	—	1	—	3	3	194
Wellington	68	—	—	—	1	12	—	—	1	9	—	—	10	5	106
Aruvankadu	36	—	3	—	—	1	—	—	—	—	—	—	1	1	42
Kotagiri	25	—	—	—	—	2	1	—	—	—	—	—	1	1	30
Kullakamby	6	—	—	—	—	1	—	—	—	—	—	—	—	—	7
Devashola	2	—	—	—	—	—	—	—	—	—	—	—	—	—	2
Gudalur	...	—	—	—	—	1	—	—	—	—	—	—	—	—	1
Naduvattam	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Coimbatore District															
Coimbatore	3	—	—	—	—	—	—	—	—	—	—	—	—	—	3
Valparai	8	—	—	—	—	2	—	—	—	—	—	—	—	—	10
Paralai	2	—	—	—	—	—	—	—	—	—	—	—	—	—	2
Tiruchirapalli District															
Tiruchirapalli	2		—	—	—	2	—	—	—	—	—	—	—	—	4
Mysore	2	1	1	—	—	—	—	—	—	—	—	—	—	—	1
Kerala	...	...	—	—	—	—	—	—	—	—	—	—	—	—	1
Place not specified	1		—	—	—	1	—	—	—	—	—	—	1	1	6
Total ...	500	1	4	5	10	66	3	2	1	11	2	2	25	12	644

14. EPIDEMIC DIARRHOEA

During 1961, at the request of the Director of Public Health, Madras State, an investigation was undertaken to determine the aetiological agent responsible for the epidemic diarrhoea in Kallakurichi taluk of South Arcot district in the Madras State. Due to lack of facilities for storing rectal and throat swabs collected from cases in a deep-freeze, the team detailed for investigation could spend only two days in the special temporary wards attached to the local hospital. The average daily admissions from 1 to 10 April 1961, numbered 15 men, 15 women and 2 children.

Majority of the cases were malnourished and had suffered from the disease for a variable period before admission. The sheet anchor of treatment was the administration of sulphaguanidine tablets.

Specimens were collected from fresh cases or relapses only. In addition to fresh specimens of faeces collected in buffered glycerol–saline, throat swabs and rectal swabs were collected from suitable cases for virological investigations. All the 21 rectal swabs and 15 throat swabs collected were transported on ice and salt mixture and inoculated into HeLa and human amnion cell cultures. No viral agents were isolated.

Microscopic examination of the fresh specimens on the spot revealed a picture characteristic of bacillary dysentery. The faecal specimens collected in buffered glycerol-saline were plated on MacConkey's bile-salt-neutral red-lactose-agar, desoxycholate-citrate-agar,and on special media for staphylococci. No staphylococci were isolated. One strain of *Shigella flexneri* A1 was isolated. On testing the drug sensitivity of the strain to various antimicrobial agents, it was found to be insensitive to sulphadiazine, sulphaguanidine and phthalylsulphathiozole. Streptomycin and chloromycetin were found to be effective in inhibiting the growth of this strain *in vitro*. This finding explained the observation of the medical officers in charge of these cases that treatment with chloromycetin gave very good results compared with sulphaguanidine

It must be emphasized that only one strain of dysentery bacillus was isolated. Nevertheless, considering reports from different parts of the world regarding the emergence of dysentery organisms resistant to sulpha drugs, and the resistance of the strain isolated to these drugs, the possiblity existed that the epidemic might have been due to a resistant strain of *Shigella flexneri*.

REFERENCE

Sci. Rep. Pasteur Institute, Coonoor. 1961.

15. SYPHILIS

Examination of sera by 'battery' of tests

Since 1957 investigations have been in progress to compare the sensitivity and the specificity of the complement fixation and flocculation tests carried out with different antigens and methods.

All specimens of sera received at the Institute for serological testing were subjected to examination by different tests (Wassermann Reaction, cardiolipin complement fixation test, Kahn flocculation test and VDRL slide test). A total of 22,743 sera were examined during the years 1957–65.

It was found that cardiolipin CF antigen appeared to be the least sensitive with 90% negatives. The Wassermann and VDRL antigens were next in order with 85.7% and 84.5% negatives respectively. The Kahn antigen was the most sensitive with only 83.7% negatives.

The data presented above were in respect of all the sera received at the Institute without selection. Among them, 1,971 sera received during 1958–65 were from cases clinically diagnosed as syphilis. The number of cases studied is obviously small for drawing conclusions. Nevertheless, it was found that the percentage of negatives among all the cases as well as the different groups was the maximum with the cardiolipin complement fixation test and minimum with Kahn test. The results of Wassermann Reaction and VDRL tests were intermediate.

An analysis of the percentage of agreement and difference in the four tests is given below.

Total number of sera tested	22,743	
No. of sera giving positive strong and positive results with all four tests	1,457	(6.4%)
No. of sera giving negative results with all four tests	17,491	(78.4%)
Total number of sera showing agreement	18,948	(84.8%)
No. of sera showing disagreement	3,795	(15.2%)

Studies on the Nichol's strain of *T. pallidum*

(i) Maintenance of the Nichol's strain of T. pallidum in rabbits

The Nichol's strain of *T. pallidum* is being maintained at the Institute since 1955 by intratesticular passage in rabbits.

(ii) Treponema agglutination test

Treponema agglutinating antigens were prepared on several occasions from the infected testes of rabbits using different methods of concentration, purification, heat treatment and preservatives. Some of them proved satisfactory in tests on doubtful sera. However, extensive investigations showed that it was difficult to produce batches of antigen of reproducible standards with regard to spirochaetal concentration, freedom from tissue material and keeping qualities.

(iii) Fluorescent treponemal antibody test

The fluorescent treponemal antibody (FTA) test has been developed as a diagnostic test using the specific treponemal antigen derived from the treponemes extracted from rabbit syphilomas infected with the Nichol's strain of *T. pallidum*. The sensitivity and specificity of this test is said to compare favourably with the *T. pallidum* immobilization (TPI) test. The advantage of the FTA test is the relative simplicity of its performance compared with the TPI test.

Since 1963 the FTA test is being used at the Institute to examine the sera showing discrepant results with the standard serological tests for syphilis.

The results of examination of 1,448 specimens of discrepant sera during 1963 – 66 were as follows:

It was found that 406 out of 1,448 (28%) discrepant sera were reactive in FTA test. Of the 406 sera, 342 (84%) were reactive in VDRL test in varying dilutions. Out of 406 sera reactive in VDRL test, 10 could not be titrated on account of non-availability of sufficient amount of serum and 165 sera were reactive in dilutions, neat to 1 in 4, while the remaining 167 sera were reactive in dilutions 1 in 8 and above.

Out of 259 sera reactive in all the four tests, only 3 were negative in FTA test and in these sera VDRL titre was 1 in 4.

Of 338 sera non-reactive in all the four tests, 27 were positive in FTA test. All these were from early cases of syphilis or from those who had been treated.

Though the number of sera examined is too small to draw definite conclusions; the following observations seem warranted: (a) If the serum is reactive in all the four tests, it is likely to be a specific result. (b) The VDRL slide test, if performed under standard conditions with standard reagents, is a reliable screening test. A VDRL titre of 1 in 8 and above is highly significant. (c) The FTA test appears to be a good, technically simple, and reliable verification test.

(iv) *Studies on resistance to reinfection*

Investigation were carried out to determine the resistance of rabbits to reinfection with the Nichol's strain of *T. pallidum*. Rabbits were rechallenged intradermally after varying periods. Their blood was collected at weekly intervals after infection and examined.

It was found that chancre-like lesions were not produced in any of the animals rechallenged intradermally. The serological findings were, however, not consistent. Although the serological reactions of some of the animals changed from negative to positive on reinfection, the findings were not regular. The relation of this observation to the problem of reinfections in human beings, who do not show clinical signs, is difficult to assess.

REFERENCES

Sci. Rep. Pasteur Institute, Coonoor. 1957–66.

16. DIPHTHERIA

(1) CULTIVATION OF *C. DIPHTHERIAE*

A comparative study of different media used in the isolation of *C. diphtheriae*.

C. diphtheriae has been known to grow well on media containing blood or serum and scantily on those without enrichment. For isolation of *C. diphtheriae* several media have been elaborated, both selective and enriched. In devising such selective media, potassium tellurite and copper sulphate have been used by the majority of workers for the inhibition of the commensal organisms of the throat.

A comparative study of the different media was made using the strains of *C. diphtheriae* isolated in the laboratory from clinical cases of diphtheria. The media tried included nutrient agar, blood agar, Leoffler's serum medium, cystine tellurite agar, Hoyle's tellurite agar and Muller's tellurite agar.

On comparison, it was evident that blood agar prepared with 10% rabbit blood, gave luxuriant growth of *C. diphtheriae* in a short period of incubation. Leoffler's serum medium gave abundant growth of this organism with characteristic development of metachromatic granules, in the same interval of incubation. Potassium tellurite had an inhibitory action on *C. diphtheriae*—this being well evidenced by the longer period of incubation required to get a limited growth. Moreover, organisms like *Staph. aureus*, streptococcus non-haemolyticus, enterococcus and *Kl. pneumoniae*, which form the common flora of the throat, were not inhibited. Further, the colonies of staphylococci on tellurite media resembled those of *C. diphtheriae* by the colony appearance, thereby rendering the differentiation of *C. diphtheriae* by the colony appearance alone difficult.

For a quick diagnosis it is necessary that the colonies of *C. diphtheriae* should appear early and their appearance should be characteristic. Selective media employing potassium tellurite, by their ability to give rise to growth only on prolonged incubation, do not appear to be the most suited. The colonies on blood agar developed in a comparatively short time and were easily differentiated from those of staphylococci, streptococci, enterococci, *Kl. pneumoniae* and *N. catarrhalis* and, therefore, in combination with

Leoffler's serum medium, which gave rise to well defined metachromatic granules, blood agar appeared to be the medium of choice in isolation and identification of *C. diphtheriae*.

Cultivation of *C. diphtheriae* in serum-free media

In view of the finding that *C. diphtheriae* grows in peptone water and broth, the effect of adding some compounds to plain nutrient agar was investigated. The results are briefly summarized below.

The addition of 0.5% glucose or 0.5% casein hydrolysate favoured the growth of *C. diphtheriae*. Nutrient agar containing 0.25% glucose, 0.025% cystine, and 50% 'Periston' gave remarkably good growth of *C. diphtheriae*. The addition of 'Marmite' (0.1%) and yeast extract (20 mg.%) to nutrient agar containing 0.5% glucose and 0.5% casein hydrolysate gave rise to luxuriant growth of *C. diphtheriae*. But, the colonies were indistinguishable from those of staphylococcus. The addition of 10% egg albumin to plain nutrient agar promoted the growth of *C. diphtheriae* producing, white, opaque, glistening colonies. The addition of starch in 1% concentration favoured the growth of the organism.

Sodium lactate agar medium

The influence of adding several organic acids as sodium salts to nutrient agar was studied. Of all the compounds tested, the addition of sodium lactate in 1% concentration was found to give the best results. Though it afforded good growth of *C. diphtheriae*, the production of metachromatic granules was inhibited. So, the effect of reducing the concentration of sodium lactate from 1 to 0.1% was tried. It was found that even at a concentration of 0.1% sodium lactate, a very luxuriant growth was obtained. Further, at this concentration, metachromatic granules were very well defined.

Enrichment of this medium was attempted by adding 5% horse serum. The growth of *C. diphtheriae* was found to be equally good in both the plain and the enriched media. But, the production of metechromatic granules was better in the medium without horse serum.

Toxicity tests with recently isolated strains from clinical cases grown in 0.1% sodium lactate agar for 18 hours were performed on rabbits. It was found that strains grown in sodium lactate agar produced more toxic symptoms compared with the same strains grown in either Leoffler's serum medium or 5% horse serum sodium lactate agar, as evidenced by the intensity of the inflammatory and dermonecrotic reactions.

These results indicated that sodium lactate could be used as a serum substitute in media for the cultivation of *C. diphtheriae*. Plain nutrient agar containing 0.1% sodium lactate gave luxuriant growth of *C. diphtheriae* with well defined metachromatic granules and the strains were able to elaborate toxin readily on this medium.

Attempts to improve the sodium lactate agar medium

Experiments were undertaken to see if the sodium lactate agar medium could be rendered selective for *C. diphtheriae* by the addition of certain chemicals.

It was found that in serum-free media *C. diphtheriae* could tolerate only up to 5 mg.% concentration of potassium tellurite. Moreover, the appearance of the colony ceased to be characteristic and the morphology of the organism was found to be altered. Also, there was marked growth of the commensal organisms of the throat.

Thymol has been found to have bacteriostatic action on several organisms. Therefore, the effect of incorporating

varying concentrations of thymol into the basal medium was investigated. It was found that *C. diphtheriae* was inhibited at concentrations of thymol far below those required to suppress the growth of other organisms of the throat.

The effect of incorporating different concentrations of mercuric chloride in the basal medium was investigated. It was found that 100 mg% concentration failed to inhibit *Staph. aureus*, *E. coli* and *B. proteus*.

Sodium lauryl sulphate in 100 mg.% concentration, inhibited the growth of *C. diphtheriae*, while other organisms like *Staph. aureus* and enterococcus were partially inhibited.

The effect of incorporating glycerine with sodium lactate agar medium in concentrations varying from 1% to 5% was studied. It was found that a concentration of 4% or 5% glycerine inhibited the growth of *C. diphtheriae*. There was no inhibition at 3% concentration but there was a change in morphology of the organism from long slender forms to short stumpy ones bereft of metachromatic granules.

It was found that the incorporation of crystal violet, 1% malachite green or 1% methylene blue into the medium was not useful in selectively growing *C. diphtheriae*.

(2) HAEMOLYTIC ACTIVITY

When *C. diphtheriae* was isolated from clinical cases it was found that majority of the strains showed from fair to well defined zones of haemolysis around the colonies on blood agar plates. As the property of haemolysis by microorganisms depends on several factors a study was made of the haemolytic property of *C. diphtheriae* on guinea-pig, rabbit, sheep, calf and human red blood cells.

It was found that red blood cells of rabbits were more susceptible to lysis by *C. diphtheriae* than guinea-pig, sheep, calf or human cells.

Equal volumes of 1000 units per ml. of diphtheria antitoxin and broth cultures of the different strains were mixed and the antigen-antitoxin mixtures tested for haemolytic activity. These mixtures gave variable results. Some strains exhibited haemolytic activity while others did not.

Filtrates of broth cultures were prepared and their haemolytic activity tested against different red cells. There was no lysis of the cells by any of the filtrates even after 48 hours incubation.

The finding that some strains of *C. diphtheriae* cause haemolysis of rabbit red blood cells on solid and liquid media while their filtrates do not suggest the possibility that the haemolytic activity is associated with the organism and not its exotoxin.

REFERENCE

Sci. Rep. Pasteur Institute, Coonoor. 1964, 1965.

17. TUBERCULOSIS

Comparison of media for isolation

An investigation was undertaken to compare the media used for isolation of *M. tuberculosis*. These included Lowenstein-Jensen medium, Lowenstein-Jensen medium with glycerol, glycerol-6 medium, modified Kirchner's medium and the pyruvate medium.

Specimens of sputa from 67 clinically and radiologically diagnosed cases of tuberculosis were studied.

Of the 67 sputa examined, the direct smear was positive in 29 cases and the smear of the concentrate in 35 cases. Both the direct and concentrate smears were negative in 32 cases.

M. tuberculosis was isolated from the sputa of 17 patients. The number of isolation in different media were as follows: Lowenstein-Jensen, 4; Lowenstein-Jensen with glycerol, 10; glycerol–6, 9; modified Kirchner, 1; and pyruvate, 1.

Sensitivity of strains to streptomycin

The sensitivity to streptomycin of the 17 strains of *M. tuberculosis* isolated was investigated. Seven of the strains were found to be sensitive to streptomycin while the rest were resistant in varying degrees.

REFERENCE

Sci. Rep. Pasteur Institute, Coonoor. 1964.

18. LEPROSY

Reactivity of newer antigens

Among the diseases which give rise to false positive serological reactions, leprosy is one of the most important. In the course of investigations carried out at the Institute on 181 sera of patients from one leprosy sanatorium, it was found that while 37% of the sera reacted with bullock heart antigen in a complement fixation test and 44% reacted with Kahn antigen in a standard Kahn test, only 9% of specimens reacted with the cardiolipin antigen of Pangborn. Experiments were undertaken to evaluate the reactivity of the newer antigens like VDRL and cardchol in leprosy and compare them with the standard cardiolipin and Kahn antigens.

The antigen used in the cardchol CF test was of the same composition as the cardiolipin antigen but without lecithin. Both the antigens were kindly made available by Dr. H. Schmidt of the State Serum Institute, Copenhagen.

Sera were obtained from different leprosy sanatoria in South India. Majority of them were from cases of lepromatous leprosy of long duration. The age of patients varied from 6 to 60 years and most of them were males.

Out of the 271 sera examined, VDRL slide test was positive in 23%, cardiolipin CF test 27. 8%, standard Kahn test in 30.2% and cardchol CF test in 55.3%. It was found that cardchol CF test proved to be the most sensitive in leprosy.

REFERENCE

Sci. Rep. Pasteur Institute, Coonoor. 1960.

19. SNAKE VENOMS

Action of ethylenediaminetetraacetic acid, polyvinylpyrrolidone and iodine in cobra and Russell's viper venom poisoning

Studies were undertaken to determine whether chelating agents, detoxifying agents and iodine had any action against poisoning by snake venoms. The value of disodium ethylenediaminetetraacetic acid (EDTA), polyvinylpyrrolidone (mean molecular weight, 12,600) and collosol iodine (Crookes) in cobra and Russell's viper venom poisoning was investigated.

It was found that EDTA appeared to have some protective value against cobra venom poisoning as judged by the survival rates in animals. The results, however, were neither consistent nor statistically significant. It seemed to have no action against Russell's viper venom poisoning. Polyvinylpyrrolidone did not appear to confer any protection against either cobra or Russell's viper venom poisoning. Collosol iodine did not afford any protection against cobra venom poisoning.

REFERENCE

Sci. Rep. Pasteur Institute, Coonoor. 1959.

20. PUBLICATIONS

(1907 – 57)

RABIES

1. CORNWALL, J. W. (1907) Recent advances of knowledge in connection with rabies. *Indian Med. Gaz.*, **42**, p. 121.

2. *Idem.* (1915) Native treatment of dog bite. *Indian Med. Gaz.*, **50**, p. 72.

3. *Idem.* (1917) A note on the occurrence of Negri bodies. *Ind. Jour. Med. Res.*, **5**, p. 478.

4. *Idem.* (1918) Anaphylactic reactions in the course of antirabic treatment. *Ind. Jour. Med. Res.*, **6**, p. 237.

5. *Idem.* (1919) The pathology of experimental rabies: I. Kidneys, adrenals, liver, pancreas, spleen. *Ind. Jour. Med. Res.*, **7**, p. 148.

6. *Idem.* (1919) An unusual case of hydrophobia lasting 8 days. *Indian Med. Gaz.*, **54**, p. 140.

7. *Idem.* (1923) Statistics of antirabic inoculations in India. *Brit. Med. J.*, **2**, p. 298.

8. *Idem.* (1924) Rabies in South India. *Brit. Med. J.*, **2**, p. 1135.

9. *Idem.* (1925) Estimation of blood sugar in normal rabbits and rabbits inoculated subdurally with rabies fixed virus. *Ind. Jour. Med. Res.*, **12**, p. 581.

10. *Idem.* (1925) On Negri bodies. *Ind. Jour. Med. Res.*, **12**, p. 601.

11. *Idem.* (1925) Rabies in Southern India. *Brit. Med. J.*, **2**, p. 765.

12. CORNWALL, J. W. and BEER, W. A. (1925) On the occurrence of paralysis after treatment with antirabies vaccine. *Ind. Jour. Med. Res.*, **13**, p. 467.

13. CORNWALL, J. W. and BEER, W.A. (1925) On the rate of multiplication of rabies fixed virus in rabbits. *Ind. Jour. Med. Res.*, **13**, p. 475.

14. *Idem.* (1926) The assessment of the infectivity of rabies fixed virus by means of albino rats. *Ind. Jour. Med. Res.*, **13**, p. 803.

15. *Idem.* (1926) The effect of etherised normal brain substance injected subcutaneously into rabbits. *Ind. Jour. Med. Res.*, **13**, p. 807.

16. CORNWALL, J. W. and KESAVA MENON, T. (1917) On the experimental immunization against rabies by the methods of Phisalix. *Ind. Jour. Med. Res.*, **4**, p. 688.

17. CORNWALL, J W. and RAMASWAMY AIYAR, S. (1915) Arneth index and antirabic treatment. *Ind. Jour. Med. Res.*, **3**, p. 332.

18. *Idem.* (1916) Arneth index in laboratory rabies. *Ind. Jour. Med. Res.*, **3**, p. 738.

19. CRUICKSHANK, J. A. and WRIGHT, R. E. (1914) A note on some experiments performed with a view to finding out the period before symptoms during which the saliva of an animal incubating rabies is infective. *Ind. Jour. Med. Res.*, **1**, p. 532.

20. GLOSTER, T. H., BEER, W.A., NAMBIAR, M. R. and SASTRY., S.S. (1929) Experiments on preinfectional immunization against rabies with carbolised and etherised vaccines. *Ind. Jour. Med. Res.*, **17**, p. 286.

21. IYENGAR, K. R. K. (1925) Case of hydrophobia with the longest recorded incubation period. *Indian Med. Gaz.*, **70**, p. 562.

22. IYENGAR, K. R. K. and BEER, W. A. (1930) The effect of ether on "Fixed Virus" in infected brain of sheep. *Ind. Jour. Med. Res.*, **18**, p. 1.

23. MCCARRISON, R., SANKARAN, G. and BEER, W. A. (1934) Electrophoresis experiments with the virus of rabies. *Ind. Jour. Med. Res.*, **21**, p. 917.

24. SANKARAN, G. and BEER, W. A. (1935) The effect of exposure of suspensions of rabies infected brain to radiation from a quartz mercury vapour lamp. *Ind. Jour. Med. Res.*, **22**, p. 581.

25. SANKARAN, G., IYENGAR, K. R. K. and BEER, W. A. (1934) A preliminary note on the electrical charges carried by the rabies virus. *Ind. Jour. Med. Res.*, **21**, p. 909.

26. VEERARAGHAVAN, N. (1941) Elimination of excess nerve tissue from antirabic vaccine. *Ind. Jour. Med. Res.*, **29**, p. 303.

27. *Idem.* (1944) A protozoan parasite of the central nervous system. *Ind. Jour. Med. Res.*, **32**, p. 207.

28. *Idem.* (1945) Cultivation of a protozoal parasite of the central nervous system *in vitro* and its relationship to rabies. *Ind. Jour. Med. Res.*, **33**, p. 285.

29. *Idem.* (1945) A rapid method for the diagnosis of rabies in animals. *Ind. Jour. Med. Res.*, **33**, p. 295.

30. *Idem.* (1946) Studies on the cultivation of the aetiological agent of rabies *in vitro* and its nature. *Ind Jour. Med. Res.*, **34**, p. 207.

31. *Idem.* (1946) Studies on antirabic immunization with culture vaccine. *Ind. Jour. Med. Res.*, **34**, p. 225.

32. *Idem.* (1947) Further observations on the cultivation of rabies virus *in vitro*. *Ind. Jour. Med. Res.*, **35**, p. 237.

33. VEERARAGHAVAN N. (1947) Cultivation of rabies virus *in vitro*. *Nature*, **159**, p. 782.

34. *Idem.* (1951) A note on the occurrence of Negri bodies. *Ind. Jour. Med. Res.*, **39**, p. 261.

35. VEERARAGHAVAN, N. and BALASUBRAMANIAN, A. (1954) A note on the action of ACTH in rabies. *Ind. Jour. Med. Res.*, **42**, p. 9.

36. VEERARAGHAVAN, N., BALASUBRAMANIAN, A. and SUBRAHMANYAN, T. P. (1954) Action of receptor destroying enzyme of *V. cholerae* in rabies. *Ind. Jour. Med. Res.*, **42**, p. 1.

37. VEERARAGHAVAN, N. and PHILIPSZ, G.L.C. (1938) Cultivation of rabies virus on the chorioallantoic membrane of the developing egg. *Ind. Jour. Med. Res.*, **26**, p. 493.

38. VEERARAGHAVAN, N. and PHILIPSZ, G.L.C. (1940) The susceptibility of domestic fowl to a strain of rabies virus obtained from a jackal. *Ind. Jour. Med. Res.*, **28**, p. 81.

Q FEVER

39. VEERARAGHAVAN, N. and SUKUMARAN, P. K. (1954) Q fever survey in the Nilgiris and Coimbatore Districts of Madras State. *Ind. Jour. Med. Res.*, **42**, p. 5.

CHOLERA

40. VEERARAGHAVAN, N. (1949) A simple medium for cultivation of *V. cholerae*. *Nature*, **163**, p. 138.

TYPHOID FEVER

41. CORNWALL, J. W. (1923) The possibility of latent infections with *B. typhosus*. *Indian Med. Gaz.*, **58**, p. 374.

42. *Idem.* (1924) Changes caused by *B. typhosus* in the reaction of some media. *Ind. Jour. Med. Res.*, **11**, p. 839.

43. *Idem.* (1925) On the variation in amount of soluble protein contained in old broth cultures of *B. typhosus*. *Ind. Jour. Med. Res.*, **13**, p. 483.

44. CORNWALL,, J.W. and BEER, W. A. (1925) On the persistence of *B. typhosus* in rabbits after intravenous injection. *Ind. Jour. Med. Res.*, **13**, p. 491.

45. *Idem.* (1926) On the cessation of multiplication of *B. typhosus* in broth culture. *Ind. Jour. Med. Res.*, **13**, p. 789.

46. CORNWALL, J. W. and LAFRENAIS, H.M. (1924) The mechanism of infection by *B. typhosus*. *Ind. Jour. Med Res.*, **11**, p. 883.

PLAGUE

47. CORNWALL, J. W. and KESAVA-MENON, T. (1917) On the possibility of the transmission of plague by bed bug. *Ind. Jour. Med. Res.*, **5** p. 137.

OTHER BACTERIA

48. CORNWALL, J. W. (1908) Remarks on the vaccine treatment of colicystitis and some errors which may arise in the determination of the opsonic index with *B. coli*. *Indian Med. Gaz.*, **43**, p. 82.

49. CORNWALL, J.W. and LAFRENAIS, H. M. (1921) Bacilluria. *Indian Med. Gaz.*, **56**, p. 325.

FUSOSPIROCHAETOSIS

50. MENON, I. G. K. (1945) Intestinal fusospirochaetosis. *Indian Med. Gaz.*, **80**, p. 454.

51. *Idem.* (1947) Intestinal fusospirochaetosis. *Brit. Med. J.*, **1**, p. 948.

52. *Idem.* (1954) Gastro-intestinal fusospirochaetosis. *Brit. Med. J.*, **1**. p. 761.

TROPICAL EOSINOPHILIA

53. MENON, I. G. K. (1945) Tropical Eosinophilia. *Indian Med. Gaz.*, **80**, p. 24.

54. *Idem.* (1946) Tropical Eosinophilia Some further observations. *Indian Med. Gaz.*, **81**, p. 70.

MYCOLOGY

55. CORNWALL, J. W. and LAFRENAIS, H. M. (1922) A new variety of streptothrix cultivated from mycetoma of the leg. *Ind. Jour. Med. Res.*, **10**, p. 239.

MALARIA

56. DONOVAN, C. (1920) Malaria of monkeys at the foot of the Nilgiris *Ind. Jour. Med. Res.*, **7**, p. 717.

57. MULLIGAN, H.W. and RUSSELL, P.F. (1940) Agglutination of sporozoites of *P. gallinaceum*. *J. Mal. Inst. Ind.*, **3**, p. 199.

58. MULLIGAN, H. W., RUSSELL, P. F. and MOHAN, B. N. (1940) Specific agglutination of sporozoites. *J. Mal. Inst. Ind.*, **3**, p. 515.

59. *Idem.* (1941) Active immunisation of fowls against *Plasmodium gallinaceum* by injections of killed homologous sporozoites. *J. Mal. Inst. Ind.*, **4**, p. 25.

60. MULLIGAN, H.W. and SOMMERVILLE, T. (1947) Malaria in the Malabar Squirrel. *Ind. J. Mal.*, **1**, p. 329.

61. MULLIGAN, H. W., SOMMERVILLE, T. and SWAMINATH, C. S. (1940) Cellular and humoral agencies in defence against malaria. *J. Mal. Inst. Ind.*, **3**, p. 563.

62. *Idem.* (1940) Attempts to control malarial infections in monkeys by administration of spleen extracts. *J. Mal. Inst. Ind.*, **3**, p. 581.

63. *Idem.* (1940) The effects of splenectomy on natural and acquired immunity in monkey malaria. *J. Mal. Inst. Ind*, **3**, p. 591.

64. MULLIGAN, H. W. and SWAMINATH, C. S. (1940) Natural infection with *Plasmodium inui* in *Silenus sinicus* from South India. *J. Mal. Inst. Ind*, **3**, p. 603.

65. PASSMORE, R. and SOMMERVILLE, T. (1940) An investigation of the effect of diet on the course of experimental malaria in monkeys. *J. Mal. Inst. Ind.*, **3**, p. 447.

66. RUSSELL, P. F., MULLIGAN, H. W and MOHAN, B. N. (1941) Specific agglutinogenic properties of inactivated sporozoites of *P. gallinaceum* *J. Mal. Inst. Ind.*, **4**. p. 13.

67. *Idem.* (1941) Active immunisation of fowls against sporozoites but not trophozoites of *Plasmodium gallinaceum* by injection of homologous sporozoites. *J. Mal. Inst. Ind.*, **4**, p. 311.

FILARIASIS

68. CRUICKSHANK, J. A. and WRIGHT, R. E. (1914) Filariasis in Cochin. *Ind. Jour. Med. Res.*, **1**, p. 741.

KALA-AZAR

69. CORNWALL, J. W. (1916) A contribution to the study of kala-azar. *Ind. Jour. Med. Res.*, **4**, p. 105.

70. *Idem.* (1922) Note on the histopathology of a nonulcerated oriental sore. *Ind. Jour. Med. Res.*, **9**, p. 545.

71. *Idem.* (1924) Resistant forms of *Leishmania donovani* found in NNN cultures. *Ind. Jour. Med. Res.*, **11**, p. 587.

72. CORNWALL, J. W. and KESAVA MENON, T. (1917) A contribution to the study of kala-azar, III. *Ind. Jour. Med. Res*, **4**, p. 672.

73. *Idem.* (1918) A contribution to the study of kala-azar, IV. *Ind. Jour. Med. Res.*, **5**, p. 541.

74. CORNWALL, J. W and LAFRENAIS, H. M. (1916) A contribution to the study of kala-azar. *Ind. Jour. Med. Res.*, **3**, p. 698.

75. CORNWALL, J. W. and LAFRENAIS, H. M. (1922) A contribution to the study of kala-azar. *Ind. Jour. Med. Res.* **9**, p. 533.

76. PATTON, W. S. (1921) Some reflections on kala-azar and oriental sore problems. *Ind. Jour. Med. Res.*, **9**, p. 496.

PROTOZOOLOGY

77. PATTON W. S. (1921) Studies on the flagellates of the *genera* Herpetomonas, Crithidia and Rhynchoidomonas, No. 3. *Ind. Jour. Med. Res.*, **8**, p. 603.

78. *Idem.* (1921) Studies on the flagellates of the *genera* Herpetomonas, Crithidia and Rhynchoidomonas, No. 4. *Ind. Jour. Med. Res.*, **8**, p. 613.

79. *Idem*, (1921) Studies on the flagellates of the *genera* Herpetomonas, Crithidia and Rhynchoidomonas No. 7. *Ind. Jour. Med, Res.*, **9**, p. 230.

80. PATTON, W. S., LAFRENAIS, H. M., and SUNDARA RAO. (1921) Studies on the flagellates of the *genera* Herpetomonas, Crithidia and Rhynchoidomonas, No. 6. *Ind. Jour Med. Res.*, **8**, p. 629.

81. *Idem.* (1921) Studies on the flagellates of the *genera* Herpetomonas, Crithidia and Rhychoidomonas, No. 8. *Ind. Jour. Med. Res.*, **9**, p. 240.

82. *Idem.* (1921) Studies on the flagellates of the *genera* Herpetomonas, Crithidia and Rhynchoidomonas, No. 9. *Ind. Jour. Med. Res.* **9**, p. 252.

83. PATTON, W. S., and SUNDARA RAO. (1921) Studies on the flagellates of the *genera* Herpetomonas, Crithidia and Rhynchoidomonas, No. 2. *Ind. Jour. Med. Res.*, p. 593.

84. *Idem.* (1921) Studies on the flagellates of the *genera* Herpetomonas, Crithidia and Rhychoidomonas, No. 5. *Ind. Jour. Med. Res.*, **8**, p. 621.

VENOMS

85. AHUJA, M. L., BROOKS, A. G., VEERARAGHAVAN, N. and MENON, I. G. K. (1946) A note on the action of heparin on Russell's viper venom. *Ind. Jour. Med. Res.* **34**, p. 317.

86. AHUJA, M. L., VEERARAGHAVAN, N. and MENON, I. G. K. (1947) Effect of heparin on the action of the venoms of some American viperine snakes. *Ind. Jour. Med. Res.*, **35**, p. 227.

87. *Idem.* (1948) Action of heparin on the venom of *Echis carinatus*. *Nature*, **158**, p. 878.

88. CORNWALL, J. W. (1916) Some centipedes and their venom. *Ind. Jour. Med. Res.*, **3**, p. 541.

89. VEERARAGHAVAN, N. (1951) Action of some sulfhydryl compounds on cobra venom. *Ind. Jour. Med. Res.*, **39**, p. 267.

COAGULATION OF BLOOD

90. MENON, I. G. K. (1946) Studies on coagulation of blood. *Ind. Jour. Med. Res.*, **34**, p. 193.

ENTOMOLOGY

91. CORNWALL, J. W. (1915) *Lepisma Sacchrina* (*?*): Its life history and anatomy and its gregarine parasites *Ind. Jour. Med. Res.*, **3**, p. 116.

92. *Idem.* (1923) Note on the salivary glands and sucking apparatus of *Cimex rotundatus*. *Ind. Jour. Med. Res.*, **10**, p. 687.

93. *Idem.* (1923) On the structure of salivary pump in certain blood sucking and other insects. *Ind. Jour. Med. Res.*, **10**, p. 996.

94. PATTON, W.S. (1920) Some notes on the arthropods of medical and veterinary importance in Mesopotamia and on their relation to disease, I. *Ind. Jour. Med. Res.*, 7, p. 735.

95. *Idem.* (1920) Some notes on the arthropods of medical and veterinary importance in Mesopotamia and on their relation to disease, II. *Ind. Jour. Med. Res.*, 7, p. 751.

96. *Idem.* (1920) Some notes on the arthropods of medical and veterinary importance in Mesopotamia and on their relation to disease, III. *Ind. Jour. Med. Res.*, 8, p. 1.

97. *Idem.* (1920) Some notes on Indian Calliphorinae, I. *Ind. Jour. Med. Res.*, 8, p. 17. .

98. *Idem.* (1920) Note on the occurrence of Passeromyia Heterochaeta Villineure in India. *Ind. Jour. Med. Res.*, 8, p. 30.

99. *Idem.* (1920) Some notes on the arthropods of medical and veterinary importance in Mesopotamia and on their relation to disease IV. *Ind. Jour. Med.*, 8, p. 245.

100. PATTON, W.S. (1920) Some notes on the arthropods of medical and veterinary importance in Mesopotamia and on their relation to disease, V. *Ind. Jour. Med. Res.*, 8, p. 253.

101. *Idem.* (1920) Cutaneous myiasis in man and animals in India. *Indian Med. Gaz.*, 55, p. 455.

102. WRIGHT, R.E. and PATTON, W.S. (1921) A case of myiasis of the frontal and ethmoidal sinuses and orbit. *Indian Med. Gaz.*, 56, p. 58.

PHARMACOLOGY

103. McCARRISON, R. and CORNWALL, J.W. (1918) Pharmaco-dynamics, of Quinine, I. *Ind. Jour. Med. Res.* 6, p. 248.

104. CORNWALL, J.W. (1919) Quinine in the treatment of malaria. *Ind, Jour. Med. Res., Spl. Ind. Sci. Con. No.* 83.

105. CORNWALL, J.W. (1919) The pharmacodynamics of Quinine, II. *Ind. Jour. Med. Res.*, 7, p. 160.

MISCELLANEOUS

106. CORNWALL, J.W. (1920) Organisation of medical research in India. *Ind. Jour. Med. Res., Spl. Ind. Sci-Con. No.* p. 1.

107. CORNWALL, J.W. (1923) The training of public health officials. *Indian Med. Gaz.*, 58, p. 285.

108. *Idem.* (1923) What are the departures from health and disease which arise from the negligence, ignorance and self-indulgence of man? *Indian Med. Gaz.*, 58, p. 15.

109. CORNWALL. J.W. and RAMSWAMY-AIYAR, S. (1916) Pedigrees of two families showing many members with supernumerary digits. *Indian Med. Gaz.*, 15, p. 407.

RABIES

1. VEERARAGHAVAN, N. (1957) Preparation of lyophilized rabies street virus material from infective submaxillary glands for challenge purposes. *Bull. Wld Hlth. Org.*, **17**, p. 937.

2. *Idem.* (1959) Improvement of the antigenicity of antirabies vaccine by pooling checked by post-challenge vaccination of guinea-pigs. *Bull. Wld Hlth. Org.*, **20**, p. 381.

3. VEERARAGHAVAN, N. and BALASUBRAMANIAN, A. (1958) A comparative study of the antigenic value of 5 per cent Semple vaccine prepared in distilled water and normal saline. *Ind. Jour. Med. Res.*, **46**, p. 514.

4. VEERARAGHAVAN, N. and SUBRAHMANYAN, T. P. (1958) The value of 5 per cent Semple vaccine prepared in distilled water in human treatment: comparative mortality among treated and untreated. *Ind. Jour. Med. Res.*, **46**, p. 518.

5. *Idem.* (1960) Value of antirabies vaccine with and without serum against severe challenges. *Bull. Wld Hlth. Org.*, **22**, p. 381.

6. *Idem.* (1961) Antigenic value of lyophilized phenolized antirabies vaccine. *Bull. Wld Hlth. Org.*, **25**, p. 115.

7. *Idem.* (1963) Value of duck embryo vaccine and high egg passage Flury vaccine in experimental rabies infection in guinea-pigs. *Bull. Wld Hlth. Org.*, **29**, p. 323.

8. *Idem.* (1963) Duration of immunity to reinfection in guinea-pigs treated with antirabies serum and vaccine and the value of booster doses of vaccine in retreatment. *Bull. Wld Hlth. Org.*, **29**, p. 511.

9. VEERARAGHAVAN, N. BALASUBRAMANIAN, A. and RANGASAMI, R. (1958) Virus content of brains and salivary glands and occurrence of Negri bodies in animals suspected of having died of natural rabies infection. *Bull. Wld Hlth. Org.*, **18**, p. 469.

10. VEERARAGHAVAN, N. BALASUBRAMANIAN, A. and SUBRAHMANYAN. T. P. (1957) Advances in rabies treatment: An experimental evaluation. *Bull. Wld Hlth. Org.*, **17**, p. 943.

INFLUENZA

11. MENON, I. G. K. (1956) Influenza epidemics and pandemics: a historical review. *Eleventh Madras State Med. Conf. Souvenir*, p. 1.

12. MENON, I. G. K. (1959) The 1957 pandemic of influenza in India. *Bull. Wld Hlth. Org.*, **20**, p. 199.

13. MENON, I. G. K. and SHAW, C. W. (1957) Iodine in the prophylaxis of influenza. *Antiseptic*, **54**, p. 692.

14. VEERARAGHAVAN, N. (1961) Influenza isolations at Government of India Influenza Centre, Coonoor, during 1950–60. *Bull. Wld Hlth. Org.*, **24**, p. 679.

15. *Idem.* (1962) Cultivation of pure influenza virus in CAM cultures *in vitro*. *Bull. Wld Hlth. Org.*, **27**, p. 808.

16. VEERARAGHAVAN, N. and KIRTIKAR, M. W. (1961) Biological characteristics of influenza virus strains isolated at the Government of India Influenza Centre, Coonoor, during 1950–60. *Bull. Wld Hlth. Org.*, **24**, p. 684.

17. VEERARAGHAVAN, N. and SREEVALSAN, T. (1961) Evaluation of some methods of concentration and purification of influenza virus *Bull. Wld Hlth. Org.*, **24**, p. 695.

18. VEERARAGHAVAN, N. and SREEVALSAN, T. (1961) Attempts to increase the yield of influenza virus from stored infected allantoic fluids. *Bull. Wld Hlth. Org.*, **24**, p. 703.

19. VEERARAGHAVAN, N., KIRTIKAR, M. W. and SREEVALSAN, T. (1961) Studies on the cultivation of influenza virus *in vitro*. *Bull. Wld Hlth. Org.*, **24**, p. 711.

20. VEERARAGHAVAN, N., NATARAJAN, S. and SREEVALSAN, T. (1962) Attempts to obtain pure influenza virus in chorioallantoic membrane cultures *in vitro*. *Ind. Jour. Med. Res.*, **51**, p. 1.

ADENOVIRUSES

21. KURIAN, P. V., MUKUNDAN, P. and KARPAGAGANAPATHY, P. R. (1962) Isolation of adenovirus type 21. *Lancet*, **2**, No. 7251, p. 355.

22. KURIAN, P. V., ROSHANLAL and PANDIT, V. (1966) Adenovirus infections in Indian army personnel *Ind. Jour. Med. Res.*, **54**, p. 812.

23. ROSHANLAL and KURIAN, P.V.(1964) Acute respiratory infections amongst army personnel. *Armed Forces. Med. J.*, **20**, p. 61.

VACCINIA

24. SUBRAMANYAN, P., DIVAKARAN, S. and VINOD RAJ, P. (1961) Cultivation of vaccinia virus in sheep kidney cell cultures. *Bull. Wld Hlth. Org.*, **25**, p, 33.

CHOLERA

25. VEERARAGHAVAN, N., KALYANARAMAN, V. and VISALAKSHI, V. K. (1966) Role of pentamethylenediamine (cadaverine) in experimental cholera infection. *Ind. Jour. Med. Res.*, **54**, p. 117.

MISCELLANEOUS

26. KURIAN, P. V. (1959) The hospital staphylococcus. *Madras Clin. J.*, **26**, p. 149.

27. *Idem.* (1960) Aetiology of tropical eosinophilia *Madras Clin.* J., **27**. p. 7.

28. KURIAN, P. V. and MUKUNDAN, P. (1960) Human amnion cells: their preparation and use in virology. *J. I. M. A.*, **35**, p. 405.

Section IV

TO-DAY AND TO-MORROW

The Institute started in 1907 under humble beginnings. It has since its inception had the good fortune to have a series of distinguished Directors to guide its destinies and to their extraordinary ability, scientific talent and vision, it owes its present position to-day. The enormous number of patients who have been treated with vaccine prepared at the Institute and the numerous papers of scientific interest and importance which have been published during the past 60 years indicate the important part which the Institute has played in medical and health work in India.

The Institute as stated in the objects of the Association was started primarily to make available effective means of preventing the occurrence of rabies, to spread the knowledge of such means among the public and to undertake research work on rabies. Even to-day this continues to be its important activity and the Institute is recogised as an important centre for rabies research in the world. But, what is not realised is that the Institute, since its inception, thanks to the varied interests of its successive Directors, has been continuously engaged in and made outstanding contributions to problems other than rabies. These include subjects like malaria, Kala-azar, snake venoms, typhoid fever, entomology etc.

During recent years the activities of the Institute have increased considerably. The routine work has more than trebled. The demands for antirabies vaccine have reached an all-time record. There has been a great increase in the number of specimens received for bacteriological, biochemical and pathological examinations. The work in connection with the study of epidemics on behalf of the Public Health Departments of the different States has considerably increased.

The past 10 years have witnessed a steady expansion of the research activities of the Institute. It is internationally recognised for its research work on rabies and is now serving as the International Reference Centre on Rabies of the WHO. It is the main laboratory for work on influenza in the country and is functioning as the Government of India Influenza Centre. The contributions made by the Centre during the pandemic of influenza in 1957 and subsequently are well known It is doing a considerable amount of work on other respiratory virus infections in the country the knowledge about which is very meagre. Since 1957, the Institute has been serving as an important centre for research on intestinal viruses. It helped in the investigations of the epidemics of encephalitis among children in Delhi and Nagpur and the polio epidemics in Andhra Pradesh, Palghat, Coimbatore and Dhalavoipuram. It has collected basic data on the epidemiology of poliomyelitis in this part of the country which should prove useful in the programme for immunization of children against poliomyelitis. The Institute was chosen by Dr. Albert Sabin for the production of live poliovirus vaccine for the protection of about 50 million susceptible children in the country and is actively engaged in this very responsible task. Its studies on vaccinia indicate the possibility of producing an elegant tissue culture vaccine for immunization against smallpox. In addition considerable amount of work is in progress on important public health problems like trachoma, cholera, syphilis, typhoid fever, diphtheria etc.

All these activities would not have been possible but for the generous assistance being provided by the Ministry of Health, Government of India. As mentioned earlier, since 1959 the financial commitments in respect of all the research programmes are being met by the Ministry of Health. The expenditure in connection with the construction, furnishing and equipping of the new

laboratories was entirely borne by the Ministry of Health. It is also providing finances for the poliovirus vaccine project. The Association of the Pasteur Institute of Southern India is deeply indebted to the Ministry of Health and individually to the late Rajkumari Amrit Kaur, Sri D.P. Karmakar, Dr. Sushila Nayar, the former Union Ministers for Health, and to Dr. S. Chandrasekhar, the present Minister for Health, for their generous assistance which made the expansion of the activities of the Institute during the past 10 years possible. The Association is very grateful to the Indian Council of Medical Research for the financial assistance provided for the various enquiries in the beginning. It is also greatly indebted to the WHO and the USAID Mission, New Delhi, for the valuable equipment provided which makes the Institute one of the best equipped centres for virus research in this part of the world.

It would be idle to pretend that the Institute has no problems. Its greatest handicap all along has been paucity of funds. The income of the Institute, which is a charitable organization, is derived mainly from the sale of antirabies vaccine. It is obvious that the finances of an institution which depends for its revenue on the sale of vaccine for a preventable disease like rabies could never be satisfactory. There is no denying the fact that in the past the rate of progress has been considerably hampered by the paucity of funds and there have been occasions when the position was desperate. As such it has had to depend greatly on organizations like the Indian Council of Medical Research and the Madras State Research Committee for the conduct of its research programmes during the early stages. With the taking over by the Ministry of Health the financial commitments in respect of the various enquiries the position has considerabty eased. But, the staff problem still continues. All the enquiries sponsored by the Ministry of Health are temporary and on a year to year basis. In view of the absence of security of service and the fact that there is a considerable demand outside for workers trained at the Institute in specialized disciplines like tissue culture and virology, it has become increasingly difficult to retain trained staff. Unfortunately, the meagre resources of the Institute do not permit absorbing the temporary staff employed in the enquiries on a permanent basis. It is earnestly hoped that this important problem will be solved by the Ministry of Health in order not only to help the Institute to retain the staff but enthuse them to give of their best. Any further expansion of the activities of the Institute will not be feasible till this question is settled.

The slow but steady development of the Institute would not have been possible but for the vision of the successive Presidents and the members of the Association and the Central Committee. As a result of their enlightened policy, the Institute, apart from developing excellent facilities for work, has a band of talented and enthusiastic workers devoted to research. The Association is particularly grateful to Dr. (Kumari) A. B. Marikar for her guidance, understanding and active help.

Virus research in the country for various reasons is still in its infancy. Although work is being done on some specific viruses, so far no systematic attempt has been made to map out the various virus diseases prevalent in the country. This, obviously is of prime importance. For any rational approach to their control it is essential that we should have an idea regarding their nature, incidence, distribution, natural history, etc. A study of this type is difficult as techniques for the isolation, cultivation and typing of different viruses vary and it is not possible to have all the facilities in a single set up. But, with the facilities provided by the new laboratories together with those already existing it should be possible for the Institute to undertake work of this nature. Such a study, apart from giving an idea of the viruses prevalent in a locality, may give us a clue to the aetio-

logy of at least some of the pyrexias of unknown origin. It may also help us to find out what happens to even common viruses like smallpox, chickenpox, measles, etc., during inter-epidemic periods. The Institute could play a very important role in seeking an answer to some of these problems.

Sufficient attention has not been paid in our country to the training of personnel required to carry out ambitious programmes of research work. It is hardly necessary to emphasize that it is of great importance. The Institute in addition to having very good facilities for virus work has an equally good set up for bacteriological, biochemical and high grade pathological work. It has an excellent library and very good animal houses. Most important of all it is not divorced from clinical medicine in view of its close association with the hospitals, civil and military, private practitioners, residential schools, orphanages, etc., in the Nilgiris and surrounding districts, who for years have been availing for themselves of the clinical laboratory facilities at the Institute. It is obvious that such an institution should be an ideal place for training in virology and general bacteriology. It is heartening to learn that international organizations like the WHO, the Indian Council of Medical Research, the Central and State Governments and various institutions in the country are availing themselves of the facilities for training. These could be readily extended. The Institute could also serve as a first class post-graduate training centre.

At present there is no other good reference laboratory and diagnostic centre to which all problems on virus diseases of public health importance pertaining to this region could be referred. During the past few years the Institute has been serving this very useful purpose. This activity could be extended.

With encouragement and sustained financial assistance there is no doubt that the Institute with its traditions and achievements during the past 60 years is bound to play a leading role in the development of medical research in the country.

HAFFKINE INSTITUTE
LIBRARY
BOMBAY

PRINTED BY: C. D. DHODY & SONS, WELLINGTON, NILGIRIS.