



सत्यमेव जयते

**REPORT**  
OF  
**THE HAFFKINE INSTITUTE**

For the Year 1955

BY

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BOMBAY

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# REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1955.

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# REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1955.

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## PART I.

### GENERAL.

#### I. Staff.

##### DIRECTOR.

Dr. P. M. Wagle, M.D. (Bom.), D.Bact. (Manch.), D.P.H. (Lond.), D.T.M. (Liverpool), upto 12th August 1955 (A. N.) inclusive.

Dr. D. W. Soman, M.D. (Bom.), B.Hy. (Bom.), D.P.H. (R.C.P.S. Engl.), D.T.M. & H (Lond.), from 13th August 1955 (F. N.).

##### ASSISTANT DIRECTORS.

Dr. D. W. Soman, M.D. (Bom.), B.Hy. (Bom.), D.P.H. (R.C.P.S. Engl.), D.T.M. & H. (Lond.), B.M.S. (I), Assistant Director, I/c Department of Virus Diseases including Rabies.

Dr. N. K. Dutta, M.B. (Cal.), D.Phil. (Oxon.), B.M.S. (I), Assistant Director, I/c Department of Pharmacology.

Dr. P. J. Deoras, B.Sc., M.Sc. (Nagpur), Ph.D. (Durham), Assistant Director, I/c Department of Entomology.

Dr. A. K. Hazra, M.B., B.S. (Bom.), D.T.M. (Cal.), B.M.S. (I), Assistant Director, I/c Department of Antitoxins and Sera.

Dr. C. V. Deliwala, M.Sc., Ph.D., Officiating Assistant Director, I/c Department of Chemotherapy.

Dr. B. G. Modi, M.B., B.S. (Bom.), F.C.P.S., D.P.B., B.M.S. (II), holding the charge of current duties, I/c Department of Clinical Pathology.

## II. General Review.

In general, the activities of the Institute continued to be the same as in previous years. Production of sufficient quantities of prophylactic vaccines, viz. plague, cholera, typhoid and antirabic vaccine, prophylactic and curative antitoxins and toxoids, sulpha drugs, vitamin tablets, parenteral solutions and diagnostic work for hospitals and practitioners claimed its resources in funds and personnel. For some time, the Institute had housed a centre under the Employees' State Insurance Scheme to afford medical aid. The Institute also prepared various kinds of sulpha drugs as per schedule of the Employees' State Insurance Scheme. Mention may be made of Multivitamin Tablets which were specially prepared at the Institute for supply to all the Chemists and Druggists for distribution under the same scheme. Comparatively, demands for plague vaccine are going down year by year and demands for cholera and T. A. B. vaccines were lower than that of the last year, which is a sure sign that due to public health preventive measures the incidence of these diseases is showing a downward trend. However, production of antirabic vaccine, antitoxins and sera, parenteral solutions etc. has considerably increased. The Institute also served as an Analytical Laboratory for enforcement of the Drugs Act, for the Bombay State and other States in the Indian Union and as a Provincial Bacteriological Laboratory for the Bombay State. It rendered valuable assistance to the Department of Prohibition and Excise by analysing the samples sent by them under Prohibition Scheme. A large number of samples had to be analysed and additional staff for this scheme was, therefore, sanctioned by the Government. The pressing demands on the biological products prepared at this Institute is a distinct sign of its popularity and the confidence that it enjoys at the hands of public as well as medical profession. Despite the pressure of routine work, most of the senior members of the staff carried out research work detailed in Part II of the report. Some of the research problems bearing on the various activities of the Institute were also carried out with the generous assistance of the State Government, I. C. M. R., C. S. I. R., and Fellowships granted by the Unichem Laboratories and the Raptakos Board of Medical Research, Bombay. During the year under review, more post-graduate workers and fresh science graduates were admitted in the various departments of the Institute and were entrusted with the problems in which the Institute is interested.

The sales amounted to about Rupees 24 lakhs while the expenditure was about Rupees 19 lakhs during the financial year 1954-55.

The demands for Plague and Cholera Vaccines were low as in previous years. This is mainly due to the country being fortunately free from serious epidemics during this year. A total of about 3.5 million millilitres of plague and cholera vaccines were issued during the year. This has enabled the Vaccine Department personnel to devote more time for various scientific investigations, such as fractionation of antigens of *P. pestis*, *S. typhi* and *V. cholera*.

The demand for media from various departments of the Institute is increasing year by year, especially from the Diagnostic Department, regarding the supply of Leoffler's serum. Due to increased popularity of parenteral solutions, the sale has gone up considerably since 1946; this year, 84,360 bottles were prepared as compared with 72,000 of last year. The material is at present issued to Government Hospitals as well as private hospitals in Bombay and other States.

The Section of Public Health Chemistry and Bacteriology was mainly engaged in carrying out bacteriological and chemical examination of water samples received from Bombay State and other States in the Indian Union for ascertaining the potability of water for drinking purposes. In addition, investigation was undertaken in this section to evaluate the role of Eijkman's Test in bacteriological analysis of water samples.

The Department of Antitoxins and Sera has been receiving demands for various antitoxins, particularly antivenin and tetanus antitoxin, for the last few years, far in excess of what could be met with or is possible to produce with the existing resources. The supplies of antitoxins and sera this year were worth over 13 lakhs of rupees. With the advent of Health Insurance Scheme of the Government of Bombay and the mass prophylaxis campaign for the Triple Vaccine by the Bombay Municipal Corporation, there have been considerable demands for diphtheria and tetanus prophylaxis, which has put a heavy strain on the departmental working. It now appears inevitable that until the Pimpri Farm in Poona is developed and utilised as a full production unit, it would not be possible to step up the production further, to meet increasing demands, both from the States of the Indian Union as well as from the Armed Forces and even from abroad.

The pressure of work in the Department of Pharmacology continued to be very heavy, there being 72 per cent. increase in the number of samples tested. Under the Drugs Act, 1642 samples were tested. These were received from the States of Delhi, East Punjab, Uttar Pradesh, Bihar, Himachal Pradesh, Madhya Pradesh, besides our own. Three hundred and eighteen samples were tested on behalf of commercial firms. A large number of samples were tested for the different departments of the Government of Bombay, *e.g.* Prohibition and Excise, Police, etc. The number of samples tested under "Biological and Special Products" and "Other Special Products" was 1218.

Instances of faked drugs have been brought to light. The bulk of the samples that were received from the Department of Prohibition and Excise were spirituous medicinal preparations. Majority of these tinctures were nothing but coloured alcohol mixed in some cases with flavouring agent.

About 42 per cent. of the drugs, out of the total of 4,055, were of non-standard quality. Under the Drugs Act, about 45 per cent. of the samples failed to comply with the required standards.

Investigations were carried out on hydrolysate from horse fibrin regarding its suitability for intravenous medication in human beings. Clinical trials of this product at the K. E. M. Hospital showed that it was non-toxic and seemed to be quite effective in raising the serum protein values to the normal level.

A method for detection and determination of saccharin in "Prepared Tea" has been further modified to suit the examination of other pharmaceutical preparations to detect its presence. The work on *Wagatia spicata* Dalzell (Vakeri, Vagati) was continued under the Ayurvedic Research Scheme and the crystalline substance that was obtained was investigated for its pharmacological and toxicological properties.

Further work was carried out on the plant *Scilla indicā* which grows abundantly in Bombay State. Samples were examined biologically and none of the samples was found to contain the active principle which has action on heart.

During the year, the routine work in the Department of Entomology connected with the identification of fleas, insects, and arthropods of medical importance, rats, insecticides, rodenticides and rat traps was carried out. A course of lectures in plague preventive measures was held in February 1955. Assistance was rendered in setting up two exhibitions regarding this Institute. The research work on the pulicidal value of *Pyrethrum*, collection of rat-ectoparasites and biometrical studies on Bombay rats was continued. The work connected with the breeding of snakes in the snake farm, venom output of snakes and their comparative morphology was continued.

The Department of Viruses including Rabies has reported that the demand for antirabic vaccine is increasing year by year. 27,340 treatments were supplied during the year as compared with 21,555 in the year 1954. The number of antirabic centres in the State and outside have increased from 163 to 176, out of which 79 are within the Bombay State. The antirabies hyperimmune serum prepared at the Institute was tried for rabies prophylaxis at a few centres in the Bombay State. In view of the small number of selected cases receiving such treatment, the trials will have to be further extended. The importance of this additional treatment could well be realised, since Virus Diseases Advisory Committee under I. C. M. R. has taken up this question with a view to extend such field trials to different States all over the country. Researches were carried out on rabies with a view to devise suitable serological technique for a quick diagnosis on specimens of brain and salivary glands from suspected rabid animals and also to study the comparative frequency of infectivity of these specimens, which would throw some light on the transmission of rabies in nature. The World Health Organization Influenza Centre established in the Virus Section is doing excellent work. In the absence of any large-scale epidemic or outbreaks of influenza during the last five years, a dozen strains have been isolated and typed and reported by the centre. Three strains of influenza virus have been isolated and typed this year and have been confirmed by the World Influenza Centre, London. Efforts were also directed towards the

development of a method for a large-scale manufacture of influenza vaccine to meet any emergency and devising a suitable method for the biological assay, of this product.

The Department of Chemotherapy continued to manufacture Sulphathiazole Powder, Tablets and injectibles, Vitamin Tablets of different composition and Sulphetrone Injectibles. The production showed considerable rise. The sale value amounted to Rs. 5,81,716 as against the previous year's figure of Rs. 2,85,363, *i.e.* more than double. Several new products were introduced during the year such as Sulphadiazine, Quinine-sulphate and Multivitamin Tablets, which were primarily taken up for the Employees' State Insurance Scheme. In addition to the manufacture of sulphathiazole from starting raw materials, investigations into the possibility of large-scale manufacture of other important synthetic drugs such as Iso-nicotinic acid hydrazide, Dapsone, PAS, Sulphadiazine, Sulphaguanidine, Paludrine, Chloroquin, etc. were continued and important data collected.

During the year 1955, the Department of Clinical Pathology carried out, as in previous years, bacteriological, serological, biological and histopathological examinations of clinical materials received from Government as well as private institutions, hospitals medical practitioners, etc. The supply of diagnostic reagents continued to show a steady increase. The free diagnostic aid for clinical cases of typhoid fever entered its 8th year with substantial usefulness and popularity. During the year under review, 23,585 clinical materials were examined. The number of specimens examined for histopathology was 2,498 and the number of calls attended to under the Typhoid Scheme was 731. The department continued to co-operate with Indian Council of Medical Research on Inter-laboratory Evaluation of Serological Tests for Syphilis and 191 samples of sera were examined. The survey of the prevalence of Hook Worm in Kaira District was completed during the year. In all, 10,699 samples of stools were examined out of which 3,447 showed ova of Hook Worm. The overall incidence of the disease was found to be 35 per cent.

The Department of Biochemistry rendered excellent service to the various Government, Municipal and private hospitals and the medical practitioners in the City. Special assistance was extended to the Seamen's Medical Examination Scheme to examine some seamen for their Glucose Tolerance Test. Analysis of milk samples on behalf of the public has become a regular feature of the department.

The work of the Blood Bank Department showed increased activity in all spheres. The demand for dried plasma was more than the last year and so its production had to be increased. This year, 2,198 bottles were supplied as against 1,232 in the last year. However, the position regarding blood donors remained unsatisfactory, as only vagrant and under-nourished type of persons usually come up for blood donation. Facilities were offered to Hospitals and medical men to have their patients examined for Rh investigation.

Besides the research work done by the members of the staff, facilities were provided to young Science Graduates to do research work for their post-graduate degrees. Six students are working in the Institute for their post-graduate degrees and 8 have already completed and taken their post-graduate degrees during the year.

### III. DEPARTMENT OF VACCINES.

During the year under review the demand for Plague Vaccine was practically the same as of the last year. The demand for Cholera and T. A. B. Vaccine was slightly lower than that of the last year.

1.1 million millilitre of Plague Vaccine, 2.7 million millilitre of Cholera Vaccine and 2,74,099 millilitre of T. A. B. Vaccine were issued during the year as against 1.1 million millilitre of Plague, 3.8 million millilitre of Cholera and 3,90,082 millilitres of T. A. B. Vaccine last year.

The total quantity of Vaccines issued during the year 1955-56 is 41,18,638 millilitres. The demands for Vaccines were received from all parts of the country, and for Plague Vaccine from some Foreign countries also.

#### A. PLAGUE VACCINE.

During the year under report the quantities of plague vaccine issued are detailed below :—

|                   | Quantities in<br>millilitres. |
|-------------------|-------------------------------|
| January .. .. .   | 1,20,506                      |
| February .. .. .  | 2,72,955                      |
| March .. .. .     | 2,28,040                      |
| April .. .. .     | 65,363                        |
| May .. .. .       | 41,160                        |
| June .. .. .      | 2,020                         |
| July .. .. .      | 73,655                        |
| August .. .. .    | 21,500                        |
| September .. .. . | 2,06,500                      |
| October .. .. .   | 26,244                        |
| November .. .. .  | 55,740                        |
| December .. .. .  | 21,640                        |
| Total ..          | <u>11,35,323</u>              |



Issue of plague vaccine from the Institute since its beginning in quinquennial periods are given below :—

| Year.     |    |    |    | Quantities in millilitres. |
|-----------|----|----|----|----------------------------|
| 1898-1901 | .. | .. | .. | 8,601,123                  |
| 1902-1906 | .. | .. | .. | 10,366,611                 |
| 1907-1911 | .. | .. | .. | 10,752,786                 |
| 1912-1916 | .. | .. | .. | 13,702,098                 |
| 1917-1921 | .. | .. | .. | 20,003,682                 |
| 1922-1926 | .. | .. | .. | 21,607,908                 |
| 1927-1931 | .. | .. | .. | 17,787,729                 |
| 1932-1936 | .. | .. | .. | 19,909,176                 |
| 1937-1941 | .. | .. | .. | 20,078,811                 |
| 1942-1946 | .. | .. | .. | 44,528,208                 |
| 1947-1951 | .. | .. | .. | 65,757,991                 |
| 1952-1955 | .. | .. | .. | 8,213,179                  |
| Total     |    |    |    | 261,309,302                |

The plague vaccine is issued in India and to other countries from this Institute. The demands within and outside India met during the year under report are shown below :—

*India—*

|                  |    |    |    | Quantities in millilitres. |
|------------------|----|----|----|----------------------------|
| Bombay           | .. | .. | .. | 42,953                     |
| Bihar            | .. | .. | .. | 60,580                     |
| U. P.            | .. | .. | .. | 5,00,320                   |
| M. P.            | .. | .. | .. | 2,500                      |
| Hyderabad        | .. | .. | .. | 21,300                     |
| Madras           | .. | .. | .. | 61,650                     |
| V. P.            | .. | .. | .. | 11,940                     |
| East Punjab      | .. | .. | .. | 2,520                      |
| Delhi            | .. | .. | .. | 280                        |
| West Bengal      | .. | .. | .. | 1,75,000                   |
| Himachal Pradesh | .. | .. | .. | 50,760                     |
| Kashmir          | .. | .. | .. | 100                        |
| Defence Services | .. | .. | .. | 5,040                      |
| Total            |    |    |    | 9,34,943                   |

*Foreign countries—*

|             |    |    |    |           |
|-------------|----|----|----|-----------|
| Burma       | .. | .. | .. | 2,00,000  |
| Malaya      | .. | .. | .. | 380       |
| Total       |    |    |    | 2,00,380  |
| Grand Total |    |    |    | 11,35,323 |

## B. CHOLERA VACCINE.

During the year under report the quantities of Cholera Vaccine despatched monthly are as given below :—

| Months.   |    |    |    |    | Quantities in millilitres. |
|-----------|----|----|----|----|----------------------------|
| January   | .. | .. | .. | .. | 59,371                     |
| February  | .. | .. | .. | .. | 25,999                     |
| March     | .. | .. | .. | .. | 2,23,683                   |
| April     | .. | .. | .. | .. | 3,08,674                   |
| May       | .. | .. | .. | .. | 2,54,222                   |
| June      | .. | .. | .. | .. | 6,28,009                   |
| July      | .. | .. | .. | .. | 4,65,008                   |
| August    | .. | .. | .. | .. | 97,887                     |
| September | .. | .. | .. | .. | 4,68,176                   |
| October   | .. | .. | .. | .. | 41,713                     |
| November  | .. | .. | .. | .. | 84,706                     |
| December  | .. | .. | .. | .. | 51,768                     |
| Total     |    |    |    |    | 27,09,216                  |

## C. T. A. B. VACCINE.

The quantities of T. A. B. Vaccine despatched monthly during the year under report are as given below :—

| Months.   |    |    |    |    | Quantities in millilitres. |
|-----------|----|----|----|----|----------------------------|
| January   | .. | .. | .. | .. | 10,243                     |
| February  | .. | .. | .. | .. | 7,407                      |
| March     | .. | .. | .. | .. | 13,657                     |
| April     | .. | .. | .. | .. | 16,547                     |
| May       | .. | .. | .. | .. | 16,126                     |
| June      | .. | .. | .. | .. | 26,284                     |
| July      | .. | .. | .. | .. | 54,628                     |
| August    | .. | .. | .. | .. | 16,660                     |
| September | .. | .. | .. | .. | 33,251                     |
| October   | .. | .. | .. | .. | 35,270                     |
| November  | .. | .. | .. | .. | 23,949                     |
| December  | .. | .. | .. | .. | 20,077                     |
| Total     |    |    |    |    | 2,74,099                   |

## D. MEDIA SECTION.

The Media Section's activities comprise :—

(1) Preparation of culture media for the production of Plague, Cholera, and Typhoid vaccines.

(2) Preparation of various culture media and stains for routine diagnostic and research work in the Institute.

(3) Manufacture of Parenteral Solutions.

During the year, due to fall in demand for Plague and Cholera vaccines, the production of Casein hydrolysate medium was less as compared to the previous year. 34,64,000 ccs. of the medium were prepared during 1955. 2,784 Roux bottles of meat digest agar were prepared for the production of Typhoid vaccine.

Sixty-four thousand one hundred and twenty-four bottles of sterility media were prepared as required by the various Departments of the Institute and also by the Hindustan Antibiotics Ltd.

The media required for diagnostic work especially Loeffler's serum agar were also in great demand.

The parenteral solutions are enjoying increased popularity as can be seen from the following figures. The requirements from the various Hospitals in the State and also outside have been fully met with :—

*Sale of Glucose Saline etc., during 1955.*

| Serial No. | Name of Product.                             | Number of Bottles sold. | Amount.  |    |    |
|------------|----------------------------------------------|-------------------------|----------|----|----|
|            |                                              |                         | Rs.      | a. | p. |
| 1          | Glucose Saline 500 cc. ..                    | 39,906                  | 69,835   | 8  | 0  |
| 2          | 5 per cent. Glucose Solution 500 cc..        | 17,693                  | 30,962   | 12 | 0  |
| 3          | Protein Hydrolysate 400 cc. ..               | 7,583                   | 30,332   | 0  | 0  |
| 4          | Normal Saline 500 cc. ..                     | 4,317                   | 7,554    | 12 | 0  |
| 5          | 25 per cent. Glucose Solution 500 cc.        | 2,352                   | 5,880    | 0  | 0  |
| 6          | 25 per cent. Glucose Solution 100 cc.        | 2,204                   | 2,204    | 8  | 0  |
| 7          | A. C. D. Solution (Anti-Coagulant Solution). | 1,314                   | 2,299    | 8  | 0  |
| 8          | Hypertonic Saline 500 cc. ..                 | 1,120                   | 1,960    | 0  | 0  |
| 9          | 50 per cent. Glucose Solution 100 cc.        | 796                     | 796      | 0  | 0  |
| 10         | Sodium Citrate 500 cc. ..                    | 578                     | 1,011    | 8  | 0  |
| 11         | Sodium Sulphate 500 cc. ..                   | 428                     | 749      | 0  | 0  |
| 12         | Distilled Water 500 cc. ..                   | 6,069                   | 6,069    | 0  | 0  |
| Total      |                                              | 84,360                  | 1,59,654 | 8  | 0  |

The following table gives comparative figures for previous years :—

| Year.        | No. of bottles. | Amount.  |
|--------------|-----------------|----------|
|              |                 | Rs.      |
| 1947 .. ..   | 10,250          | 15,986   |
| 1948 .. ..   | 15,995          | 20,781   |
| 1949 . . . . | 31,718          | 46,542   |
| 1950 .. ..   | 47,111          | 88,833   |
| 1951 .. ..   | 54,892          | 1,03,232 |
| 1952 .. ..   | 67,942          | 1,28,930 |
| 1953 .. ..   | 1,00,073        | 1,94,934 |
| 1954 .. ..   | 72,174          | 1,38,139 |
| 1955 .. ..   | 84,360          | 1,59,654 |

#### E. SECTION OF PUBLIC HEALTH CHEMISTRY AND BACTERIOLOGY.

This section is engaged mainly in carrying out the bacteriological and chemical examination of samples of water for ascertaining their fitness for drinking, sanitary and industrial purposes.

These samples were received from Bombay State as well as from other States.

A special reference may be made to the sample of Katadyn filter received for ascertaining its water sterilizing capacity. This filter was kept in operation over a period of one month and heavily contaminated water samples were filtered through at regular intervals and tested bacteriologically. All the samples were found to be free from contaminating organism.

The following table gives the summary of various examinations carried out during the year :—

| Sample examined. | Nature of examination.                        | No. of samples examined. |
|------------------|-----------------------------------------------|--------------------------|
| Water ..         | Fitness for drinking purposes ..              | 102                      |
| Water ..         | Fitness for Sanitary and Industrial purposes. | 36                       |
| Water ..         | Fitness for swimming Pool purposes ..         | 2                        |
| Milk ..          | Fitness for use in Baby Food ..               | 1                        |
| Filter ..        | Fitness for Sterilizing Polluted water ..     | 1                        |
| Total            |                                               | 142                      |

#### IV. Department of Antitoxins and Sera.

During the year under review the demands for the products of the department continued to show an increase. The sale value of the products during 1955 was Rs. 13,15,777 which is about four and half lakhs more than in 1954, though the sales in 1954 was the highest in the history of the department. The sale value of the products of the department sold to the Defence Forces during the year was Rs. 96,712. Lyophilised antivenin worth Rs. 29,745 was sold to foreign countries.

There was an unusually heavy demand for diphtheria antitoxin during the year. Inspite of a 70 per cent. increase in the production of diphtheria antitoxin the demands from outside the State could not be met in full. The demands for all other products of the department were met in full. Efforts are being made to increase the production of diphtheria antitoxin still further.

During the year the demands for prophylactic toxoids against diphtheria and tetanus for human immunization increased immensely. The sale of diphtheria Toxoid (P. T. A. P.) in 1955 was about 6 times more than in the previous year. The sale of tetanus toxoid for human use was 8 times more than in 1954. Efforts are being made to increase the production of these two prophylactics.

As the demands for the products of serum department were very heavy during the year, all efforts were mainly directed to produce as much as it was possible to produce within the limits of efficiency. This unfortunately resulted in no newer scientific investigations being pursued. With the facilities available for production at present, substantial increase in production will not be possible until steps are taken to develop the Pimpri Stud Farm and at the same time expand the existing capacity at the Institute also.

1. *Horses*.—The total number of horses used for producing antitoxins and sera during the year was 172. Besides these, there were 43 horses at Pimpri Stud Farm undergoing preliminary immunization at the end of the year. The horses under immunization were grouped as follows :—

| Antitoxins.                                  | Number of<br>Horses used. |
|----------------------------------------------|---------------------------|
| (1) Tetanus antitoxin .. .. .                | 75                        |
| (2) Diphtheria antitoxin .. .. .             | 48                        |
| (3) Antivenin .. .. .                        | 32                        |
| (4) Gas gangrene antitoxin (welchii) .. .. . | 9                         |
| (5) Anti-dysentery .. .. .                   | 2                         |
| (6) Anti-rabic .. .. .                       | 4                         |
| (7) Normal Horse Serum .. .. .               | 2                         |
| Total .. .. .                                | <hr/> 172 <hr/>           |

2. *Preparation of Toxins and Toxoids.*—During the year, diphtheria, tetanus and welchii toxins were produced. The reserve of the other toxins sufficed during the year. A part of the diphtheria and tetanus toxins were converted into toxoids for immunization of horses. A portion of the toxoids were also purified for human use.

| Types of Toxins and Toxoids.           |    |    |    |    | Quantities in<br>millilitres. |
|----------------------------------------|----|----|----|----|-------------------------------|
| Tetanus                                | .. | .. | .. | .. | 568,950                       |
| Diphtheria                             | .. | .. | .. | .. | 424,050                       |
| Welchii Toxin                          | .. | .. | .. | .. | 18,100                        |
| Septique                               | .. | .. | .. | .. | Nil.                          |
| Oedematiens                            | .. | .. | .. | .. | Nil.                          |
| Shiga                                  | .. | .. | .. | .. | Nil.                          |
| Tetanus toxin converted into toxoid    | .. | .. | .. | .. | 142,850                       |
| Diphtheria toxin converted into toxoid | .. | .. | .. | .. | 173,750                       |

3. *Preparation of Antitoxins and Sera.*—The quantity of each concentrated sera and natural sera produced during the year are as follows:—

#### I. CONCENTRATED SERA.

|                            |    |    |    |    | Amount in<br>millilitres. |
|----------------------------|----|----|----|----|---------------------------|
| (1) Tetanus Antitoxin      | .. | .. | .. | .. | 685,400                   |
| (2) Diphtheria Antitoxin   | .. | .. | .. | .. | 308,370                   |
| (3) Antivenin              | .. | .. | .. | .. | 254,100                   |
| (4) Gas Gangrene Antitoxin | .. | .. | .. | .. | 110,000                   |
| (5) Anti-dysentery         | .. | .. | .. | .. | Nil.                      |
| (6) Anti-rabic serum       | .. | .. | .. | .. | 6,000                     |
| Total                      |    |    |    |    | 1,363,870                 |

#### II. NATURAL SERA.

| No.   | Name.                |    |    |    | Amount of<br>concentrated serum<br>in millilitres. |
|-------|----------------------|----|----|----|----------------------------------------------------|
| 1     | Normal Horse Serum   | .. | .. | .. | 31,400                                             |
| 2     | Anti-dysentery Serum | .. | .. | .. | 12,100                                             |
| Total |                      |    |    |    | 43,500                                             |

4. *Lyophilisation of Sera*.—Only polyvalent anti-snake-venom serum was issued in lyophilised form during the year. The amount of serum issued was 2,89,830 c. c.

5. *Supply*.—The sale value of the products of this department in 1955 was about Rs. 13,15,777 as compared with Rs. 8,73,425 in 1954. Sale value of our products to Defence Forces was Rs. 96,712 during the year. Lyophilised antivenin worth Rs. 29,745 was sold to foreign countries in 1955. The following quantities of antitoxins, toxoids and sera were issued during the year :—

| Name of Sera.                                                                                                                                                            | Quantities<br>in millilitres. |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------|
| (1) <i>Tetanus Antitoxin</i> —                                                                                                                                           |                               |
| (a) Prophylactic 3,000 I. U. (1928) in 2 c. c. ..                                                                                                                        | 6,51,816                      |
| (b) Curative 10,000 I. U. (1928) in 5 c. c. ..                                                                                                                           | 47,095                        |
| (c) Curative 20,000 I. U. (1928) in 10 c. c. ..                                                                                                                          | 1,55,440                      |
| (d) Highly concentrated 20,000 I. U. (1928) in<br>4 c. c. .. .. .                                                                                                        | 1,16,552                      |
| (2) <i>Diphtheria Antitoxin</i> —                                                                                                                                        |                               |
| (a) Prophylactic 1,000 I. U. in 2 c. c. ..                                                                                                                               | 5,340                         |
| (b) Curative 5,000 I. U. in 5 c. c. ..                                                                                                                                   | 3,095                         |
| (c) Curative 10,000 I. U. in 10 c. c. ..                                                                                                                                 | 2,65,420                      |
| (d) Highly concentrated 10,000 I. U. in 5 c. c. ..                                                                                                                       | 8,515                         |
| (3) <i>Gas-Gangrene Antitoxin</i> —                                                                                                                                      |                               |
| (a) 3,000 I. U. Welchii Antitoxin in 10 c. c. ..                                                                                                                         | 97,330                        |
| (b) 9,000 I. U. Welchii 4,500 I. U. septique and<br>9,000 I. U. Oedematiens Antitoxins, in<br>a volume not exceeding 15 cc., highly<br>concentrated and purified .. .. . | 2,640                         |
| (4) <i>Anti-Plague Serum</i> , 20 c. c. ..                                                                                                                               | 740                           |
| (5) <i>Lyophilised Polyvalent Anti-snake-venom Serum</i> ,<br>10 c. c. .. .. .                                                                                           | 2,89,830                      |
| (6) <i>Anti-dysentery Serum</i> —                                                                                                                                        |                               |
| (a) Natural, 20 c. c. .. .. .                                                                                                                                            | 60                            |
| (b) Concentrated 4,000 I. U. in 5 c. c. ..                                                                                                                               | 225                           |
| (7) <i>Tetanus Toxoid</i> —                                                                                                                                              |                               |
| (a) 10 c. c. .. .. .                                                                                                                                                     | 1,260                         |
| (b) 5 c. c. .. .. .                                                                                                                                                      | 1,370                         |
| (c) 1 c. c. .. .. .                                                                                                                                                      | 41,005                        |
| (8) <i>Diphtheria Toxoid</i> —                                                                                                                                           |                               |
| (a) 5 c. c. .. .. .                                                                                                                                                      | 28,380                        |
| (b) 1 c. c. .. .. .                                                                                                                                                      | 2,076                         |
| (9) <i>Normal Horse Serum</i> in 10 c. c. ..                                                                                                                             | 2,480                         |
| (10) <i>Lyophilised Schick Test Toxin</i> for 25 tests ..                                                                                                                | 33 boxes.                     |

Total 16,46,578 ml. of antitoxins and sera, and 74,091 ml. of toxoids.

In addition to the above, 37,975 ml. of natural horse serum was supplied to local hospitals.

*Other Activities of the Department.*—The Department, in view of the available facilities, undertook to test samples of Antitoxin and other biologicals as well as sterility tests for surgical cat-guts, submitted either directly or on behalf of the Drug Control Organization. There have been many requests to test the efficacy of so-called sure-cures for snake bites. These “drugs” are usually tested on mice, and in one case, as desired by the sponsorer, facilities were given and experiment arranged to demonstrate if protection to a cobra-bitten dog was possible by the drug. In no case any such drug was found to achieve neutralization of snake-venom. In all cases experimental animals died with usual symptoms of snake-venom poisoning.

## V. Department of Entomology.

Technical assistance was rendered to different states of the Indian Union and local bodies in connection with identification of fleas, flies and mosquitoes. A large number of enquiries were attended pertaining to the information about ticks, mites, spiders, scorpions, insects of medical importance, coelenterates, nematodes and amphibia. Information and guidance was supplied with reference to rats, rat-poisons and rat-traps.

A course in Plague preventive measures was held from 28th February 1955 to 16th March 1955. Twenty-two candidates deputed by the local bodies, port-trust, port-health department, and the Director of Public Health, Bombay attended the same. These epidemeological officers and Sanitary Inspectors were given a theoretical and practical training in the subject of prevention and control of Plague.

More than 4,000 persons belonging to various academic Institutions in India and private bodies were given a talk on snakes, venom extraction demonstration and taken round the Institute. This department assisted in setting up exhibits regarding the Institute at two exhibitions; one in Poona and the other at Nasik.

Work has been started on the statistical analysis of the collection of ecto-parasites of the different kinds of rats brought alive to the Institute. Biometrical studies on the rat body measurements were conducted. These studies would supplement the characteristics meant for the identification of Bombay rats.

The studies of the Pulicidal value of Pyrethrum were continued. It has been noted that even deteriorated Pyrethrum extract when kept in dark gave a residual effect in knock-down up to 75 days. When mixed with Piperonyl butoxide this was enhanced. The powders have been tested for residual effect in rat-burrows in some of the villages of Madras State and in Satara District of Bombay State.

Three post-graduate students in Entomology and one in herpetology were guided for their degrees of the University of Bombay. One of the former obtained his M.Sc. degree during the year.



## A. SNAKES.

Forty specimens of snakes were referred to this department for identification during the year. These snakes were either from the city of Bombay or from the districts. The following number of snakes were produced for Venom extraction—Cobra—49, Russels Viper—71, Krait—24 and Echis 1959. A number of non-poisonous snakes handled were—*Ptyas mucous*, *Coluber arnensis*, *Eryx conicus*, *Eryx Johni*, *Python Molurus*, *Dryophis nasutus*, *Lycodon aulicus*, *Oligodon sp*, *Micropisthodon plumbicolor*, and *Natrix sp*. A large number of enquiries pertaining to the information about snake-habits, snake poisons, snake-food, snake enemies and the utility of snake products were attended to.

The venom was collected each month. A comparative account was also kept of the Venom output of equal number of snakes housed in the snake farm and in cages in the snake room. The following table No. 1 gives the detail of the venom produced and supplied during the year. Table No. 2 gives the average venom output of snakes in the farm as compared to those kept in the room.

TABLE 1.

| Cobra           |                 | Russels viper   |                 | Krait           |                 | Echis           |                 |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Venom produced. | Venom supplied. | Venom produced. | Venom supplied. | Venom produced. | Venom supplied. | Venom produced. | Venom supplied. |
| 75·9333 gms.    | 67·7077 gms.    | 53·6201 gms.    | 61·4259 gms.    | 14·7934 gms.    | 13·8360 gms.    | 20·2505 gms.    | 20·4042 gms.    |

TABLE 2.

| Cobra  |        | Russels Viper |        | Krait  |        | Echis     |      |
|--------|--------|---------------|--------|--------|--------|-----------|------|
| Farm   | Room   | Farm          | Room   | Farm   | Room   | Farm      | Room |
| 0·1906 | 0·1480 | 0·1176        | 0·2050 | 0·0254 | 0·0172 | Not taken |      |

(Average output of venom in Grams per snake.)

Field observations were done at Deogad on the food habits of *Echis carinatus*. It has been observed that the maximum number of animals recovered from under the stones in the locality and from the gut contents of *Echis carinatus*, were insects. The majority of these insects were Dermaptera (particularly Forficula Sp :) and Hymenoptera (particularly ants.).

## B. EXAMINATION OF RATS FROM BOMBAY CITY AND PORT TRUST AREA.

A permanent organisation is maintained by the Municipality of Bombay for catching rats. On an average 2,820 rats were received every day at the Institute from the different wards within the Municipal area. These rats were either trapped alive or found dead in the various parts of the City—including the Port Trust area. All the rats were classified according to the species. Only the dead ones were dissected and examined for post-mortem signs of plague infection. In doubtful cases, diagnosis was confirmed by smear and cultural examination. All the rats received daily were finally destroyed by incineration. Besides providing valuable information on the subject of epidemiology, this work is intended to give the Municipal authorities, the first signal of the plague epizootic in any part of the City with a view to controlling plague infection in time. During the year under review, no plague cases were reported in Greater Bombay.

Experiments on the susceptibility of Bombay City rats to plague infection by injecting a standard test infective dose were continued during this year also. The following results were obtained:—

| Species of rats.        | No. of rats used<br>in experiments. | No. of rats<br>dead. | Mortality<br>per cent. |
|-------------------------|-------------------------------------|----------------------|------------------------|
| <i>R. rattus</i> ..     | 109                                 | 11                   | 10·0                   |
| <i>R. norvegicus</i> .. | 75                                  | 2                    | 2·6                    |
| <i>Gundmys kok</i> ..   | 78                                  | 55                   | 70·0                   |

The results do not show any significant difference as compared to the results of the last year.

## C. EXAMINATION OF RATS FROM STEAMERS AND OTHER SOURCES.

During the year under review, there was a plague suspected case in Andheri. An intensive search was, therefore, undertaken to detect the infection if any in the area. Not only dead rats caught in the area were thoroughly examined but live rats in and around the plague suspected area were caught in large numbers and kept under observation. Besides, nearly 100 rats caught in and around the suspected area were used for determining the flea index and also for the detection of infection in the fleas by flea-pool inoculation in mice. This latter method did not prove useful as the area was almost free from fleas probably due to heavy dusting with flea killing agents. Fortunately no plague infection could be detected either in rats or in fleas. To corroborate this, the plague suspected case also was found to be bacteriologically negative and hence declared as 'not plague'.

In all 17 dead rats, 30 smears from dead rats and 19 fleas were received from different towns of the Bombay State. No plague infection was detected. In the same year, 952 killed rats were received from the Health Officer of the Port of Bombay. Of these 547 belonged to the species of *Rattus rattus rufescens*, 18 *Rattus norvegicus*, 83 *Mus dubius*, 1 *Suncus cæruleus* and 303 *putrid*. None of them was found plague infected.

During the year under review, 6 rats were received from the City of Bombay through other agencies or private persons. None of them was found plague infected.

TABLE 1.—*Showing rats received at the Institute from the Bombay Municipality during 1955.*

| Month.       | Alive.    | Dead. | Total.    |
|--------------|-----------|-------|-----------|
| January ..   | 90,512    | 49    | 90,561    |
| February ..  | 79,516    | 23    | 79,539    |
| March ..     | 82,481    | 25    | 82,506    |
| April ..     | 85,712    | 27    | 85,739    |
| May ..       | 79,767    | 14    | 79,781    |
| June ..      | 81,672    | 20    | 81,692    |
| July ..      | 89,724    | 10    | 89,734    |
| August ..    | 89,154    | ..    | 89,154    |
| September .. | 82,430    | 6     | 82,436    |
| October ..   | 87,185    | 2     | 87,187    |
| November ... | 85,543    | 3     | 85,546    |
| December ..  | 95,797    | 1     | 95,798    |
| Total ..     | 10,29,493 | 180   | 10,29,673 |

TABLE 2.—*Showing dead rats examined for plague during 1955.*

| Month.          | Dead rats examined. | Found plague infected. |
|-----------------|---------------------|------------------------|
| January .. ..   | 39                  | Nil.                   |
| February .. ..  | 17                  | Nil.                   |
| March .. ..     | 18                  | Nil.                   |
| April .. ..     | 19                  | Nil.                   |
| May .. ..       | 14                  | Nil.                   |
| June .. ..      | 16                  | Nil.                   |
| July .. ..      | 8                   | Nil.                   |
| August .. ..    | ..                  | Nil.                   |
| September .. .. | 6                   | Nil.                   |
| October .. ..   | 1                   | Nil.                   |
| November .. ..  | 3                   | Nil.                   |
| December .. ..  | 1                   | Nil.                   |
| Total ..        | 142                 | Nil.                   |

In addition to dead rats, 333 live rats from plague suspected localities and 8,067 live rats from the Bombay Port Trust area were examined during the year. The total number of live and dead rats examined was 8,542.

TABLE 3.—Showing the number and percentage of different species of rats trapped alive in the City of Bombay (including the suburbs) during 1955.

| Species.                          | January |             | February |             | March   |             | April   |             | May     |             | June    |             |
|-----------------------------------|---------|-------------|----------|-------------|---------|-------------|---------|-------------|---------|-------------|---------|-------------|
|                                   | Number. | Percentage. | Number.  | Percentage. | Number. | Percentage. | Number. | Percentage. | Number. | Percentage. | Number. | Percentage. |
| <i>Rattus rattus rufescens</i> .. | 23,991  | 26.5        | 18,490   | 23.3        | 17,285  | 21.0        | 18,464  | 21.5        | 16,109  | 20.2        | 16,239  | 19.9        |
| <i>Rattus norvegicus</i> ..       | 16,409  | 18.1        | 14,890   | 18.7        | 13,789  | 16.7        | 15,655  | 18.2        | 14,196  | 17.8        | 14,384  | 17.6        |
| <i>Gunomys kok</i> ..             | 35,543  | 39.3        | 34,119   | 42.9        | 38,514  | 46.7        | 38,717  | 45.2        | 37,466  | 47.0        | 37,933  | 46.4        |
| <i>Bandicota malabarica</i> ..    | 943     | 1.0         | 708      | 0.9         | 633     | 0.8         | 472     | 0.6         | 616     | 0.7         | 734     | 0.9         |
| <i>Mus dubius</i> ..              | 4,571   | 5.1         | 3,477    | 4.4         | 3,340   | 4.0         | 3,422   | 4.0         | 3,049   | 3.8         | 2,992   | 3.7         |
| <i>Suncus caeruleus</i> ..        | 9,055   | 10.0        | 7,832    | 9.8         | 8,920   | 10.8        | 9,002   | 10.5        | 8,331   | 10.5        | 9,390   | 11.5        |
| Total ..                          | 90,512  | 100.0       | 79,516   | 100.0       | 82,481  | 100.0       | 85,712  | 100.0       | 79,767  | 100.0       | 81,672  | 100.0       |

| Species.                          | July    |             | August  |             | September |             | October |             | November |             | December |             | Whole year. |             |
|-----------------------------------|---------|-------------|---------|-------------|-----------|-------------|---------|-------------|----------|-------------|----------|-------------|-------------|-------------|
|                                   | Number. | Percentage. | Number. | Percentage. | Number.   | Percentage. | Number. | Percentage. | Number.  | Percentage. | Number.  | Percentage. | Number.     | Percentage. |
| <i>Rattus rattus rufescens</i> .. | 19,909  | 22.2        | 20,881  | 23.4        | 19,952    | 24.2        | 20,574  | 23.6        | 23,520   | 27.5        | 25,351   | 26.5        | 2,40,765    | 23.4        |
| <i>Rattus norvegicus</i> ..       | 15,626  | 17.4        | 16,482  | 18.5        | 14,975    | 18.2        | 14,633  | 16.8        | 13,482   | 15.8        | 15,634   | 16.3        | 1,80,135    | 17.5        |
| <i>Gunomys kok</i> ..             | 39,849  | 44.4        | 38,024  | 42.6        | 34,452    | 41.8        | 38,495  | 44.1        | 36,083   | 42.2        | 42,251   | 44.1        | 4,51,446    | 43.9        |
| <i>Bandicota malabarica</i> ..    | 816     | 0.9         | 636     | 0.7         | 425       | 0.5         | 497     | 0.6         | 452      | 0.5         | 619      | 0.6         | 7,551       | 0.7         |
| <i>Mus dubius</i> ..              | 3,573   | 4.0         | 3,627   | 4.1         | 3,946     | 4.8         | 3,897   | 4.5         | 4,398    | 5.1         | 4,417    | 4.6         | 44,709      | 4.3         |
| <i>Suncus caeruleus</i> ..        | 9,951   | 11.1        | 9,504   | 10.7        | 8,680     | 10.5        | 9,089   | 10.4        | 7,608    | 8.9         | 7,525    | 7.9         | 1,04,887    | 10.2        |
| Total ..                          | 89,724  | 100.0       | 89,154  | 100.0       | 82,430    | 100.0       | 87,185  | 100.0       | 85,543   | 100.0       | 95,797   | 100.0       | 10,29,493   | 100.0       |

TABLE 4.—Showing the number and percentage of different species of rats found dead in the City of Bombay  
(including the suburbs) during 1955.

| Species.                       | January |             | February |             | March   |             | April   |             | May     |             | June    |             |
|--------------------------------|---------|-------------|----------|-------------|---------|-------------|---------|-------------|---------|-------------|---------|-------------|
|                                | Number. | Percentage. | Number.  | Percentage. | Number. | Percentage. | Number. | Percentage. | Number. | Percentage. | Number. | Percentage. |
| <i>Rattus rattus rufescens</i> | 22      | 44.9        | 7        | 30.4        | 7       | 28.0        | 7       | 25.9        | 6       | 42.8        | 8       | 40.0        |
| <i>Rattus norvegicus</i>       | 3       | 6.2         | 4        | 17.4        | 1       | 4.0         | 3       | 11.1        | 4       | 28.6        | 2       | 10.0        |
| <i>Gunomys kok</i>             | 14      | 28.5        | 5        | 21.7        | 5       | 20.0        | 9       | 33.4        | 4       | 28.6        | 6       | 30.0        |
| <i>Bandicota malabarica</i>    | ..      | ..          | 1        | 4.4         | 1       | 4.0         | ..      | ..          | ..      | ..          | ..      | ..          |
| <i>Mus dubius</i>              | ..      | ..          | ..       | ..          | 4       | 16.0        | ..      | ..          | ..      | ..          | ..      | ..          |
| <i>Suncus cruleus</i>          | ..      | ..          | ..       | ..          | ..      | ..          | ..      | ..          | ..      | ..          | 4       | 20.0        |
| <i>Putrid</i>                  | 10      | 20.4        | 6        | 26.1        | 7       | 28.0        | 8       | 29.6        | ..      | ..          | ..      | ..          |
| Total                          | 49      | 100.0       | 23       | 100.0       | 25      | 100.0       | 27      | 100.0       | 14      | 100.0       | 20      | 100.0       |

  

| Species.                       | July    |             | August  |             | September |             | October |             | November |             | December |             | Whole year |             |
|--------------------------------|---------|-------------|---------|-------------|-----------|-------------|---------|-------------|----------|-------------|----------|-------------|------------|-------------|
|                                | Number. | Percentage. | Number. | Percentage. | Number.   | Percentage. | Number. | Percentage. | Number.  | Percentage. | Number.  | Percentage. | Number.    | Percentage. |
| <i>Rattus rattus rufescens</i> | 3       | 30.0        | ..      | ..          | 5         | 83.3        | ..      | ..          | 3        | 100.0       | 1        | 100.0       | 69         | 38.3        |
| <i>Rattus norvegicus</i>       | 2       | 20.0        | ..      | ..          | 1         | 16.7        | ..      | ..          | ..       | ..          | ..       | ..          | 20         | 11.1        |
| <i>Gunomys kok</i>             | 3       | 30.0        | ..      | ..          | ..        | ..          | ..      | ..          | ..       | ..          | ..       | ..          | 46         | 25.6        |
| <i>Bandicota malabarica</i>    | ..      | ..          | ..      | ..          | ..        | ..          | ..      | ..          | ..       | ..          | ..       | ..          | 2          | 1.1         |
| <i>Mus dubius</i>              | ..      | ..          | ..      | ..          | ..        | ..          | 1       | 50.0        | ..       | ..          | ..       | ..          | 5          | 2.8         |
| <i>Suncus cruleus</i>          | ..      | ..          | ..      | ..          | ..        | ..          | ..      | ..          | ..       | ..          | ..       | ..          | 4          | 2.2         |
| <i>Putrid</i>                  | 2       | 20.0        | ..      | ..          | ..        | ..          | 1       | 50.0        | ..       | ..          | ..       | ..          | 34         | 18.9        |
| Total                          | 10      | 100.0       | ..      | ..          | 6         | 100.0       | 2       | 100.0       | 3        | 100.0       | 1        | 100.0       | 180        | 100.0       |

# EXAMINATION OF RATS FROM THE BOMBAY PORT TRUST DOCKS.

These rats were received along with those of the other wards of the Bombay Municipality, and have been already accounted for in the monthly totals given in previous tables. A separate record of these rats, giving the number and percentage of the different species, is also maintained at the instance of the Public Health Commissioner.

TABLE 5.—Showing the number and percentage of different species of rats caught alive from the Bombay Port Trust area in 1955.

| Species.                          | January |             | February |             | March   |             | April   |             | May     |             | June    |             |
|-----------------------------------|---------|-------------|----------|-------------|---------|-------------|---------|-------------|---------|-------------|---------|-------------|
|                                   | Number. | Percentage. | Number.  | Percentage. | Number. | Percentage. | Number. | Percentage. | Number. | Percentage. | Number. | Percentage. |
| <i>Rattus rattus rufescens</i> .. | 355     | 51·8        | 311      | 58·3        | 274     | 50·6        | 337     | 54·3        | 222     | 37·2        | 317     | 47·1        |
| <i>Rattus norvegicus</i> ..       | 56      | 8·2         | 70       | 13·3        | 72      | 13·3        | 100     | 16·1        | 187     | 31·3        | 67      | 10·0        |
| <i>Gunomys kok</i> ..             | 182     | 26·5        | 51       | 9·5         | 70      | 12·9        | 78      | 12·6        | 94      | 15·7        | 185     | 27·5        |
| <i>Bandicota malabarica</i> ..    | 3       | 0·4         | 5        | 0·9         | 1       | 0·2         | 2       | 0·3         | 1       | 0·2         | 12      | 1·8         |
| <i>Mus dubius</i> ..              | 64      | 9·3         | 80       | 15·0        | 55      | 10·2        | 72      | 11·6        | 77      | 12·9        | 71      | 10·5        |
| <i>Suncus aeruleus</i> ..         | 26      | 3·8         | 16       | 3·0         | 69      | 12·8        | 32      | 5·1         | 16      | 2·7         | 21      | 3·1         |
| Total ..                          | 686     | 100·0       | 533      | 100·0       | 541     | 100·0       | 621     | 100·0       | 597     | 100·0       | 673     | 100·0       |

| Species.                          | July    |             | August  |             | September |             | October |             | November |             | December |             | Whole year |             |
|-----------------------------------|---------|-------------|---------|-------------|-----------|-------------|---------|-------------|----------|-------------|----------|-------------|------------|-------------|
|                                   | Number. | Percentage. | Number. | Percentage. | Number.   | Percentage. | Number. | Percentage. | Number.  | Percentage. | Number.  | Percentage. | Number.    | Percentage. |
| <i>Rattus rattus rufescens</i> .. | 578     | 61.9        | 366     | 50.7        | 394       | 48.3        | 353     | 42.5        | 326      | 46.4        | 299      | 35.6        | 4,132      | 48.6        |
| <i>Rattus norvegicus</i> ..       | 110     | 11.8        | 77      | 10.7        | 108       | 13.2        | 219     | 26.4        | 103      | 14.5        | 171      | 20.4        | 1,340      | 15.8        |
| <i>Gunomys kok</i> ..             | 125     | 13.4        | 167     | 23.2        | 181       | 22.2        | 160     | 19.3        | 154      | 21.9        | 223      | 26.5        | 1,670      | 19.7        |
| <i>Bandicota malabarica</i> ..    | 12      | 1.3         | 5       | 0.7         | 3         | 0.4         | 5       | 0.6         | 7        | 1.0         | 5        | 0.6         | 61         | 0.7         |
| <i>Mus dubius</i> ..              | 69      | 7.4         | 73      | 10.1        | 114       | 14.0        | 40      | 4.8         | 58       | 8.3         | 91       | 10.8        | 864        | 10.2        |
| <i>Suncus coruleus</i> ..         | 39      | 4.2         | 33      | 4.6         | 16        | 1.9         | 53      | 6.4         | 55       | 7.9         | 51       | 6.1         | 427        | 5.0         |
| Total ..                          | 933     | 100.0       | 721     | 100.0       | 816       | 100.0       | 830     | 100.0       | 703      | 100.0       | 840      | 100.0       | 8,494      | 100.0       |

TABLE 6.—*Showing the different localities in the Bombay Province from which smears, rats and fleas were received for examination during 1955.*

| Name of Town.  | No. of smears. | No. of rats | Fleas           |               |
|----------------|----------------|-------------|-----------------|---------------|
|                |                |             | No. of samples. | No. of fleas. |
| Ahmednagar ..  | 8              | 1           | 2               | 19            |
| Ambernath ..   | ..             | 5           | ..              | ..            |
| Baramati ..    | 4              | 1           | ..              | ..            |
| Dangs, Ahwa .. | 3              | ..          | ..              | ..            |
| Jath ..        | 2              | ..          | ..              | ..            |
| Karad ..       | 2              | ..          | ..              | ..            |
| Karwar ..      | 2              | ..          | ..              | ..            |
| Kolaba ..      | 5              | 1           | ..              | ..            |
| Panvel ..      | ..             | 7           | ..              | ..            |
| Satara ..      | 2              | ..          | ..              | ..            |
| Surat ..       | 2              | ..          | ..              | ..            |
| Thana ..       | ..             | 2           | ..              | ..            |
| Total          | 30             | 17          | 2               | 19            |

No plague infection was detected from the examination of smears, rats and fleas mentioned above.

TABLE 7.—*Bombay City rats supplied to different Institutions during the year 1955.*

| Name of the Institution.                       | No. of rats supplied. |
|------------------------------------------------|-----------------------|
| (1) Wilson College ..                          | 429                   |
| (2) Ramnarain Ruia College ..                  | 201                   |
| (3) Institute of Science ..                    | 251                   |
| (4) Jai Hind College ..                        | 180                   |
| (5) Ruparel College ..                         | 52                    |
| (6) Siddharth College ..                       | 28                    |
| (7) Seth G. S. Medical College ..              | 26                    |
| (8) Khalsa College ..                          | 18                    |
| (9) Prince of Wales Museum of Western India .. | 37                    |
| (10) Topiwala National Medical College ..      | 24                    |
| (11) D. E. Society's Bombay College ..         | 33                    |
| (12) Kishinchand Chellaram College ..          | 10                    |
| Total                                          | 1,289                 |



## VI. Department of Clinical Pathology and Diagnostic Reagents.

The Department of Clinical Pathology and Diagnostic Reagents continued to (1) conduct bacteriological, serological, microscopic and biological tests on clinical materials obtained from Government hospitals, and dispensaries as well as from private practitioners and institutions; (2) prepare, stock and issue diagnostic reagents and stains; (3) carry out histological examinations of research and biopsy material and report on pathological tissues; (4) prepare autogenous vaccines; (5) conduct special tests such as Rideal Walker Test, tests for pregnancy; (6) train science graduates and others for laboratory duties; (7) afford facilities for post-graduate research students; and (8) conduct research.

### A. GENERAL BACTERIOLOGICAL WORK.

Clinical material from various Government hospitals and dispensaries from the city of Bombay and outside stations as well as from private practitioners and institutions continued to be received for examination during the year under review. All essential tests for the diagnosis of infectious diseases such as diphtheria, cerebro-spinal fever, enteric group of fevers, typhus, plague, cholera, tuberculosis (pulmonary), etc., were carried out free of charge.

(i) *The Government of Bombay Typhoid Scheme* was continued for the eighth year and found very popular and useful. Calls from 731 private practitioners were attended to. As before, blood samples were collected for blood culture and triple Widal tests. Some practitioners required additional laboratory tests which were carried out for their convenience on payment. All cases positive for typhoid group were reported to the Executive Health Officer, Bombay Municipality, Bombay, to enable him to take necessary preventive measures at the earliest opportunity. From examination of 720 blood samples for culture and Widal 235 i.e. 33 per cent. were positive.

An analysis of records from this Section showed the following interesting points:—

(a) *Seasonal Incidence of Typhoid in Bombay.*—The largest number of calls were during the month of June to October with lower incidence in the months of January to March.

(b) *Results of blood culture and triple Widal test.*—Samples examined during 1953, 1954 and 1955 are tabulated to show the type and extent of typhoid infection amongst cases of fevers of unknown origin. Arthur Road Hospital discontinued sending samples for clot culture from April 1955.

| Year.   | Total Samples. | Hospital Samples. | Typhoid Scheme Samples. | Widal — Culture+ | Widal+ Culture* | Widal+ Culture+ | Total Cultures done. | B. typhosus isolated. | B. paratyphoid A isolated. |
|---------|----------------|-------------------|-------------------------|------------------|-----------------|-----------------|----------------------|-----------------------|----------------------------|
| 1953 .. | 3,416          | 2,651             | 765                     | 113              | 680             | 62              | 3,385                | 161                   | 46                         |
| 1954 .. | 3,503          | 2,776             | 727                     | 111              | 597             | 69              | 3,476                | 196                   | 52                         |
| 1955 .. | 2,798          | 2,078             | 720                     | 157              | 572             | 155             | 2,773                | 261                   | 70                         |

(ii) *Routine Examinations*.—The number of routine examinations carried out during the years 1953, 1954 and 1955 are shown in the following table for purposes of comparison. It will be observed that in majority of items there was an increase in the number of samples received during the year under review :—

| (I) <i>Serological Examinations</i> —                                                  |    | 1953. | 1954. | 1955. |
|----------------------------------------------------------------------------------------|----|-------|-------|-------|
| (1) Wassermann Test ..                                                                 | .. | 3,004 | 3,664 | 3,199 |
| (2) Kahn Test ..                                                                       | .. | 2,935 | 2,620 | 2,992 |
| (3) Triple Widal Test ..                                                               | .. | 2,568 | 2,415 | 2,630 |
| (4) Agglutination Test for—                                                            |    |       |       |       |
| (a) Typhus ..                                                                          | .. | 2,568 | 2,415 | 2,634 |
| (b) Brucellosis ..                                                                     | .. | 2,573 | 2,422 | 2,630 |
| (c) Leptospirosis ..                                                                   | .. | 144   | 58    | 66    |
| (d) Paul-Bunnell Test ..                                                               | .. | 9     | 9     | 9     |
| (II) <i>Bacteriological and Biological Tests</i> —                                     |    |       |       |       |
| (1) Blood culture for enteric group.                                                   |    | 3,385 | 3,476 | 2,780 |
| (2) Urine culture for pathogenic organisms.                                            |    | 123   | 78    | 112   |
| (3) Throat swabs for diphtheria .                                                      |    | 281   | 405   | 556   |
| (4) Stools and rectal swabs for culture.                                               |    | 1,680 | 65    | 6     |
| (5) Sputum and body fluid for A.F.B. .                                                 |    | 961   | 870   | 1,111 |
| (6) C. S. F. for culture ..                                                            | .. | 425   | 504   | 231   |
| (7) Material from suspected plague cases.                                              |    | Nil.  | Nil.  | 1     |
| (8) Material from Medico-Legal Cases.                                                  |    | 25    | 13    | 14    |
| (9) Blood Culture—Routine ..                                                           | .. | ..    | ..    | 53    |
| (III) <i>General Microscopic Examinations</i> —                                        |    |       |       |       |
| (1) Blood Films for M. P. and Filariasis.                                              |    | 25    | 27    | 47    |
| (2) Blood cell count ..                                                                | .. | 1,942 | 1,763 | 1,500 |
| (3) Bone Marrow ..                                                                     | .. | 26    | 8     | 15    |
| (4) Semen for fertility ..                                                             | .. | 43    | 83    | 79    |
| (5) Material for smear examination (Presence of pathogenic organisms like G. C. etc.). |    | 55    | 46    | 44    |
| (6) Stool—Routine Test ..                                                              | .. | 1,019 | 1,062 | 973   |
| (7) Urine—Routine Test ..                                                              | .. | 1,114 | 1,203 | 914   |
| (IV) <i>Pregnancy Test</i> ..                                                          | .. | 691   | 841   | 884   |
| (V) <i>Rideal Walker Test</i> ..                                                       | .. | 68    | 53    | 59    |

(iii) *Autogenous Vaccines*.—Use of autogenous Vaccines is still in vogue but to a lesser extent amongst the practitioners in Bombay and outstations. Cases of chronic inflammatory conditions and allergic states such as sinusitis, follicular pharyngitis, gingivitis bronchial asthma, etc., etc., applied for such vaccine. The number of courses issued during 1955 were 46 as against 28 and 35 in the years 1953 and 1954 respectively.

## B. DIAGNOSTIC REAGENTS.

(i) *Supply of diagnostic reagents*.—The work in connection with the production, stocking and supply of various reagents for diagnostic purposes to laboratories all over the State of Bombay and outside the State was continued. The demands for these reagents are increasing in most commonly used items as may be seen from the following annual supplies for the last three years :—

| <i>Reagents—</i>                                      | Quantity supplied in ml. |         |         |
|-------------------------------------------------------|--------------------------|---------|---------|
|                                                       | 1953.                    | 1954.   | 1955.   |
| (1) Killed suspension for Widal and Weil-Felix Tests. | 164,895                  | 160,215 | 162,115 |
| (2) Blood grouping sera ..                            | 7,776                    | 8,751   | 10,638  |
| (3) High Titre Sera and Amboceptor ..                 | 686                      | 676     | 768     |
| (4) Kahn Antigen .. ..                                | 4,932                    | 4,980   | 7,000   |
| (5) W. R. Antigen .. ..                               | 180                      | 380     | 420     |
| (6) Russel's Viper and Cobra Venom Solutions.         | 1,436                    | 2,545   | 2,229   |

(ii) *Supply of Stains*.—In addition to the above diagnostic reagents, the supply of various biological stains was also continued. The demand for these stains had considerably increased as is evident by the supply of 478,030 mls. this year as against a total of 161,555 mls. last year.

(iii) *Collection of Standard Strains*.—Collection and maintenance of standard strains of different pathogenic organisms, is continued. A number of demands for such strains, for demonstration and manufacturing purposes from various teaching and pharmaceutical institutions were met with during the year.

## C. HISTOPATHOLOGY SECTION.

*Pathological Specimens*.—During the year under review, 2,388 pathological specimens received from different hospitals in the State of Bombay and a few from outside the State were examined and reported upon. These included brain tissues from suspected rabid animals, neoplasms, gynæcological conditions and some rare and interesting pathological specimens. In addition, 110 research specimens from experimental animals were processed. These were mostly from different departments of the Institute as well as different research enquiries conducted at the Institute. In all 2,498 specimens were subjected to histological examination.

The classification of the specimens of tissues received from hospitals in the city of Bombay and outstations during the years 1953, 1954 and 1955 is tabulated below :—

|                                                        | 1953. | 1954. | 1955. |
|--------------------------------------------------------|-------|-------|-------|
| <i>Classification :—</i>                               |       |       |       |
| (1) Number of samples found to be normal.              | 265   | 185   | 241   |
| (2) Number of samples unfit for examination.           | 157   | 140   | 104   |
| (3) Brain samples for Negri bodies ..                  | 83    | 64    | 52    |
| (4) Samples for miscellaneous pathological conditions. | 1,172 | 1,366 | 1,563 |
| (5) Sample for tuberculosis infection ..               | 138   | 95    | 143   |
| (6) Nonmalignant neoplasms ..                          | 95    | 125   | 144   |
| (7) Malignant tumours .. ..                            | 146   | 158   | 141   |
| Total                                                  | 2,056 | 2,133 | 2,388 |

It will be seen that there has been a steady and marked increase in the number of samples received in the department of histological examination.

Some of the important and interesting pathological conditions encountered are grouped and tabulated below :—

#### I. NEOPLASMS.

##### (a) *Malignant :—*

|                            |    |    |    |
|----------------------------|----|----|----|
| (1) Carcinoma Primary .. . | .. | .. | 83 |
| (2) Carcinoma Secondary .. | .. | .. | 14 |
| (3) Sarcoma .. ..          | .. | .. | 25 |
| (4) Sarcoma Secondary ..   | .. | .. | 1  |
| (5) Malignant Lymphoma ..  | .. | .. | 6  |
| (6) Retinoblastoma .. ..   | .. | .. | 1  |
| (7) Seminoma .. ..         | .. | .. | 7  |
| (8) Teratoma .. ..         | .. | .. | 4  |

|                                         |    |    |     |
|-----------------------------------------|----|----|-----|
| (b) <i>Nonmalignant Neoplasms</i> .. .. | .. | .. | 144 |
|-----------------------------------------|----|----|-----|

|                                         |    |    |     |
|-----------------------------------------|----|----|-----|
| II. TUBERCULOSIS IN DIFFERENT ORGANS .. | .. | .. | 143 |
|-----------------------------------------|----|----|-----|

#### D. SPECIAL INVESTIGATIONS.

(i) *Inter Laboratory Evaluation of Serologic Tests for Syphilis.*—This department continued to co-operate in this Enquiry of the Indian Council of Medical Research. The Enquiry was discontinued from the 1st April 1955 and during this period, 191 samples of sera were received in 12 batches. As before, each batch of sera was subjected to quantitative and qualitative Wassermann, Kahn and V. D. R. L. tests,

Analytical report on the correlation of various tests with clinical findings is awaited.

(ii) *Hook-worm Survey in Kaira District.*—A laboratory unit sent under the guidance of Dr. B. G. Modi and Dr. B. D. Wani concluded the survey of Hook-Worm infestation in Kaira District. This investigation was taken in response to report sent by local practitioners who suspected about 34 per cent. incidence of Hook-worm disease in various villages of Kaira District.

In all, 10,699 samples of stool were examined from the whole district and 3,447 showed Hook-Worm ova, thus giving an overall incidence of Hook-Worm infestation in Kaira District of 35 per cent.

The infestation was prevalent in all talukas although the degree of infection varied from taluka to taluka. Balasinor taluka showed the least percentage of infection, viz., 13 per cent., while Nadiad and Cambay showed the highest percentage, i.e. 50 per cent.

Blood samples of 2,340 cases out of 3,447 positive cases were examined for incidence of anæmia. Four hundred and twenty-five cases showed evidence of severe anæmia, i.e. Hæmoglobin was less than 9 gm. per cent.

Certain signs and symptoms considered to be pathognomonic of this infection were also studied in positive cases. They were spots on the sclera, pigmentation of knuckles, tenderness over duodenal area, tendency to flattening of nails, dislike for milk, neurotic symptoms, etc. 88 per cent. of positive cases showed one or more of these signs.

## VII. Department of Pharmacology.

In 1955, the Institute accepted the responsibility of testing all medicinal preparations sent by the Department of Prohibition and Excise. This additional burden which the Department has shouldered was reflected in the sharp rise of the total number of samples reported during the year under review. In 1955, 4,055 samples were reported as against 2,361 during the preceding year. In spite of the heavy pressure of the routine work, the Department continued to investigate problems of interest.

*Research Work.*—One of the problems which has been taken in hand about a couple of years back was to prepare a hydrolysate from horse fibrin and to find out its suitability for intra-venous medication in human beings. The nutritive value of fibrin hydrolysate and its non-antigenic character have already been reported. The clinical trial of this product was undertaken at the K. E. M. Hospital in co-operation with Dr. P. Raghavan, M.D., Visiting Physician to the hospital. Fibrin hydrolysate proved to be non-toxic and seemed to be quite effective in raising the serum protein values to the normal level.

A method for detection and determination of Saccharin in "Prepared Tea" has already been reported previously. This method has now been employed for determination of Saccharin in pharmaceutical preparations. In some cases the method had to be modified to exclude the possible interference caused by the presence of preservatives like p-hydroxybenzoic acid and flavouring agent like vanillin.

*Wagatia spicata* Dalzell (Vakari, Vagati) is a robust woody climber growing abundantly in Bombay State. The root of this plant was investigated chemically and pharmacologically. From it, a crystalline substance which had a yield of nearly 2 per cent. and an amorphous substance (obtained in small quantity) were isolated. The crystalline substance called X has been investigated for some of its pharmacological and toxicological properties. This is being reported elsewhere. The work is being continued.

It has been reported previously that tinctures prepared from bulbs of *Urginea scilla* and *Scilla indica* grown in India were in no way inferior to the tinctures prepared from *Urginea scilla* from the Mediterranean coast (imported). *Scilla indica* grows abundantly in Bombay State. Samples of the bulb of *Scilla indica* were collected from different parts of the country and were examined biologically. None of the samples contained cardiac glycosides.

The abstracts of the research work are incorporated in the Part II of the Report.

*Analytical Work.*—The number of samples tested during the year under review showed an increase to the extent of about 72 per cent. over the preceding year. The table below shows the figures of the last three consecutive years :—

|                          | 1953. | 1954. | 1955. |
|--------------------------|-------|-------|-------|
| No. of samples tested .. | 1,909 | 2,361 | 4,055 |

Under the Drugs Act, 1642 samples were tested. These were received from the different parts of India. Samples were tested for the different Government (Bombay) Departments and commercial firms. These are shown in the following table :—

| Samples received under the Drugs Act |        |              |                |        |                   |                 | Samples received from     |                   |        |
|--------------------------------------|--------|--------------|----------------|--------|-------------------|-----------------|---------------------------|-------------------|--------|
| Bombay.                              | Delhi. | East Punjab. | Uttar Pradesh. | Bihar. | Himachal Pradesh. | Madhya Pradesh. | Government. Departments.* | Commercial firms. | Total. |
| 1,321                                | 133    | 16           | 120            | 15     | 1                 | 36              | 2,095                     | 318               | 4,055  |

\*Include those of Haffkine Institute.

The bulk of the samples that were received from the Department of Prohibition and Excise was spirituous medicinal preparations. Majority of these tinctures were nothing but coloured alcohol mixed in some cases with flavouring agents. Lack of adequate pharmacopoeial standard prescribed for certain tinctures, is being taken advantage of by some unscrupulous persons to market non-standard or spurious preparations in the name of genuine drugs. The Institute is quite alive to this situation and has devised special tests to find out the real nature of these preparations in most cases.

About 42 per cent. of the drugs, out of the total of 4,055 was of non-standard quality. It showed an increase of 6 per cent. over the figure of the last year. This has been attributed to the increase in the category of tinctures of substandard quality.

Out of the 1,642 samples reported under the Drugs Act, 739 were found to be not of standard quality, the percentage being 45. In 1954, about 44 per cent. of the drugs failed to comply with the required standard.

The number of samples tested under "Biological and special products" and "Other special products" showed a slight rise. The figures for 1954 and 1955 were 1,123 and 1,218 respectively. The quality of these products showed no improvement as 67 per cent. of them were of standard quality as compared to 76 per cent. reported in 1954.

Many commercial firms situated far and wide had their products tested at this Institute. Seventy-two per cent. of these drugs were under the category mentioned above as "Biological and special products" and "other special products". These samples were rather complex in nature and took a great deal of our time and attention.

It would be out of place to mention the detailed reasons for failure of various samples to comply with the required standards as they are too numerous. A brief review of the selected cases is given below:—

#### *Examination of Drugs by Chemical Methods.*

Two thousand one hundred and twenty samples of "Spirituos" preparations were tested. They included tinctures like Alstonia, Benzoin Co., Myrrh et Boracis, Rauwolfia, Tolutana, Saussurea, Zingiberis, Carminative, Cubebæ, Buchu, etc., extracts like Taraxaci, Kolæ, etc., concentrated waters like Anisi, chloroform, Anethi, etc. spirits like Cajuputi, Aetheris Nitrosi, Juniperi, etc., and essences like Raspberry, Vanilla, etc.

A very large number of the drugs (mentioned above) which were declared to be of non-standard quality appeared to be simple alcoholic (ethyl) preparations containing lower percentage of extractives and to which a little color or flavouring essence were added. In some cases sugars and glycerine were added, to make them palatable or to increase the bulk.

Tr. Myrrh et Boracis contains Myrrh, Borax, Glycerine, Krameria and essential oils. In 53 samples out of 58, one or two ingredients were missing. Fifty-two samples of spiritus cajuputi, out of 61 examined, did not give characteristic odour of the essential oil, in fact, they did not contain any. About 90 per cent. of the samples of Tr. Benzoin failed to show the presence of gum benzoin. In the case of Tr. Benzoin Co., the content of total Balsamic acids was low in as many as 92 per cent. of the samples. Not less than 159 samples of Tr. Senegæ were examined out of which only a small percentage showed the presence of hæmolytic saponins, the active principle. Out of the 42 samples of Tr. Rauwolfia only two of them did not show the presence of alkaloids.

Out of 84 samples of Distilled Water for parenteral administration, 9 were found to be of non-standard quality, most of which contained suspended matter and others failed to comply with the limit for Ammonia or Oxidisable matter. Only 3 samples of Glucose Solutions (injectable), out of 82, failed in respect of clarity. Out of the 15 samples of Normal Saline, 2 were rejected on the ground that one of them contained more Sodium Chloride than the prescribed limit while the other had less amount.

Several samples of Dextrose failed to comply with the B. P. tests for limit of less soluble sugars and dextrans and that of sulphate.

From among the 34 samples which were classified as "Gripe Mixture", 21 were found to be of standard quality. The remaining 13 of them failed to comply with the stated amount of Sodium Bicarbonate. Some of them contained Saccharin in place of sugars.

The instability of Ergot alkaloids in liquid extracts has again been observed as it was pointed out in the last report. Out of 9 samples, 1 did not contain any alkaloids and others contained less than the B. P. C. requirements.

In some cases the quality of Mercurochrome used for the preparation of Mercurochrome Solutions, was found to be inferior. The presence of free mercury and bromide ions could be detected in the drug.

Twenty-one out of 36 samples of Acetylsalicylic Acid, did not comply with the Pharmacopœial test for the limit of free Salicylic Acid. The storage of such preparations seems to be a problem.

A sample of Iodoform was found to contain only a small proportion of the pure substance, the rest of it being some inert filling materials.

Five samples of Sandal Wood Oil out of 15, did not conform to B. P. C. specifications for optical rotation, refractive index and weight per ml. at 20°C.

Out of 7 samples of Calcium Lactate, 3 failed to comply with the B. P. C. test for the limit of alkalinity. More than half the samples of surgical dressings failed to comply with the B. P. C. requirements. Four samples of Absorbent Cotton Wool could not satisfy the B. P. C. standards for absorbency, water soluble extractive and ash. One sample claimed to be sterile, was grossly contaminated.

All the samples of Absorbent Lint were declared as non-standard quality for either they failed to conform to B. P. C. specifications for absorbency and superficial area per ounce or their nap was raised from weft yarns and not from warp yarns.

Four samples of Absorbent Gauze or Bandage out of 11, were not of required standard.

Several samples which were claimed to be "Substitute for Olive Oil" were tested. They were, in fact, Arachis Oil and not Olive Oil.

Out of the 3 samples of Cocaine Hydrochloride, 2 contained mostly Calcium Carbonate and the remaining one was Procaine Hydrochloride instead.



*Examination of Drugs by Biochemical and Microbiological Methods.*

A large number of multivitamin preparations were examined. Testing of these preparations very often presented difficult problems as most of them were complex in nature and contained various ingredients of diverse character. The following vitamins were generally encountered. Vitamin A, Aneurine hydrochloride, Riboflavine, Ascorbic acid, Folic acid, Nicotinic acid, and its amide, Pantothenic acid and its Calcium and Sodium salts; Vitamin B<sub>12</sub> etc.

Several enzymatic preparations and amino acids were also analysed.

Out of the 560 vitamin preparations examined, it was observed that 53 per cent. of them failed to conform to the claim made on the label. Six samples showed heavy deposits which could not be brought into solution in spite of vigorous shaking. Two of the injectible preparations had to be rejected as the pH of the solutions did not comply with the official specifications.

Microbiological technique was followed whenever colorimetric and fluorimetric methods were not applicable. It was mostly used in cases of Riboflavin, Nicotinic acid and its amide, Pantothenic acid and its salts, Pteroylglutamic acid and Vitamin B<sub>12</sub>. Most of the antibiotics were assayed microbiologically but they are reviewed under "Bacteriological examination of drugs."

One hundred and twenty preparations containing Vitamin A were analysed. These included Cod Liver Oil, Shark Liver Oil, Vitamin A concentrates and a few samples of water soluble Vitamin A. In 45 samples, the vitamin content did not agree with the declared potency; in most of them it was lower.

Vitamin B<sub>1</sub> when dispensed in a liquid preparation is one of the most unstable vitamins known. Out of the 339 samples examined 103 were not of standard quality. In most cases the vitamin content was as low as 50 per cent. of the declared potency and in some it was even less.

Vitamin B<sub>2</sub> is very stable and does not deteriorate in potency easily. Among 177 preparations examined for B<sub>2</sub> content, only 25 did not comply with the required strength.

Out of 134 samples of Vitamin C, 37 were found not to conform to the declared potency. Most of them had deteriorated in potency.

In nearly 57 per cent. of the samples containing Folic acid, the amount found did not comply with the stated amount. In oral multivitamin preparations, in which this vitamin was frequently dispensed it did not seem to preserve its potency for a long time.

Niacin seemed to be the most stable substance encountered in our routine examination. Out of the 213 preparations containing this vitamin, only 7 showed lower strength.

It is noteworthy to mention that all the 20 samples of Methionine were of standard quality and strength.

The proteolytic activity of Pepsin was determined in 5 samples wherein it was dispensed along with vitamins. Only in 1 sample proteolytic activity was low.

As many as 148 samples of Pantothenic acid (or its salts) were examined, out of which 66 did not conform to the declared amounts.

About 60 per cent. of the samples tested for B<sub>12</sub> activity showed full potency but most of the remaining ones had lower strength. Some samples showed higher potency than the declared amount and in these excess amounts of the vitamin were added to counteract the loss of potency.

Ten samples of Insulin were biologically standardised. Only 1 failed to pass. It contained less than 10 per cent. of Insulin.

#### *Examination of Drugs by Pharmacological Methods.*

Biological standardisation of Inj. Liver Ext. for its histamine content has become a routine procedure. Several manufacturing firms had their products tested regularly at this Institute. The amounts of histamine or histamine-like substances present in the 24 samples examined here, did not exceed 30/ug/ml. This was well within the limits of safety.

Out of the 49 samples of adrenaline tested, 11 were of non-standard quality. Most of them had turned pink but there were two products which were perfectly colourless. It was noticed that samples of adrenaline meant for parenteral injection were sometimes not properly labelled. The difference between the two preparations of the British Pharmacopœia (1948) viz., "Inj. Adrenaline" and "Liq. Adrenaline Hydrochloride" was not properly appreciated. Liq. Adrenaline Hydrochloride was wrongly dispensed for injectible purposes.

The rat uterus technique as well as guinea-pig uterus method continued to be used for biological assay of pituitary. Out of the 10 samples examined, 2 failed to comply with the required standard. One sample of Vasopressin was tested on cat's blood-pressure and proved to be of substandard quality.

In all, 11 samples of Digitalis were assayed, out of which a few did not show any glycosidal activity. Several samples were received from commercial firms for standardisation purposes. Out of 8 samples of Tr. Scillæ 6 did not show the presence of the active principles.

Saponin is the active constituent of Senegæ B. P. It has the property of hæmolysing red cells of the blood. This was taken advantage of for detecting the presence of saponins in Tr. Senegæ B. P. As mentioned previously a large number of these tinctures failed to satisfy this test.

A very large number of injectibles (Distilled Water, Glucose Saline, Protein Hydrolysate, Calcium gluconate, etc.), was examined for the absence of pyrogenic substances. As compared with the previous year, the year under review showed about 10 per cent. rise in the number of samples (401) tested. Only 3 samples produced abnormal rise in temperature in the rabbit. One of them was contaminated.

Seventy-seven samples were tested for the absence of undue toxicity. Mice, rats and rabbits were mainly used. The samples tested were synthetic organic compounds, antibiotics like Procaine Penicillin, Streptomycin, Phenathemate hydriodide, etc.

*Examination of Drugs by Bacteriological and Serological Methods.*

Among the 110 samples of antibiotics tested only 6 [Penicillin G (crystalline), 3; Penicillin ointment, 2; Chloramphenicol, 1] failed to comply with the required standards. There were 47 samples of Penicillin G (crystalline), 30 of Procaine-Penicillin, 14 of Streptomycin, 6 of Penicillin and Streptomycin, 10 of Chloramphenicol, 4 of Chlorotetracyclin, and 1 of Oxytetracyclin (ointment). Out of the 47 samples of Penicillin, 22 were ointments. Only 2 of the latter preparations were of substandard quality. Both microbiological and chemical methods were used wherever possible. In cases of Chloramphenicol, Chlorotetracyclin and Oxytetracyclin chemical methods were used.

Six samples of Anti-toxin (Diphtheria, Tetanus) were biologically assayed, only 1 of them (Tetanus) was of sub-standard quality.

Two hundred seventy-six samples were tested for their sterility. About 98 per cent. of them proved to be sterile.

### VIII. Department of Chemotherapy.

The manufacturing activities of the department showed a considerable rise during the period under review, which is reflected in the sale value of the products, amounting to Rs. 5,81,716 as against the previous year's figure of Rs. 2,85,363. In addition to all the products manufactured in the previous years, several new ones were introduced, *viz.*, sulphaguanidine, sulphadiazine and quinine sulphate tablets as well as multivitamin tablets. These new products were taken up primarily for the Employees State Insurance Scheme but with the availability of additional facilities such as extra staff, some addition to equipment etc., for further increase in the production, these products could also be made available to State and Municipal hospitals and other charitable institutions. With the increase in the output it is also possible to bring down the prices of the various products and a proposal to this effect has already been forwarded to the Government for their approval.

In the matter of the manufacture of Sulphathizole starting from basic raw materials, which has been continued for the past several years on the pilot plant, a detailed analysis of the various factors affecting the cost of production has clearly indicated that no further reduction could be effected in the cost of production unless (1) the output is stepped up from the present pilot-scale to full scale production (2) Various sulfa drugs taken up together (3) the anomaly in the matter of import duty on raw materials as against finished products is remedied. This matter has been already discussed in the previous annual reports but these problems have yet to be resolved.

In addition to the manufacturing activities, investigations into the possibility of manufacturing other important synthetic drugs were continued. In particular, various methods for the manufacture of Iso-nicotinic hydrazide, DDS and its derivatives, and P-A-S were studied to work out the most suitable method for their economic production. Similar studies have also been started in the matter of Chloromycetin. Thus the department has been able to get

valuable data and know-how on the large scale production of the sulpha-drugs such as Sulphathiazole, sulphaguanidine, sulphadiazine; antimalarials like paludrine, chloroquin; anti-tubercular agents like P-A-S, Iso-nicotinic acid hydrazide and anti-leprosy drugs like DDS and derivatives which would be of great value whenever it is proposed to take up the large scale manufacture of these important drugs.

In addition to the above-mentioned normal activities, the department also undertook special problems, such as recovery of sulphanilamide from spoilt tablets on behalf of Armed forces medical stores depot, and conversion of sub-standard pink quinine sulphate to high purity Quinine Sulphate B. P. About 1,000,000 sulphanilamide tablets which had deteriorated and discoloured were treated by the department and the sulphanilamide B. P. obtained therefrom was returned to the Armed forces medical stores. Similarly the large quantity of pink-coloured sub-standard quinine sulphate which is in the custody of the Government of Bombay and which could not be disposed of in the existing condition could now be processed and thus better price for this stock could be obtained. These two instances would indicate how the department could be of immense service in tackling any other problems in connection with salvage of drugs and chemicals, and thus conserving Government goods which otherwise would either be lost or disposed of at very insignificant value.

*Table indicating the Sales of the various products manufactured by the Department of Chemotherapy during the years 1953, 1954 and 1955.*

| Serial No.                     | Kind of Product.                  | Number of tablets sold during the period |             |             |
|--------------------------------|-----------------------------------|------------------------------------------|-------------|-------------|
|                                |                                   | 1953.                                    | 1954.       | 1955.       |
| 1                              | Vitamin A-D tablets ..            | 32,91,150                                | 37,37,475   | 50,72,618   |
| 2                              | Vitamin A-D Special tablets.      | 1,32,000                                 | 88,000      | 1,25,000    |
| 3                              | Vitamin A Special tablets.        | 6,000                                    | 1,000       | 2,000       |
| 4                              | Vitamin B-C tablets ..            | 50,95,350                                | 54,71,100   | 74,71,755   |
| 5                              | Vitamin B-C No. 3 tablets.        | 4,82,250                                 | 8,68,050    | 9,64,475    |
| 6                              | Vitamin B <sub>1</sub> tablets .. | 1,47,150                                 | 2,06,950    | 4,61,760    |
| 7                              | Vitamin B <sub>2</sub> tablets .. | 31,450                                   | 65,300      | 1,22,100    |
| 8                              | Vitamin C tablets ..              | 2,14,900                                 | 3,33,450    | 5,67,560    |
| 9                              | Vitamin B-C Special tablets.      | Nil.                                     | Nil.        | Nil.        |
| 10                             | Acid Nicotinic tablets ..         | 97,700                                   | 1,49,400    | 2,66,200    |
| 11                             | Vitamin C Special tablets.        | 2,000                                    | Nil.        | Nil.        |
| 12                             | Multi-vitamin tablets ..          | Nil.                                     | Nil.        | 7,21,980    |
| Total sale of Vitamin tablets. |                                   | 94,99,950                                | 1,09,20,725 | 1,57,75,448 |

| Serial No. | Kind of Product.                                  | Number of tablets sold during the period |               |               |
|------------|---------------------------------------------------|------------------------------------------|---------------|---------------|
|            |                                                   | 1953.                                    | 1954.         | 1955.         |
| 13         | Sulphathiazole tablets ..                         | 35,20,600                                | 19,99,550     | 23,55,825     |
| 14         | Sulphadiazine tablets ..                          | Nil.                                     | Nil.          | 18,41,500     |
| 15         | Sulphaguanidine tablets.                          | Nil.                                     | Nil.          | 5,44,000      |
| 16         | Quinine Sulphate tablets.                         | Nil.                                     | Nil.          | 2,83,300      |
|            | Total sale of above ..                            | 35,20,600                                | 19,99,550     | 50,24,625     |
|            | Total sale of all kinds of tablets.               | 1,30,20,550                              | 1,29,20,275   | 2,08,00,073   |
| 17         | Femon ..                                          | 4,778 lbs.                               | 6,000 lbs.    | 7,754 lbs.    |
| 18         | Sulphathiazole Injectable                         | 19,500 ccs.                              | 9,300 ccs.    | 12,720 ccs.   |
| 19*        | Sulphatrone Injectable .                          | 1,35,650 ccs.                            | 1,34,250 ccs. | 1,27,300 ccs. |
|            | Total sale value of all Products mentioned above. | Rs. 3,00,117 7 0                         | 2,85,362 4 0  | 5,81,724 8 9  |

\*Free Supply.

### IX. Department of Biochemistry.

During the year under review the department continued its normal activities—clinical materials for analyses were received from Government and private hospitals and City practitioners as also a few occasional samples from district hospitals. The total number of analyses carried out on the samples, including some special tests on the patients, numbered 5,543. The accompanying table gives a comparative idea of the tests carried out during 1955 as against the previous five years:—

|                             | 1950  | 1951  | 1952  | 1953  | 1954  | 1955  |
|-----------------------------|-------|-------|-------|-------|-------|-------|
| Blood .. ..                 | 1,684 | 2,170 | 2,637 | 3,752 | 3,468 | 3,264 |
| Urine .. ..                 | 194   | 179   | 149   | 280   | 241   | 234   |
| C. S. F. and Body fluids .. | 638   | 1,236 | 1,764 | 1,324 | 1,165 | 856   |
| Stool Samples ..            | 6     | 3     | 9     | 14    | 9     | 2     |
| Milk .. ..                  | 92    | 471   | 1,596 | 1,318 | 674   | 734   |
| Special Tests ..            | 148   | 207   | 397   | 470   | 346   | 453   |
| Total ..                    | 2,762 | 4,266 | 6,552 | 7,158 | 5,903 | 5,543 |

It should be pointed out that due to lack of care in collecting and despatching, quite a number of samples are either unfit or insufficient for analysis. There has been an increased request for blood protein fractions, sugar, urea and cholesterol.

The Acworth Leprosy Home, Matunga, referred some samples of blood for the estimation "Diasone" as it was suspected that the indigenous drugs tried on the patients might have been mixed with the synthetic drug "Diasone". However, none of the samples gave the test for diamino diphenyl sulphone.

The seamen's medical examination board continued to refer some seamen with glycosuric symptoms for glucose tolerance test. 83 of them were examined at the special concessional rate allowed by the Government. With our limited staff, it has not been possible to examine more cases.

Testing of milk samples brought by the public is still being carried on, although it is felt that it is not part of the duty of this department to dissipate its interest in this line. As a general rule only unboiled milk samples are tested for their fat content and total solids and the department is not in a position to help the public in undertaking the testing of all milk products collected by the Municipality. In this connection we are sometimes summoned by the Court to give evidence on behalf of the defence.

The department also supplies some standard solutions and reagents to outside hospitals whenever requested.

#### *Research Problems :—*

(1) The study of serum protein fractions with particular reference to 'true' albumin, alpha, beta, and gamma globulins by fractionation with different concentrations of sodium sulphate, is being continued.

(2) A comparison of 'true' albumin and globulin fractions by salt fractionation and paper electrophoresis has been taken up.

(3) A study of the blood glycogen levels in normal donors from the blood bank department is under progress.

(4) Data is being collected on the blood amylase activity of normal blood donors and seamen with glycosuria referred to for G. T. T. at the Institute.

#### **X. Department of Virus Diseases including Rabies.**

The Department continued to (1) afford anti-rabic treatment to patients in Northern part of the island of Bombay, (2) prepare antirabic vaccine for issue to anti-rabic treatment centres, (3) assist in the diagnosis of rabies in animals, (4) hold stocks and conduct Yellow Fever Inoculations with the assistance of Yellow Fever Diagnostic Unit sanctioned by the Government of India, (5) conduct research in specific virus diseases like influenza, Q fever, rabies and small-pox.

(a) *Anti-rabic treatment centre at this Institute.*—During the year under report 9,581 persons applied for anti-rabic treatment; of these 8,331 completed the full course of treatment and 1,181 were 'advice cases'. In cases where there was a doubt about the condition of the dog inflicting the bite and the dog was available for observation, patients were given three injections and were advised to keep the dog under observation for 10 days. At the end of the period of observation, if the dog was healthy, no further treatment was given and the case was classified as 'advice case'. The number of patients who applied for and submitted to treatment at the Haffkine Institute Centre, present interesting data, which is shown below in Table I.

TABLE I.—*Details of treatment carried out at the treatment centre of this Institute since 1951-1955.*

| Details.                                                          | 1951. | 1952. | 1953. | 1954. | 1955. |
|-------------------------------------------------------------------|-------|-------|-------|-------|-------|
| Number of persons treated ..                                      | 6,489 | 7,779 | 8,714 | 9,051 | 9,581 |
| Advice cases .. ..                                                | 624   | 687   | 985   | 473   | 1,181 |
| Fully treated .. ..                                               | 5,865 | 7,092 | 7,722 | 8,577 | 8,331 |
| Discontinued Treatment ..                                         | Nil.  | Nil.  | 7     | 1     | 69    |
| Cases of hydrophobia among those treated at the Institute Centre. | Nil.  | Nil.  | 1     | Nil.  | Nil.  |

(b) *Preparation and supply of Anti-rabic vaccine.*—Five per cent. phenolised sheep brain vaccine (Semple) was prepared as usual and issued to various Anti-rabic Centres recognised by the Institute for supply within and outside the Bombay State. Total number of such centres increased from 163 to 176 during the year out of which 79 were in Bombay State. The vaccine was also supplied to the Veterinary Surgeons within the State and outside for treatment of animals. 12612 ml. of 20 per cent. canine vaccine was prepared and supplied for prophylactic use in dogs.

The production and supply of vaccine in ml. as well as in the form of treatments, the number of treatment centres, the number of animals used for production have all gone up considerably during the last five years as seen from the following Tables II and IV. The production and supply of Anti-rabic vaccine in millilitres month by month is shown in Table III.

TABLE II.—*Production of Carbolicised Anti-rabic vaccine and canine vaccine in ml. from 1951 to 1955.*

| Details.                     | 1951.     | 1952.     | 1953.     | 1954.     | 1955.     |
|------------------------------|-----------|-----------|-----------|-----------|-----------|
| Anti-rabic vaccine in ml. .. | 19,40,215 | 23,39,740 | 26,10,160 | 27,58,160 | 24,54,695 |
| Canine vaccine in ml. ..     | 1,475     | 3,470     | 6,185     | 9,760     | 12,612    |

TABLE III.—*Production of Anti-rabic vaccine and supplies in ml. during 1955 month by month.*

| Month.    |       | No. of ml. vaccine<br>manufactured. | No. of ml. vaccine<br>issued.                      |
|-----------|-------|-------------------------------------|----------------------------------------------------|
| January   | .. .. | 1,57,153                            | 2,40,126                                           |
| February  | .. .. | 2,64,311                            | 2,11,822                                           |
| March     | .. .. | 2,83,339                            | 2,41,359                                           |
| April     | .. .. | 2,43,631                            | 2,41,544                                           |
| May       | .. .. | 2,44,936                            | 2,22,598                                           |
| June      | .. .. | 2,41,981                            | 2,04,123                                           |
| July      | .. .. | 2,32,920                            | 1,91,769                                           |
| August    | .. .. | 1,04,407                            | 1,72,281                                           |
| September | .. .. | 1,29,644                            | 1,70,863                                           |
| October   | .. .. | 1,45,113                            | 1,62,954                                           |
| November  | .. .. | 1,87,297                            | 2,09,957                                           |
| December  | .. .. | 2,19,963                            | 2,31,204                                           |
| Total     |       | 24,54,695                           | 25,00,600<br>(includes balance<br>from last year). |

TABLE IV.—*Details showing the number of treatments sent to all centres and Veterinary Surgeons, reported cases of Hydrophobia from Centres, number of Sheep used for production of Anti-rabic Vaccine and number of treatment Centres.*

| Details.                                                | 1951.  | 1952.  | 1953.  | 1954.  | 1955.  |
|---------------------------------------------------------|--------|--------|--------|--------|--------|
| No. of human treatments                                 | 13,432 | 17,604 | 19,230 | 21,555 | 27,340 |
| No. of veterinary treatments                            | 646    | 827    | 804    | 804    | 1,244  |
| Reported cases of hydrophobia                           | Nil.   | 6      | 3      | 1      | Nil.   |
| No. of sheep used for production of anti-rabic vaccine. | 1,342  | 1,337  | 1,546  | 1,618  | 1,609  |
| No. of treatment centres                                | 134    | 145    | 153    | 163    | 176    |



A total of 24,54,695 ml. of Anti-rabic Vaccine was manufactured at this Institute and 24,58,260 ml. was supplied to various Anti-rabic Centres (including at this Institute) during the year. The total number of treatments issued during the year was 27,340 according to new classification.

|                    |    |    |    |        |
|--------------------|----|----|----|--------|
| Class I            | .. | .. | .. | 2,138  |
| Class II (a) Child | .. | .. | .. | 1,591  |
| Class II (a) Adult | .. | .. | .. | 13,700 |
| Class II (b) Child | .. | .. | .. |        |
| Class II (b) Adult | .. | .. | .. | 9,911  |

Thirteen additional centres for Anti-rabic treatment were opened during the year taking the total to 176. They are shown along with the dates of their sanction. A complete list of all anti-rabic treatment centres in Bombay is given separately. In addition there are 17 Railway centres, 18 military centres and 62 centres in other than Bombay State.

| Serial No. | Name of the Anti-rabic Centre.                                         | Date of sanction.   |
|------------|------------------------------------------------------------------------|---------------------|
| 1          | The Civil Surgeon, Bhandara (M. P.)                                    | 18th March 1955.    |
| 2          | The Medical Officer, Mahad Municipality Mahad Kolaba).                 | 31st March 1955.    |
| 3          | The Medical Officer, Main Hospital, Buldana (M. P.).                   | 18th March 1955.    |
| 4          | The Medical Officer, Jashpurnagar (M. P.)                              | 18th April 1955.    |
| 5          | The Medical Officer, Sangola Municipal Dispensary, Sangola (Sholapur)  | 31st May 1955.      |
| 6          | The Medical Officer, Bhiwandi Municipality, Bhiwandi (District Thana). | 31st May 1955.      |
| 7          | The Medical Officer, Chitgupi Hospital, Hubli.                         | 31st May 1955.      |
| 8          | The Medical Officer, Dwarka Cement Works, Dwarka.                      | 31st May 1955       |
| 9          | The Assistant Surgeon, Main Hospital, Damoh (M. P.).                   | 31st March 1955.    |
| 10         | The Medical Officer, N. R. Tata Hospital, Nargol, District Thana.      | 17th August 1955.   |
| 11         | The Medical Officer, D. M. Petit Municipal Dispensary, Bulsar.         | 24th June 1955.     |
| 12         | The District Medical Officer, Railway Hospital, Bhavnagar.             | 10th November 1955. |
| 13         | The Assistant Surgeon, Rajmamdgapm (M.P.).                             | 17th November 1955. |

(c) *Diagnosis of Rabies in Animals*.—Brain specimens of suspected rabid animals are sent to the department for diagnosis of rabies and are received from Bombay City and the districts, preserved either in formalin or glycerine. Formalin preserved material is suitable for only histological examination. When received in glycerine the diagnosis is usually made by examination of impression smears and by histological methods if necessary.. Where no negribodies could be demonstrated by either of these methods they are subjected to a biological test in mice. Out of 56 brain specimens received 37 were sent in glycerine and 18 in formalin. In addition to microscopic and histological examination the number of biological tests carried out in mice to diagnose rabies was 24.

TABLE V.—*Number of brain specimens received for diagnosis of rabies from Bombay City and out-stations during the year 1955.*

|              |    |    | Total | Positive. | Negative. | Unfit for examination. |
|--------------|----|----|-------|-----------|-----------|------------------------|
| Bombay City  | .. | .. | 32    | 20        | 12        | Nil.                   |
| Out-stations | .. | .. | 24    | 13        | 9         | 2                      |
| Total        | .. | .. | 56    | 33        | 21        | 2                      |

TABLE VI.—*Statement showing the classification of different animals with the results of their brain examination for rabies during 1955.*

| Animals.    |    |    | Total. | Positive. | Negative. | Unfit for examination. |
|-------------|----|----|--------|-----------|-----------|------------------------|
| Dog         | .. | .. | 43     | 27        | 15        | 1                      |
| Human       | .. | .. | 4      | 2         | 2         | ..                     |
| Buffal calf | .. | .. | 1      | 1         | ..        | ..                     |
| Cow calf    | .. | .. | 2      | 2         | ..        | ..                     |
| Buffalo     | .. | .. | 1      | 1         | ..        | ..                     |
| Cow         | .. | .. | 1      | ..        | 1         | ..                     |
| Monkey      | .. | .. | 1      | ..        | ..        | 1                      |
| Cat         | .. | .. | 2      | ..        | 2         | ..                     |
| Horse       | .. | .. | 1      | ..        | 1         | ..                     |
| Total       | .. | .. | 56     | 33        | 21        | 2                      |

As seen from Table VI above 4 human brains were received for diagnosis of hydrophobia this year. Out of these 4 brains one was received from Grant Medical College and the other from Topiwala National College, Bombay. Negri bodies were seen in brain smears of both the cases and the virus isolated in mice. In addition the virus was demonstrated in the saliva of the patient received from Topiwala National Medical College, 24 hours before the patient's death; both these cases had not received any anti-rabic treatment.

The remaining two brains were received from outside Bombay City preserved in formalin. Negri bodies were not found in brain sections and biological test could not be carried out due to preservation in formalin. But the case histories sent along with the specimens were not at all suggestive of the disease hydrophobia.

(d) *Researches carried out in the Department.*—Besides stepping up the production of anti-rabic vaccine to meet the growing demand, studies were continued on Influenza and Rabies viruses besides carrying out diagnostic laboratory tests for primary atypical pneumonia, mumps, and typhus with materials received from different local hospitals.

*Anti-rabic treatment centres in Bombay State.*

(1) *Bombay City.*

- |                                |                          |
|--------------------------------|--------------------------|
| 1. Haffkine Institute, Bombay. | 4. St. Georges Hospital. |
| 2. J. J. Hospital.             | 5. K. E. M. Hospital.    |
| 3. G. T. Hospital.             |                          |

(2) *Civil Hospitals.*

- |                 |                            |
|-----------------|----------------------------|
| 1. Ahmedabad.   | 15. Kaira.                 |
| 2. Ahmednagar.  | 16. Kolhapur.              |
| 3. Alibag.      | 17. Karwar.                |
| 4. Amreli.      | 18. Nasik.                 |
| 5. Baroda.      | 19. Palanpur.              |
| 6. Broach.      | 20. Patan.                 |
| 7. Belgaum.     | 21. Poona.                 |
| 8. Bijapur.     | 22. Ratnagiri.             |
| 9. Dhulia.      | 23. Surat.                 |
| 10. Dangs.      | 24. Satara (North).        |
| 11. Dharwar.    | 25. Sholapur.              |
| 12. Godhra.     | 26. Sangli (Satara South). |
| 13. Himatnagar. | 27. Thana.                 |
| 14. Jalgaon.    |                            |

(3) *Government Dispensaries.*

- |                    |                 |
|--------------------|-----------------|
| 1. Alirajpur.      | 10. Kalyan.     |
| 2. Cambay.         | 11. Nasik.      |
| 3. Chhota Udepur.  | 12. Navsari.    |
| 4. Dakor.          | 13. Petlad.     |
| 5. Dharampur.      | 14. Rajpipla.   |
| 6. Deogad (Baria). | 15. Sawantwadi. |
| 7. Dhanduka.       | 16. Upleta.     |
| 8. Jamkhindi.      | 17. Vijaynagar. |
| 9. Jawhar.         |                 |

(4) *Municipal and Local Board Dispensaries.*

- |                |                    |
|----------------|--------------------|
| 1. Anand.      | 16. Kharaghoda.    |
| 2. Akalkot.    | 17. Limbdi.        |
| 3. Ahmedabad.  | 18. Lusadia.       |
| 4. Borsad.     | 19. Modasa.        |
| 5. Barsi.      | 20. Malji.         |
| 6. Baroda.     | 21. Malegaon.      |
| 7. Broach.     | 22. Mahad.         |
| 8. Bhiwandi.   | 23. Nadiad.        |
| 9. Bulsar.     | 24. Nargol.        |
| 10. Dholka.    | 25. Poona.         |
| 11. Dohad.     | 26. Surat.         |
| 12. Hubli.     | 27. Sangola.       |
| 13. Kapadwanj. | 28. Viramgaon.     |
| 14. Khed.      | 29. Walchandnagar. |
| 15. Karad.     | 30. Wai.           |

(e) *Yellow Fever Vaccine and Inoculation.*—The Yellow Fever inoculation work and testing of potency of the yellow fever vaccine stocked here on behalf of the Government of India, was carried out by the Yellow Fever Diagnostic Unit at this Institute. Several batches of the Rockefeller Foundation Yellow Fever Vaccine and two batches of the Wellcome Yellow Fever Vaccine were tested for potency. Total number of potency tests performed was 13.

Yellow Fever inoculation figures during 1955 are shown in Table VII. The persons inoculated were air or sea passengers from Bombay proceeding to or passing through yellow fever endemic areas. Figures for 1953 and 1954 are given for comparison.

TABLE VII.

| Month.          | No. of persons inoculated |       |       |
|-----------------|---------------------------|-------|-------|
|                 | 1953.                     | 1954. | 1955. |
| January .. ..   | 598                       | 675   | 404   |
| February .. ..  | 818                       | 506   | 638   |
| March .. ..     | 916                       | 685   | 825   |
| April .. ..     | 976                       | 556   | 776   |
| May .. ..       | 1,204                     | 533   | 559   |
| June .. ..      | 846                       | 498   | 799   |
| July .. ..      | 1,011                     | 721   | 608   |
| August .. ..    | 752                       | 450   | 605   |
| September .. .. | 635                       | 439   | 645   |
| October .. ..   | 759                       | 470   | 601   |
| November .. ..  | 693                       | 716   | 666   |
| December .. ..  | 891                       | 597   | 959   |
|                 | 10,099                    | 6,846 | 8,085 |

Yellow Fever Vaccine stored at this Institute was distributed to the following yellow fever inoculation centres:—

| Centre.                                                                  | Quantity of Vaccine supplied in doses |        |
|--------------------------------------------------------------------------|---------------------------------------|--------|
|                                                                          | 1954.                                 | 1955.  |
| (1) The Chief Medical Officer, Porbunder ..                              | 300                                   | 240    |
| (2) The Port Health Officer, Jamnagar ..                                 | 400                                   | 400    |
| (3) The Director of Sanitary and Health Services, Ceylon, Colombo .. ..  | 4,000                                 | 5,000  |
| (4) The Director General of Medical and Health Services, Goa .. ..       | 300                                   | 900    |
| (5) The Delhi State Health Laboratory, New Delhi .. ..                   | 2,000                                 | 1,000  |
| (6) The King Institute, Guindy, Madras ..                                | 1,000                                 | 950    |
| (7) The All India Institute of Hygiene and Public Health, Calcutta .. .. | 6,900                                 | 2,525  |
| (8) The Air Port Health Officer, Santa Cruz ..                           | 1,100                                 | 720    |
| (9) The Port Health Officer, Visakapatnam ..                             | ..                                    | 1,375  |
| Total ..                                                                 | 16,000                                | 13,100 |

(a) *Potency of Yellow Fever Vaccine.*—Several batches of Rockefeller Foundation Yellow Fever Vaccine were repeatedly subjected to mouse potency test, and it was found that most of them had retained the requisite level of potency after 9 years cold storage at 0–4°C.

(b) *Post Vaccinal Reactions.*—Out of the 8,085 inoculations carried out during the year, no cases of post vaccinal reactions were reported.

## XI. Blood Bank Department.

During the year under report the work of the department showed an upward trend in all respects.

The demand for blood plasma from the moffussil hospitals showed an increase over that of the previous year and so the output of this product had to be increased. However, the position regarding the blood donors remains unsatisfactory as only vagrant and under-nourished type of persons came up for blood donation.

The demand for blood grouping sera is still increasing showing that blood transfusion therapy is being utilised to a greater extent and is spreading to moffussil. It is becoming difficult to meet this increasing demand as the hæmagglutination titre of the professional blood donors falls by repeated donation and the same donors cannot be utilised repeatedly for this purpose. Therefore some other means have to be thought of to meet this demand.

The lyophilisation of the antivenine also had to be carried out on a greater scale as the demand for this product showed still greater increase. It was possible to meet this demand by utilising the Desivac Plant; but for this plant it would not have been possible to meet this demand. The appointment of the engineering staff during the year enabled to utilise this machine. With the appointment of Maintenance Engineer now, it would be possible to increase this work.

As usual the routine activity of collection of blood and lyophilisation of plasma and antivenine was carried on.

The following statements show the work carried out during the year under report :—

2,604 donors were screened by  $\text{CuS}^\circ_4$  specific gravity method for their fitness to give blood, out of which 902 persons were rejected giving rejection rate of 34.0 per cent.

33,480 ampoules of Antivenine (10 cc. each) were dried as against 27,595 ampoules last year for Serum Department. 716 ampoules of Sodium Sulphathiazole were also dried. 532 ampoules of anti-rabic serum were dried for Virus Department. 29,000 cc. of blood with high hæmagglutination was supplied to the Diagnostic reagent section for preparation of blood grouping sera.

Tables showing the work carried out during the year under report :—

TABLE NO. I—*Showing the No. of Blood donors.*

| Centre.            | Type of Donor. | No. of Donors bled. | Total No. of Donors bled. |
|--------------------|----------------|---------------------|---------------------------|
| Haffkine Institute | Paid           | 1,792               |                           |
|                    | Voluntary      | 5                   |                           |
|                    | Jail           | 479                 |                           |
|                    |                |                     | 2,276                     |
| Indian Red Cross   | Paid           | 1,555               |                           |
|                    | Voluntary      | 1                   |                           |
|                    |                |                     | 1,556                     |
| Other Hospitals    |                | 169                 |                           |
|                    |                |                     | 169                       |
|                    | Grand Total    |                     | 4,001                     |

TABLE NO. II—*Showing the yield of blood plasma.*

| Product.      | Quantity in litres. | Bottles. |
|---------------|---------------------|----------|
| Blood         | 939·500             | ..       |
| Liquid Plasma | 619·750             | ..       |
| Dried Plasma  | 539·250             | 2,157    |

TABLE NO. III—*Showing the distribution of plasma.*

| Nature. | Quantity in litres. | Bottles. |
|---------|---------------------|----------|
| Sold    | 428,000             | 1,712    |
| Free    | 121,500             | 486      |
| Total   | 549,500             | 2,198    |

TABLE No. IV—*Showing the results of Kahn test carried out during the year.*

|                       |    |    |                         |
|-----------------------|----|----|-------------------------|
| No. of samples tested | .. | .. | 3,409                   |
| No. of negative cases | .. | .. | 2,602 (76·33 per cent.) |
| No. of positive cases | .. | .. | 807 (23·67 per cent.)   |

TABLE No. V—*Showing the blood grouping reports.*

Three thousand two hundred and nineteen samples were grouped according to ABO system. The following is their percentage :—

| Group.   | No. belonging to the group. | Percentage. |
|----------|-----------------------------|-------------|
| AB .. .. | 313                         | 9·72        |
| B .. ..  | 885                         | 27·49       |
| A .. ..  | 992                         | 30·82       |
| O .. ..  | 1,029                       | 31·97       |
| Total .. | 3,219                       |             |

TABLE No. VI—*Showing Rh examination carried out.*

|                                |    |    |      |
|--------------------------------|----|----|------|
| No. of individuals tested      | .. | .. | 708  |
| No. of Rh negative individuals | .. | .. | 58   |
| Percentage of Negative cases   | .. | .. | 8·19 |

## XII. Training of Health Personnel.

The Institute plays an important part in dissemination of scientific knowledge by affording facilities to persons desirous of learning different types of techniques, either for the prosecution of research problems or for routine working in their different spheres of activities. In addition short courses of training of a preliminary nature are given to public health personnel with a view to enable them to carry on their disease-control work efficiently. The following is the list of persons to whom training was given in the various departments during the year 1955 :—

| Serial No. | Name.                          | Designation and address.                                                   | Department in which trained. | Duration of training.                 |
|------------|--------------------------------|----------------------------------------------------------------------------|------------------------------|---------------------------------------|
| 1          | Shri Bansi Modi,<br>M.Sc.      | Chemist, Messrs. Uni-chem Laboratories,<br>Bombay.                         | Department of Pharmacology.  | 3rd September 1954 to 2nd March 1955. |
| 2          | Dr. P. S. Menon,<br>M.B., B.S. | Deputy Assistant Director General, Central Research Institute,<br>Kasañli. | Department of Entomology.    | 8th November 1954 to 5th March 1955.  |



| Serial No. | Name.                                         | Designation and address.                                                      | Department in which trained.                         | Duration of training.                                                |
|------------|-----------------------------------------------|-------------------------------------------------------------------------------|------------------------------------------------------|----------------------------------------------------------------------|
| 3          | Shri C. L. Wasidagama.                        | Municipal Veterinary Officer, Colombo.                                        | Department of Virus Diseases including Rabies.       | 17th January 1955 to 24th January 1955.                              |
| 4          | Shri S. V. Patwardhan, B.E. (Civil).          | Sanitary Engineering student at V. J. T. I., Matunga, Bombay.                 | Department of Vaccine.                               | 2nd February 1955 to 31st March 1955.                                |
| 5          | Shri Chait Singh Bagh, B.Sc.                  | Lab. Assistant, Western Railway Laboratories, C. & W. Shops, Ajmer.           | Department of Vaccine.                               | 1st March 1955 to 30th March 1955.                                   |
| 6          | Shri Y. R. Gawde ..                           | Chemist, Edisons Continental Laboratories, Bombay.                            | Department of Pharmacology.                          | 3rd March 1955 to 2nd September 1955.                                |
| 7          | Shri N. H. Laxmeshwar.                        | Assistant Superintendent, I/c Calf Section. Vaccine Institute, Belgaum.       | Department of Clinical Pathology and Media Sections. | 13th April 1955 to 11th June 1955.                                   |
| 8          | Dr. V. G. Shirhatti .                         | Medical Officer I/c Cottage Hospital, Dahannu (District Thana).               | Department of Virus Diseases including Rabies.       | 3rd May 1955 to 9th May 1955.                                        |
| 9          | Shri A. L. Kelkar, B.Sc., M.B., B.S., (Med.). | Junior Lecturer in Physiology, B. J. Medical College, Ahmedabad.              | Department of Pharmacology, and Biochemistry.        | 13th June 1955 to 18th June 1955.<br>16th May 1955 to 21st May 1955. |
| 10         | Shri B. Mukherjee, M.B., B.S.                 | Junior Lecturer in Physiology, B. J. Medical College, Ahmedabad.              | Department of Serum.                                 | One week from 16th May 1955.                                         |
| 11         | Shri J. H. Mathur, L.C.P. & S.                | Assistant Surgeon, Grade II, Western Railway.                                 | Blood Bank Department.                               | 27th May 1955 to 25th June 1955.                                     |
| 12         | Shri V. V. Badiger, M.Sc.                     | Member of the Staff of the Karnatak University, Dharwar.                      | Department of Clinical Pathology.                    | One year from 1st July 1955.                                         |
| 13         | Dr. P. C. Metha ..                            | Chief Medical Officer, Tata Hospital, Nargol.                                 | Department of Virus Diseases including Rabies.       | 26th July 1955 to 1st August 1955.                                   |
| 14         | Shri A. K. Khot ..                            | Assistant Technician, Grant Medical College, Pathology School, Bombay.        | Animal House Section.                                | 6th August 1955 to 5th October 1955.                                 |
| 15         | Shri D. D. Bhargava.                          | Assistant Director, Provisional Hygiene Institute, Lucknow.                   | Department of Vaccine.                               | 31st August 1955 to 6th September 1955.                              |
| 16         | Shri G. S. Murti, M.Sc., (Tech.).             | Technical Manager, Oriental Pharmaceutical Industries Ltd., Mahim, Bombay 16. | Department of Pharmacology.                          | 3rd September 1955 onwards for four months.                          |

| Serial No. | Name.                                  | Designation and address.                            | Department in which trained.                                 | Duration of training.                       |
|------------|----------------------------------------|-----------------------------------------------------|--------------------------------------------------------------|---------------------------------------------|
| 17         | Dr. K. K. Lahiri, M.B., B.S.           | Medical Officer, Barkui Hospital, Parasia.          | Department of Virus Diseases including Rabies.               | 5th September 1955 to 14th September 1955.  |
| 18         | Shri A. K. Deb, M. Sc. (Biochemistry). | Chemist, Raptakos Brett Co., Ltd., Worli, Bombay.   | Department of Vaccines.                                      | 8th September 1955 to 7th November 1955.    |
| 19         | Shri H. J. Chauhan, M.B., B.S.         | District Health Officer, Cutch.                     | General working of the various departments in the Institute. | 22nd September 1955 to 27th September 1955. |
| 20         | Dr. D. N. Trivedi                      | Medical Officer, Associated Cement Co., Dwarka.     | Department of Virus Diseases including Rabies.               | 21st October 1955 to 30th October 1955.     |
| 21         | Dr. S. Govindrajan                     | Assistant Director, King Institute, Guindy, Madras. | General working of the various departments in the Institute. |                                             |

### XIII. Critics Club.

Under the auspices of the Critics Club, 25 meetings were held during the year. The scientific staff of the different departments of this Institute presented original research work and reviews. There were four lectures by guest speakers during the year. The list is given below :—

| Subject.                                                                                          | Speaker.                                                                                                   |
|---------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|
| (1) Impressions of my Tour in U. S. S. R.                                                         | Major-General S. S. Sokhey, M.P.                                                                           |
| (2) Anaemias Associated with Malnutrition.                                                        | Prof. A. W. Woodruff, Prof. of Clinical Tropical Medicine, London School of Tropical Medicine.             |
| (3) Recent Trends in Virology with special Reference to Influenza.                                | Sir Frank M. Burnett, Director, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia. |
| (4) Prevention and Regression of same Types of Experimental Diabetes by Oestrogens or Cortecoids. | Prof. B. A. Houssay, Director, Institute of Biology and Experimental Medicine, Buenos Aires.               |

| Subject.                                                                                | Speaker.                        |
|-----------------------------------------------------------------------------------------|---------------------------------|
| (5) Influence of Fermented Foods on Fatty Livers.                                       | Shri S. R. Wagle.               |
| (6) Bionomics of <i>X-cheopis</i> ..                                                    | Shri K. V. Tonpi.               |
| (7) Avianized Rabies Vaccine ..                                                         | Dr. D. W. Soman.                |
| (8) Blood Regeneration in Indian Donors.                                                | Shri C. J. Karkhanis.           |
| (9) Studies in the Molecular Structure of Formocibazol.                                 | Shri N. J. Sardesai.            |
| (10) Biochemistry of the Thyroid Gland.                                                 | Dr. (Mrs.) Alamela Venkatraman. |
| (11) Studies on Cardiolipin Type of Phospholipids.                                      | Shri M. S. Samant.              |
| (12) Estimation of Saccharin in Prepared Tea.                                           | Dr. M. C. Sen Gupta.            |
| (13) Enzymic Digest of Casein for Media Purposes.                                       | Shri K. H. Bharucha.            |
| (14) Evolution of Diphtheria Prophylactics.                                             | Shri H. K. Mhatre.              |
| (15) Pulicidal Value of Pyrethrum ..                                                    | Dr. P. J. Deoras.               |
| (16) Laboratory Diagnosis of Rabies with Special Reference to Complement Fixation Test. | Shri Y. I. Shethna.             |
| (17) Iron Metabolism ..                                                                 | Dr. (Mrs.) Alamela Venkatraman. |
| (18) Studies in the Synthesis of Vitamin B <sub>1</sub> .                               | Shri M. Y. Mhasalkar.           |
| (19) Blood Bank Methodology ..                                                          | Dr. K. V. Kulkarni.             |
| (20) Leptospirosis and Laboratory Methods of Diagnosis.                                 | Shri A. S. Narasimham.          |
| (21) Pharmacology of Anti-hypertensive Drugs.                                           | Shri R. G. Tamhane.             |
| (22) Eijkman Test for Detection of B. Coli type-I in water analysis.                    | Shri Y. S. Nimbkar.             |
| (23) A New Technique in Tissue Culture for Studies in Immunity.                         | Dr. (Miss) M. George.           |
| (24) Essential Amino Acid Pattern of Milk during Lactation.                             | Dr. H. Raj.                     |
| (25) Studies in Nutritional Oedema ..                                                   | Shri M. R. Radhakrishnan.       |

The Haffkine Institute took part in a meeting of the Teaching Pathologists held on Saturday the 23rd July 1955. The following papers were presented by the staff of the Institute :—

- (1) Some Insects of Medical Importance Dr. P. J. Deoras.  
collected near Bombay.
- (2) Laboratory Studies in Combined Dr. A. K. Hazra and Shri M. K.  
Immunization with Tetanus Habbu.  
Toxoid and Typhoid Vaccine.
- (3) Proteolytic Activity of Rabbit brain Dr. (Mrs.) Shanta S. Rao and  
Thromboplastin and its Inhibi- Dr. S. S. Rao.  
tion by Heparin.

**B. List of distinguished Visitors visiting Institute in 1955.**

- (1) Professor E. Rothalin, Ciba Foundation, 3rd January 1955.  
Switzerland.
- (2) Dr. Linus Pauling, California Institute of 21st January 1955.  
Technology.
- (3) Sir Cecil Wakely, President of the Royal 4th March 1955.  
College of Surgeons, England.
- (4) Shrimati T. Chandrasekharan, Deputy 4th April 1955.  
Minister of Health, Government of India.
- (5) Dr. Bang, John Hopkins Institute, 4th August 1955.  
Baltimore, U.S.A.
- (6) Dr. Yvdkin, Nutrition Expert, St. May's 17th August 1955.  
Hospital, London.
- (7) General Daulat Singh, General Officer Com- 14th September 1955.  
manding, Bombay Area.
- (8) His Highness the Prinz Von Sayn-Witt- 14th October 1955.  
genstein, Deputy Leader and Physician of  
the German Scientific Expedition.
- (9) Dr. Smetana, Chief of Pathology Armed 12th October 1955.  
Forces, Institute, Washington.
- (10) Dr. Gordon Davis, Rocky Mountain Labo- 20th October 1955.  
ratory, Hamilton, Montana, U. S. A.
- (11) His Excellency Dr. M. Hatta, Vice-President 11th November 1955.  
of Indonesia.
- (12) Their Majesties Sri Maharajadhiraja 16th November 1955.  
Mahendra Bir Bikram Shah Deva and  
Maharani Ratna Rajya Laximi Devi Shah,  
King and Queen of Nepal.
- (13) Chinese Medical Delegation consisting of 28th November 1955.  
five delegates.
- (14) Gold Coast Press Delegation consisting of 15th December 1955,  
three delegates.

- (15) Sir Macfarlane Burnet, F.R.S., Director of 15th December 1955.  
Walter and Eliza Hall Institute of Medical  
Research, Melbourne, Australia.
- (16) Lady Stansgate, London .. 22nd December 1955.
- (17) Professor Bernadro A. Houssay, N.L. 30th December 1955.  
Director, Institute of Biology and  
Experimental Medicine, Buneos Aires,  
(Argentina).
- (18) Dr. Fred Soper. Yellow Fever Expert, 14th December 1955.  
World Health Organization, Geneva (from  
America).

#### XIV. Miscellaneous.

Apart from the researches carried out by the members of the staff, the following scientific enquiries sponsored by the Indian Council of Medical Research were also conducted during the year 1955 :—

- (1) Enquiry on the Pulicidal Value of Under the Director, Haffkine  
Pyrethrum. Institute, Bombay.
- (2) Enquiry on the Role of Nutritional Under Dr. M. V. Radhakrishna  
Factors in Hepatic Cirrhosis. Rao, Assistant Director,  
Department of Nutrition,  
Haffkine Institute, Bombay.
- (3) Enquiry on the study of Indian Under Dr. P. J. Deoras, Assis-  
Poisonous Snakes. tant Director, Department of  
Entomology, Haffkine Insti-  
tute, Bombay.
- (4) Enquiry on the endemic focus of Under the Director, Haffkine  
Schistosomiasis. Institute, Bombay.
- (Carried out at Grant Medical College,  
Bombay).

In addition to abovementioned scientific enquiries conducted here, the following research fellowships and scholarships were attached to this Institute during the year 1955. Particulars of sponsors, the subject of fellowship and the candidates working under them are as under :—

| Serial No. | Sponsors.                                | Subject of Fellowship.                                                                                                        | Candidates working on the Fellowship.                                                                                |
|------------|------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|
| 1.         | Unichem Laboratories, Bombay.            | Research on "Casein Hydrolysate."                                                                                             | Shri R. E. Valadares, M.Sc., upto 30th September 1955.                                                               |
| 2.         | Unichem Laboratories, Bombay.            | Research on "Indigenous Drugs."                                                                                               | Shri V. G. Dalvi, M.Sc., upto 31st March 1955 (A.N.) Shri M. Shrinavasa Shastri, M.Sc., from 5th August 1955 (F.N.). |
| 3.         | Reptakos Medical Research Board, Bombay. | Isolation and Study of Biological properties of the therapeutically important constituents of Cobra and Russel's viper venom. | Dr. (Smt.) Shanta S. Rao, M.Sc., Ph.D., upto 8th May 1955 (A.N.).                                                    |

| Serial No. | Sponsors.                                                      | Subject of Fellowship.                                                                                 | Candidates working on the Fellowship.                                       |
|------------|----------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| 4          | Council of Scientific and Industrial Research, New Delhi.      | Isolation and study of the Biologically Important Constituents of Venoms of Cobra, and Russel's Viper. | Dr. (Smt.) Shanta S. Rao, M.Sc., Ph.D., from 9th May 1955 (F.N.).           |
| 5          | Tata Industries Ltd., Department of Industrial Health, Bombay. | Entomological Survey of Kurla and its Environments.                                                    | Shri K. V. Tonpi, B.Sc., upto 9th January 1955.                             |
| 6          | Board of Research in Ayurveda, Bombay.                         | Research in Indigenous Drugs at the Haffkine Institute, Bombay.                                        | Dr. D. V. Joshi, M.Sc., Ph.D., from 1st February 1955 to 31st January 1956. |
| 7          | Government of Bombay Research Scholarships.                    | Biometrical Studies on the Bombay Rats and the Collection of Their Ectoparasites.                      | Shri M. S. Gokhale, M.Sc. from 11th November, 1953 to 10th November 1955.   |
| 8          | Government of Bombay Research Scholarships.                    | Biochemical and Immunological Analysis of Human Lens, Bovine Lens Acqueous and Vitreous Humour.        | Kumari K. A. Nadkarni, M.Sc., from 7th April 1955 to 7th April 1956.        |

The facilities were also offered to science graduates to take their post-graduate degrees by research at this Institute. During the year 1955 the following candidates were given such facilities under this scheme :—

- (1) Kumari Ranjan D. Mehta, B.Sc.
- (2) Shri Yogendra I. Shethna, B.Sc.
- (3) Kumari M. S. Savant, B.Sc.
- (4) Shri M. S. Gokhale, M.Sc.
- (5) Kumari K. C. Patel, B.Sc.
- (6) Smt. Susy Cherian, B.Sc.

Following is the list of post-graduate students who obtained their M.Sc. degrees (by research) of the Bombay University during the year 1955 :—

| Name.                | Subject.             | Guide.                         |
|----------------------|----------------------|--------------------------------|
| Shri C. J. Karkhanis | .. Biochemistry      | .. Dr. M. V. Radhakrishna Rao. |
| Shri M. S. Samant    | .. Do.               | .. Dr. S. S. Rao.              |
| Shri M. E. Kulkarni  | .. Do.               | .. Do.                         |
| Shri N. V. Bhagavan  | .. Do.               | .. Do.                         |
| Shri P. Y. Dighe     | .. Microbiology      | .. Dr. P. M. Wagle.            |
| Shri S. M. Vaidya    | .. Do.               | .. Do.                         |
| Shri K. V. Tonpi     | .. Entomology        | .. Dr. P. J. Deoras.           |
| Shri M. Y. Mhasalkar | .. Organic Chemistry | .. Dr. K. Ganapathi.           |

## XV. Income and Expenditure for 1954-55.

| Income.                                            |           | Expenditure.                     |           |
|----------------------------------------------------|-----------|----------------------------------|-----------|
|                                                    | Rs.       |                                  | Rs.       |
| <b>XXVII—Medical Bacteriological Laboratories.</b> |           | <b>38—Medical—</b>               |           |
| Sale Proceeds of Sera and Vaccines.                | 23,36,211 | I. Bacteriological Laboratories. |           |
| Other Miscellaneous Receipts                       | 42,092    | I.—1 <i>Haffkine Institute</i> — |           |
|                                                    |           | Pay of Officers                  | 1,97,946  |
|                                                    |           | Pay of Establishment             | 4,53,282  |
|                                                    |           | Allowances                       | 3,33,736  |
|                                                    |           | Contingencies                    | 8,88,450  |
|                                                    |           | Research Scholarship             | 2,550     |
|                                                    |           | H.—2 <i>Pasteur Institute</i> —  |           |
|                                                    |           | Pay of Officers                  | 12,047    |
|                                                    |           | Allowances and Honorarium        | 3,063     |
| Total                                              | 23,78,303 | Total                            | 18,91,074 |

## PART II.

### RESEARCH WORK.

#### Biological Assay of Antibiotics.

(HABBU)

During the year under review, the department continued the work of assaying the antibiotics, especially penicillin and streptomycin. In all 109 penicillin and 23 streptomycin assays were carried out.

It is gratifying to note that only 9 penicillin samples and 1 streptomycin sample were found to be below the standard.

The penicillin and streptomycin assays were carried microbiologically using *St. aureus* and *K. pneumonia* respectively as test organisms. It may be mentioned here that the results obtained by this method were fairly accurate and compared favourably with the results of the assay carried out by the chemical method in a few cases.

#### II. Friedlander Infection Simulating a case of Plague.

(SOMAN AND HABBU)

About the middle of October 1955, a patient suspected to be suffering from plague was removed to the Infectious Diseases Hospital from Andheri, a suburb of Bombay. The patient had developed buboes, had high temperature and showed marked toxæmia. His blood culture was sterile. The smears made at the hospital from the bubo material showed gram-negative bipolar staining organisms. The patient also responded well to treatment

with streptomycin. In view of these typical clinical observations, bipolar staining of gram-negative organisms in smears and favourable response to streptomycin, the case was provisionally diagnosed as plague at the Infectious Diseases Hospital.

The smears were received at this Institute for confirmation of the provisional diagnosis. Though the smears showed gram-negative cocobacilli and resembled apparently *P. pestis*, they were really not *P. pestis* organisms as some of them showed deeper staining than others, unlike *P. pestis* which show uniform staining. As the diagnosis of plague was in doubt, a culture made from the bubo material was requested. The culture was studied in detail at this laboratory and the full details of the study are given below.

The colonies appeared in 24 hours on ordinary agar at 37°C were fairly big, greyish white in appearance, mucoid, convex with regular edges. Microscopic examination showed that the organism was a small coco-bacillus, non-motile, non-sporing and gram-negative. It produced acid and gas in lactose, glucose, mannite, and saccharose; dulcitol was not fermented. Both indole and H<sub>2</sub>S were negative. Serologically typed the organism was not *P. pestis* as it was not agglutinated by anti-plague serum although it was found to be highly sensitive to streptomycin *in vitro* tests.

The culture when given subcutaneously or rubbed on the shaved abdomen of white mice produced death in 24-96 hours, with the following post-mortem appearance :

Subcutaneous and muscular congestion were observed. Spleen was enlarged and congested. The liver was not enlarged, not mottled, and not moulded. There were no buboes and no plural effusion. The organisms demonstrated in splenic smears were bipolar staining and capsulated.

Literature also mentions that organisms with exactly these characters are found in suppurative lesions, are highly sensitive to streptomycin and also highly pathogenic to white mice and they are classed under Friedlander's group.

It was, therefore, reported that the culture under reference was not plague but was a bacterium belonging to Friedlander's group. The Health authorities could not therefore declare it as a case of plague. The points of superficial resemblance to *P. pestis* were the morphology, and staining characters and the pathogenicity to white mice the streptomycin sensitivity and the presence of buboes. The organism however, considerably differed from *P. pestis* regarding its growth requirement on agar, colony appearance, biochemical reactions and serological specificity.

### III. Eijkman's Test.

(NIMBKAR, RAJADHYAKSHA AND HAZRA)

The results of application of Eijkman test to the strains of Coliform organisms isolated from water have been reported last year. In continuation of the same 20 more strains isolated during routine examination of the water samples received for bacteriological examination were tested by the same method.



Out of these 6 were typed as *B. coli* type I (faecal), 10 as *B. aerogenes* type I, 3 Intermediate type I and 1 irregular.

All the 6 *B. coli* type I strains gave a positive 44°C MacConkey test in 18 hours, while out of 10 *B. aerogenes* type I, 8 were negative for 48 hours test, while 1 produced acid and gas within 18 hours and one within 24 hours. Out of 3 Intermediate type I strains, 2 were negative for 48 hours test while one gave a positive reaction within 18 hours. The irregular strain gave a positive reaction in 18 hours.

In addition to this 20 more strains isolated from 3 normal human faeces were subjected to this test.

17 of these which belonged to *B. coli* type I group produced acid and gas in MacConkey within 18 hours. Out of 3 strains which belonged to *Aerogenes* type I, 2 were negative for 48 hours test while one gave a positive reaction within 18 hours.

It was also observed that though *B. aerogenes* could not be isolated from the faeces by direct plating the organism could be isolated from all three samples of faeces examined by initial sub-culture in MacConkey medium followed by a Subculture in Koser's mediums indicating the presence of *B. Aerogenes* in normal intestinal flora in small numbers.

#### IV. Heat Stability of Vi Antigen of *S. typhi*.

(KARBHARI, RAO AND HAZRA)

The effects of heat on the Vi antigen of *S. typhi* have been studied very extensively, but the results published by various workers have been most confusing. Felix and co-workers have shown that when typhoid Vi antigen which is heat labile is exposed to heat, its agglutinogenic property disappeared very readily, but its agglutinin-binding capacity withstood even heating at 100°C for one hour. Felix has also shown that Vi + 0 strains of *S. typhi* after heating for one hour at 60°C were completely or almost completely inagglutinable by typhoid Vi serum. On the other hand suspensions of pure Vi variant of *S. typhi* showed detectable Vi agglutination even after heating for 2½ hours at 100°C.

In view of these findings, it was thought desirable to examine the heat resistance of the Vi antigen in the two types of Vi strains by means of precipitation reaction. It has been shown by various workers that saline extracts of Vi strains of *S. typhi* contain a very high amount of Vi antigen.

Dense suspensions containing  $40,000 \times 10^6$  organisms per ml. of pure Vi strain Vi I (Bhatnagar's strain) and Vi + 0 strain Ty 2 were prepared. Portions of these suspensions were then subjected to 45°C, 55°C, 65°C, 80°C and 100°C for one hour. After cooling, the suspensions were centrifuged and the clear supernatants were used for precipitation tests. Each of the precipitation tubes contained 0.5 ml. of pure Vi serum (absorbed with H 901) of 1:5 dilution plus 0.5 ml. of the required dilution of the supernatant. They were kept at 37°C for 2 hours in water bath and then in cold for 24 hours.

It has been observed that no appreciable damaging effect of heating even at 100°C for one hour was demonstrable irrespective of whether the Vi antigen was present in the strain alone or together with O antigen. Agglutination tests carried out with residues of the above suspensions were quite in agreement with those of Felix. The Vi + O strain Ty 2 was found to lose its Vi agglutinability almost completely when subjected to heating at 80°C for one hour, but the pure Vi strain Vi I retained it even when subjected to heating at 100°C for one hour.

The results of agglutination and precipitation tests taken together also show that most of the Vi antigen in the Vi O strain is extracted in saline more easily than that from the pure Vi strain.

#### **V. A Study of Two Different Methods of Purification of Diphtheria Toxoid-Alum Precipitation and Ammonium Sulphate Fractionation Method.**

(MHATRE AND HAZRA).

During our attempt in the Department to experiment on the different methods of purifying diphtheria toxoid, *viz.*, Alum Precipitation according to Glenn, and Ammonium Sulphate Fractionation method according to Levine and NAFT (Natural Adjuvant Factor Toxoid) developed in our laboratory, we had the occasion to find out that the differently prepared toxoids showed different antigenic values in guinea pigs. Ammonium sulphate Fractionation was more antigenic (9 g. pigs showed 1.0 I.U./cc., 1 g. pig showed 0.1 I.U./cc.) than Alum Precipitation (7 g. pigs showed 1.0 I.U./cc. and 3 g. pigs showed 0.1 I.U./cc.) though both of them showed high antigenicity. But the loss of toxoid during the concentration by the Ammonium Sulfate Fractionation Method was very high (about 90 per cent.) in comparison to that by the Alum Precipitation Method (about 16 per cent.).

NAFTL (Natural Adjuvant Factor Toxoid of Lahiri) had no additional effect when added to the Ammonium Sulphate Fraction.

#### **VI. Keeping Quality of a Diphtheria Toxin.**

(MORE AND HAZRA).

We had occasion to test the Lf value of an old diphtheria toxin (Lot. No. 96B2) which was ampouled on 16th November 1949 and kept in cold at 3°C. By flocculation test with standard serum the Lf value of the toxin was found to have reduced to 73 per cent. No change was however observed in its dermonecrotic activity, the Lr dose being constant for the last 6 years.

#### **VII. Dermonecrotic Activity of Snake Venoms.**

(MORE AND HAZRA).

Dermonecrotic activity of the four common poisonous snakes of India *viz.* Cobra, Russel's viper, Common Krait, and Saw-scaled viper was investigated on rabbit skin. Saw-scaled viper had the highest necrotic

action. A venom dilution of 1 : 500,000 produced a reaction of 10 m.m. in diameter and a dilution of 1 : 50,000 gave a necrotic patch within 3 hours of the injection. Cobra venom was found less active, a 1 : 10,000 dilution of the venom produced only a faint reaction in 12 hours which faded out after 24 hours. Krait and Russel's viper venoms hardly exhibited any necrotic reaction even in 1 : 10,000 dilution of the venoms.

### VIII. Solubility of Freeze-dried Serum.

(MORE AND RAO).

Ether extraction treatment carried out on antitoxic globulin to improve its solubility in the dry state was found to result in about 15 per cent. loss of antitoxin during extraction. Methods to improve the solubility of freeze-dried sera were, therefore, reinvestigated by comparing the solubility of serum lots processed differently. 10 ml. serum from each lot, adjusted to 15 per cent. protein, was filled in ampoules and dried on Strumia machine. Solubility was expressed as the percentage of total protein dissolving in one minute after addition of 10 ml. distilled water to each ampoule.

Results of the test indicate that, serum, to which bile salt was added without ether treatment was as much soluble as that treated with ether and bile. Bile salts increased the solubility of globulins made lipid-free by extraction with a mixture of alcohol and ether, as well as non-extracted original globulins. Lipid free globulins, however, were not more soluble than the unextracted ones. Estimation of sodium taurocholate in serum extracted with ether in the presence of bile salts showed that considerable amount of bile salt was left over after ether extraction, which appears to have improved the solubility of the serum by acting as wetting agent.

### IX. The Proteolytic Activity of Rabbit Brain Thromboplastin and its Inhibition by Heparin.

(MRS. RAO AND RAO).

The proteolytic activity of 0.5 per cent. rabbit brain thromboplastin suspension prepared according to Quick was measured on heat denatured horse hæmoglobin substrate, at short intervals of pH between 2 and 10 using veronal-acetate-phosphate universal buffer. The optimum pH for proteolytic activity was about pH 3.2. There was hardly any proteolytic activity at neutral or alkaline pH. The proteolytic activity was completely destroyed on heating the thromboplastin for 15 mts. at 100°C.

Heparin which inhibits blood coagulation was then tested to see whether it has any inhibitive action on proteolytic activity. It was found that purified heparin (100 units/mg.) inhibited the proteolytic activity of thromboplastin at pH 3.2. The inhibition was of a competitive type.

## X. The Proteolytic Activity of Russel's Viper Venom and its Inhibition by Heparin.

(MRS. RAO AND RAO).

The proteolytic activity of 1 per cent. dialyzed Russel's viper venom was studied using heat denatured horse hæmoglobin substrate. The activity was measured at short intervals of pH between 2 and 10.5. Russel's Viper venom was found to have two proteolytic enzymes one active round about pH 3.6 and the other round about pH 9.0.

Ahuja had reported that heparin inhibits thromboplastic activity of Russel's viper venom *in vitro*. He had also reported that heparin inhibits the toxicity of Russel's viper venom, *in vivo*. There was also evidence to suppose that the thromboplastic activity of the venom was to a large extent due to its proteolytic activity. Hence, the effect of heparin was tried on the proteolytic activity of Russel's viper venom. It was found that 4000 units of purified heparin (100 units/mg.) completely inhibited the proteolytic activity of 2 ml. of 1/100 dilution of dialyzed Russel's viper venom at pH 3.6 whereas the proteolytic activity at pH 9.0 was not very much affected.

## XI. Vitamins as Reducing Agent for Inducing Anærobiosis.

(MHATRE AND RAO).

The distinguishing feature for ærobes and anærobes is the electrode potential range at which they are able to grow in media. The range of reducing activity of an ærobe is from about 0.4V to 0.2V, whilst that of an anærobe is from about 0.05 to 0.4V. An ærobe is freely able to reduce the medium from the level of potential of ordinary ærobic culture media while an anærobe is unable to reduce ordinary ærobic media at all. However, if the medium is subjected to preliminary partial reduction, the anærobes can proliferate intensely. There are various devices to bring about reduced conditions in a medium to make it suitable for growth of anærobic organisms. One of the methods is the addition of minute traces of reducing agents to induce anærobiosis.

In our experiments, vitamin C (5 per cent.) solution and Riboflavin (1 : 25 per cent.) solution were used as reducing agents. The minimum concentration of vitamin C required to induce anærobiosis in veal infusion broth medium was found to be 0.01 per cent. while the maximum limit was found to be 0.6 per cent. for the three strains of *C1. tetani* used in the test. For tryptic digest Media with the same Vitamin C solution, the limits found were 0.025 per cent. and 0.25 per cent. of vitamin C respectively for the same strains. Riboflavin did not prove effective either in the veal infusion broth or the trypsin digest media. Toxins prepared in the above way with vitamin C showed good toxicity in comparison with that produced by the cooked meat medium. Further work is being carried out on the above lines,

## XII. Analysis of Human Cataractus Lens and Bovine Lens Proteins

(KUM. NADKARNI AND RAO).

The ratio of soluble to insoluble proteins in human cataractus lenses supplied by Dr. R. N. Cooper was determined. So far, four incipient cataractus lenses, one Morganian cataractus lens, and five ripe cataractus lenses have been analysed. Analysis of more lenses is necessary before coming to any conclusion. However, results so far indicate an increase of insoluble matter with the maturation of the cataract.

Fresh bovine normal lenses were shaken in presence of sterile glass beads with 5 times its weight of sterile saline. The solution was centrifuged at high speed in cold to separate the soluble and insoluble matter. From the soluble part, alpha-crystallin was precipitated by adjusting isoelectric point to pH 4.8. From the supernatant, beta-crystallin was precipitated by adjusting the pH to 6.0. From the supernatant, globulins were separated by adding ammonium sulphate to 50 per cent. saturation. After filtration, albumin was precipitated from the filtrate by adding ammonium sulphate to full saturation. The purity of these fractions are being studied by electrophoresis and by gel diffusion precipitation test.

The insoluble part of bovine lens was found to be soluble in 90 per cent. phenol or in 6 M urea or in alkali at pH 12.0. By carrying out electrophoresis at pH 12.0, it was found that the proteins are not homogenous. Attempts are being made to fractionate the proteins. These results will later be extended to human lens material.

## XIII. Study on Ectoparasites from Bombay Rats.

(DEORAS AND GOKHALE).

During 1954 and 1955, 2,459 live rats belonging to the species, *Rattus rattus*, *Rattus norvegicus*, *Bandicota bengalensis*, *Bandicota malabaricus*, *Mus dubius* and *Suncus Cæruleus*, were brushed at this Institute and 2,378 fleas, 79 ticks, 90 mites, and 33 lice were collected. The fleas were *X. cheopis*, *X. astia* and a solitary *Cotenocephalides felis*. The ticks were found to be *Rhipicephalus sanguineus*, and *Rhipicephalus tricuspis*. The mites were identified as *Dermanyssus muris*, *Laelaps echidninus* and the lice was *Polyplax spinulosus*.

The predominant rat flea of Bombay was *X. cheopis*. This flea was recovered in maximum numbers from *Rattus rattus*. The male sex predominated in the total collection of *X. cheopis*. *Bandicota bengalensis* harboured the maximum number of *X. astia*. The female sex predominated in the total collection of *X. astia*. These findings are similar to those obtained by Webster and Chitre in their rat flea survey of Bombay City in 1929. We have not used any trap covers to bring the cages with the rats while Webster and Chitre relied on their utility.

#### XIV. Pulicidal Value of Pyrethrum.

(DEORAS AND KULKARNI).

Sixty nine pounds of Pyrethrum flowers were treated with petroleum ether to yield 1346.26 gm. of Pyrethrum extract. The pyrethrum content of the extract varied from 17.05 per cent. to 30.37 per cent. on the basis of the weight of the extract and from 0.68 per cent. to 1.19 per cent. on the basis of the weight of the powder.

The powders made from the extracts were tested at different Concentrations with laboratory bred *x. cheopis*. One mgm. per sq. feet of the concentration gave the best results. When analysed statistically these results show that Pyrethrum as such does have a residual effect comparable to DDT in the same concentrations. Stabilisers like Hydroquinone and Resorcinol showed chemically some check in the deterioration of Pyrethrum, extract, but in biological tests the results were not significant. Boosters like Piperonyl butoxide, sesame oil, castor oil, and Diesel oil were used; the first one gave very significant results in the improvement of knock-down of deteriorated Pyrethrins.

Field trials of the powders were done in some villages of Madras State and in Satara District of Bombay State. Pyrethrum powder as such was found to give a knock-down in the rat burrows upto 76 days, after treatment; when mixed with Piperonyl butoxide and tested on a comparative basis with 10 per cent. D.D.T. Pyrethrum powder gave a lower flea-index than DDT, in 76 days.

#### XV. Food Habits of *Echis Carinatus*.

(DEORAS AND VAD).

These observations were done at Deogad for one month at a time in November 1954, and February 1955. Specified number of stones were turned each day in specified beat to reveal any *Echis* lying there. All the animals found alive under the stones were recorded. The live *Echis* snakes, when recovered were killed and dissected in the field and the entire gut contents fixed and preserved in formalin. The gut contents were examined and the material identified. The animals recovered under the stones were Frogs, Scorpions; Spiders, Centipeds, millipeds, annelid worms, nematodes, some snakes and a large number of insects. The maximum number of animals recovered were insects particularly Dermaptera, Orthoptera, Hymenoptera, Isoptera, Coleoptera, Hemiptera and some times Lepidoptera. The most common insects being Forficula and ants. The material in the gut contents of the dissected *Echis* showed a preponderance of parts of insects, particularly of Forficula and ants.

It had been possible to keep a number of *Echis* snakes alive in the field station in cages on a diet of insects only.

## XVI. Anaerobically Grown Thypoid Organisms in Widal Test.

(NARSIMHAN AND SHAIKH).

The comparative agglutination studies with anaerobically grown strain of *S. typhi* 0-901, were continued. Phenol-killed and Formalin-killed emulsions of anaerobically grown culture were tested simultaneously with the standard emulsion used for routine Widal test of suspected enteric cases. The result have confirmed the previous findings that anaerobically grown culture-emulsions are superior to the standard or aerobically-grown culture emulsions routinely used. In all 374 comparative Widal tests were carried out during the year. The former emulsions uniformly gave higher titre and clarity of reaction.

A few representative readings are given below :—

| Widal No. | Titre obtained            |                                |                                | Culture Test. | Remarks. |
|-----------|---------------------------|--------------------------------|--------------------------------|---------------|----------|
|           | Routine Aerobic Emulsion. | Anaerobic Carbolised Emulsion. | Anaerobic Formalised Emulsion. |               |          |
| 1306      | 1/250                     | 1/250                          | 1/500                          | Negative.     |          |
| 1309      | 1/125                     | 1/125                          | 1/250                          | Positive.     |          |
| 1341      | ..                        | 1/50                           | 1/50                           | Positive.     |          |
| 2192      | 1/50                      | 1/50                           | 1/125                          | Negative.     |          |
| 2197      | 1/50                      | 1/50                           | 1/125                          | Negative.     |          |
| 2202      | 1/50                      | 1/50                           | 1/125                          | Negative.     |          |

Comparative studies with carbolised and formalised (anaerobic) emulsions show that formalin-killed emulsion is more sensitive. It gives rise to a higher titre and clarity of reaction also. In the previous report, it was stated that clarity of reaction was better with carbolised emulsion. But, with the use of chemically pure formalin, the clarity obtained is high along with a higher titre of reaction.

Further studies are in progress.

## XVII. Comparison of Toxigenicity Tests of *C. Diphtheria* by Three Different Methods.

(KUM. PATEL AND SOMAN).

Till 1928, the standard method of testing diphtheria strains for their virulence was to inoculate guineapigs intracutaneously with and without antidiphtheretic sera and watch for the reaction.

In 1938, however, Elek in Britain and Ouchterlony in Sweden introduced the in-vitro method, carried out on plates to determine the toxigenicity of strains of *C. diphtheria*. The test requires a basic medium containing peptone, agar and salt, to which is added 20 to 39 per cent. of normal horse serum. A filter-strip dipped in diphtheria antiserum is placed in the centre of the plate. Organism to be tested is streaked at right angle to the strip in the form of a narrow line. Toxin and antitoxin diffuse out in the descending concentration and a continuous line is formed where the points of optimum proportion meet. These lines appear on each side of the inoculum, inclines to an angle of about 45° to the inoculum so as to give an appearance of arrow-heads. Reactions are best observed after 48 hours.

In 1951, Evans in America, introduced yet another method of testing *C. diphtheria* strains for their toxigenicity in chick-embryos. He inoculated a pure culture of *C. diphtheria* on the chorio-allantoic membrane of 9 to 10 days old eggs. He found that toxigenic strain always killed the embryo within 96 hours.

In the present study, 35 strains of *C. diphtheria* including 3 non-toxigenic strains were tested by all these three methods. It has been observed that under standardized experimental conditions, the 'in vitro' method and the guinea-pig method showed a good correlation. Not only there was a qualitative agreement, but a strain which gave quicker and marked reaction on the plate, also showed wide cutaneous reaction on the animal. The difficulty essentially is met with in standardizing one's own technique.

As regards the chick embryo method, it has been found that a toxigenic strain injected into the allantoic cavity of 9-10-11 days old egg, kills the embryo between 24-48 hours, while in case of a non-toxigenic strain, the embryo survives until the date of hatching. Thus toxigenicity could be demonstrated by all the three methods. However the plate method and the chick-embryo method showed a distinct advantage over the guinea pig test as both were economical and simpler to perform. This preliminary work has shown that both these methods are reliable for testing the toxigenicity of *C. diphtheria* strains. Further work is in progress.

#### **XVIII. A Review of the Work of W. H. O. Influenza Centre at the Haffkine Institute, Bombay (1951-1955).**

(SOMAN).

With the introduction of the Influenza Programme of W.H.O. one of the two influenza centres in S. E. Asia was established at the Institute in 1951. The centre has to carry out a two-fold function as laid down by W.H.O. Firstly, the centre is required to report with all speed the occurrence of influenza within the country with an estimate of its extent and severity. Secondly, the centre is required to isolate and type the causative virus strains by serological tests. These strains are forwarded to the reference laboratory World Influenza Centre, London, for further comparative study.



In the absence of any large scale epidemic in Bombay city or elsewhere in the country the centre had to concentrate its attention on the isolation of virus strains from local sporadic cases. This work was attended with many difficulties. Firstly, number of cases referred to the centre were very few and in many cases the information was received very late, after the onset of attack. In others, patients refused to part with their blood for serological diagnosis.

However, during the five year period under review, 100 throat-washes were subjected to the isolation procedure in fertile chick embryos. A total number of 11 strains have been isolated out of which 10 have been typed and confirmed by W. I. C. in London. One more strain of the type A resembling A/Neth/53 has been isolated and will be forwarded to reference laboratory in due course.

It has been observed that use of only standard strains and their homologous sera in the hæmagglutination inhibition test for typing the strains isolated or for serological diagnosis of patients does not give clear-cut results. Hence some of the recently isolated strains and their corresponding sera should be included in the test. However, World Health Organization has now undertaken to supply in a lyophilised form reference antigens, their antisera for Hæmagglutination-Inhibition test for typing purposes. The first batch of these reagents received in December 1953, was found to be unsatisfactory and was reported as such. The second batch received in 1954 was found to be satisfactory and the strains isolated locally could be typed easily by use of these antigens. A third batch received recently has also given satisfactory results.

The paucity of material, referred to in the laboratory for virus isolation can be well illustrated by the fact that out of 11 strains isolated, 9 were isolated from throat-washes collected from members of the Institute staff, and their families in whose case prompt and adequate information about the nature of illness was readily available.

Serological examination of patients' sera collected during acute and convalescent phase was carried out wherever possible for diagnostic purposes.

The following table gives detailed information about the strains isolated, and the sero-diagnosis of patients for Influenza.

| Serial No. | Year of Isolation. | Patient's name.  | Nomenclature of isolated strains. | HA-I titres in patient's sera. |
|------------|--------------------|------------------|-----------------------------------|--------------------------------|
| 1          | 1951               | Kumari Hazra     | B/India/1/51                      | 20/2560                        |
| 2          | 1952               | Shri Salunke     | B/India/2/52                      | ....                           |
| 3          | 1953               | Shri Mainkar     | A/India/31/53                     | 8/32                           |
| 4          | 1954               | Shri Dighe       | A/India/26/54                     | 8/32                           |
| 5          | 1954               | Shri Jairam Babu | A/India/27/54                     | 16/64                          |

| Serial No. | Year of Isolation. | Patient's name.     | Nomenclature of isolated strains. | HA-I titres in patient's sera. |
|------------|--------------------|---------------------|-----------------------------------|--------------------------------|
| 6          | 1954               | Shri Kadam          | A/India/28/54                     | 8/64                           |
| 7          | 1954               | Shrimati Vengsarkar | A/India/29/54.                    | ....                           |
| 8          | 1954               | Dr. Vengsarkar      | A/India/30/54.                    | ....                           |
| 9          | 1955               | Dr. Shrimati Soman  | A/India/31/55                     | 16/64                          |
| 10         | 1955               | Dr. Dutta           | A/India/32/55.                    | ....                           |
| 11         | 1955               | Shri Nimbkar        | A/India/33/55                     | 16/64                          |

Numerator denotes the titre of the acute phase serum.

Denominator denotes the titre of the convalescent phase serum.

The World Influenza Centre, London, has commented on our strain as given below :—

Both the local-type-B strains resembled antigenically the strain Crawley of 1946, rather than the standard type B strain Lee.

The strains A/India/31/53, A/India/26/54, A/India/27/54, and A/India/28/54, were typed as viruses belonging to the Liverpool sub-group. These results were interesting as it looked very much as if only Liverpool viruses were among the influenza A strains that had been isolated in India and South Africa during 1950-54.

The strain A/India/29/54 was slightly different antigenically from all other Liverpool virus strains, but it was even distinct from the strain Nicholas received from Coonoor in 1953, which was antigenically different from any other strain in the possession of the W. I. C. London.

A/India/30/54 was a Liverpool virus in Q phase.

Strain A/India/31/55 resembled closely A/Eng/19/55, but had extremely low avidity for antibody (Q phase).

Strain A/India/32/55 was similar to A/India/31/55.

In addition to the above mentioned activity, attention is also being paid to the possibility of the preparation of the influenza vaccine. In this direction efforts are directed towards the development of a method for large scale manufacture of Influenza Vaccine and devising a suitable method for its biological assay.

## XIX. Studies of Influenza Antibodies

(SMT. CHERIAN AND SOMAN).

The serum antibody levels are usually measured by the hæmagglutination-inhibition test and complement-fixation test. Both methods have their own advantages and disadvantages but of the two, hæmagglutination-inhibition (H.A.I.) test has recently become the method of choice, because of its simplicity and the ease with which it can be performed. The sera, however,

required to be treated with cholera filtrate to destroy the normal serum inhibitors, besides the use of viral antigen in the test makes it only strain specific. In addition, along with standard strains, as antigens, locally isolated strains will also have to be used, which make this test more cumbersome. Complement-fixation test in which soluble antigens are used gives type-specific results. So far, no report has appeared on the study of antibody patterns present in normal sera of Indian population.

About 60 ml. of soluble antigen was collected from different batches of antigens prepared from chorio allantoic membranes of 13 day old chick embryos which had been infected with the strain PR8 of Influenza virus, by the allantoic route 2 days previously. To the pooled membranes was added 1.5 ml. of saline per membrane and the membranes were ground in a waring blender. After a preliminary centrifugation, the supernatant suspension was stored in the cold at 4°C for 4 days. Any precipitate formed was removed by another centrifugation at low speed after which the supernatant was centrifuged at 15,000 r.p.m. for 1 hour, in a refrigerated centrifuge. The supernatant liquid was removed and used as a soluble antigen. Its hæmagglutination titre was less than 4 and was non-infective in eggs. Pooled normal guinea-pigs serum preserved by Richardson's method was used as Complement. Positive control sera were collected from guinea pigs, infected intranasally and intraperitoneally with live virus and bled after two weeks. These sera were titrated by C.F. test with the soluble antigen and they showed some C.F. titre. Perhaps the method of immunization of guinea-pigs will require to be further re-inforced to obtain a high C.F. titre. Work in this direction is in progress.

## XX. Inactivation of Normal Serum Inhibitors with Trypsin.

(SOMAN AND NIMBKAR).

Normal animal sera such as those of rabbit, guinea-pigs, ferret and human, have been shown to contain two types of inhibitors which inhibit the agglutination of erythrocytes by different strains of Influenza virus. These inhibitors interfere with the hæmagglutination inhibition procedure adopted for typing the virus strains and may lead to erroneous results. Hence it is necessary to inactivate these inhibitors in the immune sera before they can be used in the Hæmagglutination inhibition (H.A.I.) test. These inhibitors can be inactivated by the action of enzymes contained in the crude vibrio cholera filtrate or by purified enzyme preparation called R.D.E. (Receptor Destroying Enzyme) prepared from the crude cholera filtrate.

Sampaio and Issacs have recently reported that crystalline trypsin is also highly effective in inactivating both the normal serum inhibitors present in the sera of different animal species they examined. Sera of these normal healthy rabbits were tested by H. A. I. test using PR8 FM1 and LEE virus strains along with the three locally isolated strains two belonging to the Type A and one to Type B before and after treatment with trypsin. In all these experiment B.D.H. Trypsin was used. Suitable controls were included in the experiment.

The results showed that the treatment of normal rabbit serum with trypsin completely destroys the normal serum inhibitors and typical agglutination patterns are obtained in as low a dilution as 1 : 8 with treated serum as against a complete inhibition observed in a dilution as high as 1 : 128 with untreated serum.

Experiments were also carried out to determine the optimum quantity B.D.H. Trypsin required for complete inactivation of the inhibitor. For this a constant volume of one of the serum samples was treated with increasing quantities of trypsin varying from 4 mg. per ml. to 40 mg. per ml. dissolved in phosphate buffer at pH 8 and heated at 56°C for  $\frac{1}{2}$  hour. The treated samples were tested by H.A.I. Test along with the untreated control using A/India/31/55 B/India/2/53 as test virus strains. It was observed that a quantity of 28 mg. per ml. completely destroyed the inhibitors while sera treated with 4 mg. per ml. showed a destruction of 25-50 per cent. of the inhibitor when tested against the strains stated above.

Experiments were also carried out with normal and immune rabbit sera using standard antiserum and antigen supplied by World Health Organisation. For this purpose one of our normal rabbit serum and immune rabbit serum A/U.S.A./47 supplied by W.H.O. was treated with quantities of trypsin varying from 4 mg. per ml. to 32 mg. per ml. and the treated sera were tested against the antigen A/U.S.A./47 also supplied by W.H.O. It was observed with this test antigen even treatment with 4 mg. of trypsin inactivated the normal inhibitor in normal as well as in immune serum. However, it was observed that treatment of immune serum with 8 mg. of trypsin per ml. destroyed the specific antibody also. The fact that no loss of antibody occurred by treatment with 4 mg. of Trypsin was confirmed by testing the untreated immune serum treated with crude cholera filtrate supplied by W.H.O.

## XXI. Studies on Influenza Vaccine.

(SOMAN AND HABBU).

The W.H.O. committee on Influenza are of the opinion that the current laboratory methods for testing the potency of Influenza vaccine are not satisfactory. In particular further research is required on the reproducibility and significance of the different tests, including the active immunity test in mice.

Studies were therefore undertaken to determine the suitability of the active immunity test in mice for the purpose.

A small batch of formalin killed vaccine was prepared with the PR 8 egg adapted strain. The virus was purified and concentrated by absorbing it on to fowl cells.

Groups of mice weighing 10-12 g. were selected and immunised with varying doses by giving the vaccine intraperitoneally in two equal doses at an interval of one week. A batch of mice selected at the same time was kept apart to serve as controls. A week after the 2nd immunising dose, the challenge dose, consisting of 10 per cent. mouse-lung suspension of a mouse adapted PR 8 strain was given intra-nasally to all groups including the controls.

The results obtained in the first two preliminary experiments are given in the table below :—

TABLE.

| Experiment No. | Dose of Vaccine |         |          |           | Controls. |
|----------------|-----------------|---------|----------|-----------|-----------|
|                | 0.5 ml.         | 0.1 ml. | 0.02 ml. | 0.004 ml. |           |
| 1              | 0/5             | 0/5     | ..       | ..        | 4/4       |
| 2              | ..              | 1/5     | 0/5      | 2/5       | 3/5       |

*Denominator*—Mice used for the test.

*Numerator*—The mortality in mice.

The results showed that the variation in response is graded with varying doses and the results seem to be fairly regular. It therefore appears that it is possible to standardise the conditions for an active immunity test for Influenza vaccine. Further work regarding the reproducibility and sensitivity of the test and hence its suitability for routine use is in progress.

## XXII. Complement-Fixation Test in the Diagnosis of Rabies.

(SHETHNA AND SOMAN).

At present the diagnosis of Rabies in animals is done by examination of brain smears for negri bodies, or when they are absent by mice inoculation of brain or salivary glands. This latter procedure takes at least 10–15 days for getting the results. Thus, an attempt has been made to shorten this period by carrying out a complement-fixation test using brain or salivary gland. The brain or salivary gland suspension is used as an unknown antigen to be tested against a known positive hyperimmune guinea-pig rabies serum.

So far 37 brains and 9 salivary glands received from suspected rabid animals have been tested by complement-fixation test. The results have agreed mostly with smear examination for negri bodies and mice inoculation. In the case of 2 brain specimens (from one dog and one rabbit) the results were at variance. In the case of dog brain 27/55 the C.F. results twice agreed with the results of mice inoculation. But when used for the third time as a positive control the test was negative. Subsequent repetition gave also the same results.

In the case of a rabbit intramuscularly inoculated with street virus, and which died of rabies after 19 days, the C.F. test was carried out in the brain but the test was negative; the mice inoculation was however positive. The cause of such discrepancies is being investigated. The C.F. test if it could be successfully applied will enable the diagnosis of Rabies to be made in 24 hours from the receipt of brain or salivary gland of a rabid animal and thus help the person bitten to undergo early treatment and relieve him from the anxiety of a long waiting period of 10–15 days to know the result of mice inoculation.

### XXIII. Further Report on the Biological Test for the Diagnosis of Rabies.

(KHEMANI AND SOMAN).

The biological test using white mice for diagnosis of rabies was continued during this year, and as in the previous year, was mainly carried out with Negri negative specimens. 56 brains were received for diagnosis of rabies out of which 37 were sent in glycerine and the rest in formol-saline. The results of 24 biological tests are summarised in the following table:—

*Table showing results of Biological Tests.*

| Year.      |     |    |    | Total No. | Smear —<br>Mouse — | Smear +<br>Mouse + | Smear —<br>Mouse + |
|------------|-----|----|----|-----------|--------------------|--------------------|--------------------|
| 1952 .. .. | 28  | 15 | 10 | 3         |                    |                    |                    |
| 1953 .. .. | 28  | 17 | 4  |           |                    |                    |                    |
| 1954 .. .. | 28  | 17 | 3  | 8         |                    |                    |                    |
| 1955 .. .. | 24  | 14 | 6  | 4         |                    |                    |                    |
| Total ..   | 108 | 63 | 23 | 22        |                    |                    |                    |

These results have again confirmed the previous observations that correct diagnosis of rabies on 4 specimens would have been missed if biological tests were not carried out. As already pointed out, these tests suffer from one drawback that the results become available after 7 to 14 days.

### XXIV. Studies on Street Virus of Rabies.

(KHEMANI AND SOMAN).

Comparative studies for the presence of virus in brain and salivary glands were continued during this year on 25 specimens. The results are summarised in the table along with those of 1953 and 1954.

TABLE.

| Year.    | Total. | Brain +<br>Salivary<br>Gland + | Brain +<br>Salivary<br>Gland — | Brain —<br>Salivary<br>Gland — | Brain —<br>Salivary<br>Gland + |
|----------|--------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| 1953 ..  | 29     | 8                              | 8                              | 13                             | 0                              |
| 1954 ..  | 28     | 16                             | 4                              | 8                              | 0                              |
| 1955 ..  | 25     | 9                              | 8                              | 8                              | 0                              |
| Total .. | 82     | 33                             | 20                             | 29                             | —                              |

Thus out of proved rabid animals, the virus could be demonstrated in both brain and salivary glands in 33 (62 per cent.) but only in brain in 20 (37 per cent.). The above results again confirm the previous years findings, that the virus may not be always present in saliva of rabid animals. As such even though a person may have been bitten by proved rabid animal with demonstration of virus in brain, he does not necessarily run the risk of contracting rabies. On epidemiological grounds, therefore, it is very important to test salivary glands of a rabid animal rather than its brain for presence of rabies virus.

## **XXV. Value of Hyperimmune Rabies Serum in Rabies Prophylaxis .**

(SOMAN AND KHEMANI).

The value of hyperimmune rabies serum in conjunction with anti-rabic vaccine for rabies prophylaxis in severe cases has been established by many workers in laboratory animals and in field trials. As already reported (Annual Report, 1954) hyperimmune rabies horse serum with high antibody content was prepared this year and its potency tested by virus neutralization test. This particular batch has been found to have a very high neutralising index. As reported in 1954, the serum was issued to five selected anti-rabic centres in Bombay State for field trials. Out of these five centres only two centres have been able to use the serum in the treatment of severely bitten cases. In all, eleven cases, (five adults and six children) have been treated. The serum dose varied between 10 to 40 cc. according to the weight of the individual. No death has been reported amongst the eleven cases during six months after treatment. In view of the few cases treated the trial would be further extended to more anti-rabic centres next year. The importance of this line of treatment could well be realized since Virus Diseases Advisory Committee of the ICMR has taken up this question with a view to extend the field trials at suitable anti-rabic centres in different States.

## **XXVI. Adaptations of Street Virus to Embryonated Eggs.**

(KHEMANI AND SOMAN)

Recent developments in rabies refer to preparation of avianized vaccine in chick embryos using egg adapted rabies virus strains. The live vaccine thus prepared has been shown to be more potent than killed virus vaccine and to confer better and longer immunity in animals which is substantiated by field trials carried out in Isreal, Malaya and U.S.A. But it has been the experience of many workers in the field that very few street virus strains could be so adapted to eggs.

During this year locally isolated street virus strains were inoculated into eggs with a view to adapt them to eggs. The method used was that advocated by Dr. Komarov of Isreal i.e. a street Virus strain was passaged five times in mice intracerebrally and the brains made into 20 per cent. suspension which was inoculated in embryonated eggs by yolksac route. Two street virus

strains have been tried this way in a number of egg passages. Before inoculating into eggs, behaviour of each strain in different laboratory animals was studied when inoculated intramuscularly and intracerebrally. All animals developed typical paralysis and died.

Both strains had thus to be discarded because the virus failed to grow in eggs after several passages. It is proposed to try some more strains next year.

## **XXVII. Estimation of Saccharin in Pharmaceutical Preparations.**

(BHATT AND MADIWALE)

Saccharin has been occasionally found to replace sugar in pharmaceutical preparations, when intake of sugar is not advisable. During routine examination, however, it was observed that its use as an adulterant for sugar is not quite uncommon. It was therefore, thought desirable to investigate into such possibilities of adulteration.

In this context, pharmaceutical syrupy preparations were divided into three main groups (i) Gripe mixtures, (ii) Cough mixtures and (iii) Oral liquid vitamin preparations.

Pharmacopoeial and other available methods for estimation of saccharin cannot be said to be very suitable for the purpose as they are not sufficiently specific, reproducible, accurate and usually demand larger amount of the substance.

The colorimetric method which has been developed in our laboratory and reported before was resorted to. Substances which are known to interfere with the basic reaction of conversion of saccharin into phenolsulphonephthalein are quite likely to occur in pharmaceutical preparations mentioned above, either as the main ingredients or preservations or flavouring agents.

After a careful study of the solubility properties of these substances,—salicylic acid p-chloro-benzoic acid, p-hydroxybenzoic acid, ethyl-p-hydroxy benzoate, vanillin, caffeine besides clove and cinnamon oils, salicylaldehyde, formaldehyde, gum benzoin, tannins etc. and peculiarly slight solubility of saccharin in chloroform and petroleum ether, elimination of their interference was planned. Thus, the original method developed for estimation of saccharin in prepared tea was modified to make it reliable for this purpose.

The work that has been done so far has shown that recovery of saccharin in such case can range from 92.0 to 99.0 per cent. Further work consisting in the examination of a large number of preparations is being pursued.

## **XXVIII. Suitability of Fibrin Hydrolysate for Intravenous Medication in Human Beings.**

(DUTTA, THADANI, VALADARES AND RAGHAVAN)

Fifteen patients were selected for intravenous medication of Fibrin Hydrolysate. There were cases of extreme malnutrition with oedema, diarrhoea, ascitis and liver cirrhosis. Prior to the commencement of the treatment, the blood of each patient was examined for the contents of total proteins, albumin,



globulin and non-protein nitrogen. Urine nitrogen was also estimated. They were periodically checked both during and after the completion of the treatment. Each patient daily received 400 ml. of Fibrin Hydrolysate along, with 100 ml. of 50 per cent. glucose solution by intravenous drip method at the rate of not exceeding 48 drops per minute. Fibrin Hydrolysate did not produce any untoward reactions and seemed to be effective in raising the protein values to the normal level.

### XXIX. *Scilla-Indica*—Grown in Bombay State.

(DESHPANDE AND DUTTA)

Chopra and De (1931) had reported that Tincture prepared from *Urginae scilla* and *Scilla indica* is in no way inferior to the Tincture prepared from *Urginae scilla* from the Mediterranean coast (imported). *Scilla indica* grows in abundance in the State of Bombay. An investigation was undertaken to find out the potency of tinctures prepared from the bulbs of *Scilla* grown at different parts of the State. Bulbs of *Scilla indica* were collected from Khanpur, Dharwar, Mahableshwar, and Badami through the Silviculturist, Government of Bombay. Tinctures were prepared according to British Pharmacopoeia from the different samples. These tinctures were tested biologically on cats and guinea-pigs. None of them showed the presence of any glycosidal activity.

### XXX. Chemical and Pharmacological Investigation of Roots of *Wagatea spicata* Dalzell.

(JOSHI, TAMHANE AND DUTTA)

The roots of *Wagatea spicata* Dalzell (N. O. Leguminosae; Vernacular, Vakeri, Vagati) growing abundantly in Bombay State, specially in Konkan jungles and on the Ghats near Mahableshwar, are known to be used in cases of pneumonia and frequently in the treatment of pulmonary tuberculosis.

A systematic investigation of the constituents of the root of the plant, has been undertaken. A crystalline substance (which will be referred to as substance "X") has been isolated from the alcoholic extract (96 per cent. ethyl alcohol) of the dried roots. The yield was 2.0 per cent. of the weight of dried roots.

A preliminary study of the crystalline substance "X" revealed that it crystallises from dilute alcoholic solution with one molecule of water. The melting point of the hydrated compound, was found to be 150°C while that of anhydrous compound it was 238°C. The molecular formulæ for the hydrated as well as for the anhydrous compounds have been found to be  $C_{15}H_{18}O_{10} \cdot 1H_2O$  and  $C_{15}H_{16}O_{10}$  respectively. The ultra violet spectra of the compound showed the presence of phenolic OH groups.

The substance was found to be slightly bitter in taste, soluble in alcohol, sparingly soluble in water and insoluble in other organic solvents.

The substance "X" was found to be non-toxic to white mice. Oral single doses of 1.2 g/kg. and intravenous doses of 400.0mg./kg. were well tolerated by white mice. The substance at a dose level of 20 mg./kg. had no effect on the blood pressure and respiration of the anaesthetised adult cat. The substance "X" had no effect on the isolated intestine of the guinea-pig or uterus of the rat. It did not block the action of histamine or acetylcholine on the isolated intestine of guinea-pig.

The substance "X" failed to inhibit the growth of *E. Coli*, *S. aureus* and *E. typhi* in a concentration of 5.0 mg./ml.

### XXXI. Studies in the Synthesis of Vitamin B<sub>1</sub>—Part IV: Synthesis of Thiamin—Like Compounds of Potential Therapeutic Value.

(MHASALKAR AND SHIRSAT)

The growing amount of literature on the subject of synthesis of anti-vitamins as products of potential therapeutic value prompted us to synthesize several thiazole compounds having one or more substituents in 2, 4, and 5 positions of the thiazole molecule. These on condensation with 4-amino-5-bromomethylpyrimidine, having a methyl or ethyl substituent in 2 position, afforded several compounds related to the Thiamin molecule. In like manner, 4-amino-5-bromomethylpyrimidine having a methyl or ethyl substituent in position 2 were successfully condensed with a few amides and tertiary amines to obtain the expected quaternary ammonium compounds. An antibacterial and pharmacological investigation of these compounds is expected to yield interesting results.

During the course of the work, approximately 50 thiamin-like compounds were synthesized of which almost all are hitherto unreported.

### XXXII. Chemotherapy of Intestinal Infections : Chemistry of Schiff Bases of Sulphonamides.

(SARDESAI AND SHIRSAT)

The study of the chemistry of Schiff bases of the potent sulfonamides (Annual Report for 1953) which were synthesized for the primary purpose of developing new chemotherapeutic agents for the treatment of bacterial diseases of the intestinal tract was further extended.

Attention was now particularly devoted to the study of the chemical constitution of the formaldehyde derivative of Sulphathiazole (Formo Cibazole) since this compound has shown the desirable property of being insoluble in cold dilute alkalis in addition to being insoluble in acids. On the basis of the results so far obtained, it has been tentatively suggested that the compound is a polymer resulting from the interaction of the equimolecular proportions of sulphathiazole and formaldehyde, the methylene group in the polymer being linked up between the anilino amino group of one molecule of sulphathiazole and the tertiary nitrogen of the thiazole ring of another molecule of sulphathiazole.

Reaction of acetyl acetone with Sulphaguanidine proved to be very interesting. It has been reported in literature that interaction of acetyl acetone with Sulphaguanidine gives rise to the formation of 2-Sulphanilamido-4:6-dimethylpyrimidine (Sulphamethazine). This result was confirmed and it was further found that two more products can be isolated from this reaction under suitable experimental conditions. These products were identified as N<sup>4</sup>-Acetyl acetone anil of Sulphaguanidine and N<sup>4</sup>-acetyl acetone anil of Sulphamethazine.

Condensation of acetonyl acetone with Sulphanilamide, Sulphathiazole, Sulphadiazine, Sulphamerazine, Sulphamethazine and Sulphaguanidine resulted in the formation of the corresponding pyrrole derivatives wherein the anilino group of the sulfonamide compounds participated in the formation of the pyrrole ring. Although the interaction of aromatic amines with acetonyl acetone to form pyrrole compounds was indeed well known, the condensation of acetonyl acetone with various potent sulfonamides does not seem to have been reported so far. The antibacterial activities of these compounds are being investigated.

### XXXIII. Protein and Lipoid Analyses of Human Sera.

(BEARUCHA AND RAO)

The serum albumin-globulin ratio in normal Indian subjects was being estimated by the method of Howe using 23 per cent. sodium sulphate fractionation. According to this method of Howe, the ratio of albumin-globulin is 2 : 1. Recent work in foreign countries showed that this method of Howe does not give a true picture of albumin-globulin ratio revealed by Electrophoretic studies. In the light of this, Howe's method has been replaced by chemical fractionation technique using 27.4 per cent. sodium sulphate. (Report of the Haffkine Institute for the year 1951 Part II). According to this method the normal ratio of albumin-globulin is 1.

This work is now being extended by checking the results obtained by chemical fractionation technique using 27.4 per cent. by paper electrophoresis. Electrophoresis is being conducted on Whatman 3MM paper using Barbiturate buffer of pH 8.6. Separation was carried out with a constant current of 3mA for 18-20 hours preferably overnight. After electrophoresis the paper strip is dried and stained with bromophenol blue and the protein fraction estimated according to the method of Jencks, W. P. et al. (The Biochem. Journal, vol. 60 No. 2, 1955. pp. 205-215).

Along with proteins, the Lipoproteins in normal as well as diseased sera are also being studied by paper electrophoresis using the same Barbiturate buffer of pH 8.6 and staining with Sudan Black according to the method of Shwan. (Scand. J. Clin. & Lab. Invest. 1952 4 98).

The cholesterol and lipid phosphorous in normal Indian subjects has also been estimated during the study of lipoproteins and it was observed that normal cholesterol for Indians is lower than the foreign standard which is 150-230 mg. per 100 cc. blood, while the lipid phosphorous in normal Indians is almost the same as the foreign standard. So far about 50 samples are analysed and the further work is in progress.

### XXXIV. Studies in Hæmagglutination Titre in Blood Grouping Sera.

(KULKARNI AND SARNIS)

Difficulties are encountered in obtaining high-titred hæmagglutination sera from regular professional blood donors. In order to determine the titre of hæmagglutinins in these donors hæmagglutination titres of over 208 donors was determined. 208 blood donors were bled and their hæmagglutination titres were determined and shown in the Table below :—

| Hæmagglutination<br>Titre. | Anti-B titre in<br>A group persons. | Anti-A titre in<br>B group persons. |
|----------------------------|-------------------------------------|-------------------------------------|
| 1 : 2                      | 1                                   | 2                                   |
| 1 : 4                      | 7                                   | 13                                  |
| 1 : 8                      | 21                                  | 30                                  |
| 1 : 16                     | 19                                  | 38                                  |
| 1 : 32                     | 25                                  | 37                                  |
| 1 : 64                     | 4                                   | 6                                   |
| 1 : 128                    | 1                                   | 4                                   |
|                            | 78                                  | + 130 = 208                         |

The hæmagglutination titres of most of them fall within a range of 1 : 8 and 1 : 32. Perhaps this is due to frequent blood donations by these donors and their low nutrition status. It is observed that the titer falls down with subsequent donation. Work is being pursued to confirm this.

### XXXV. Amino Acid Composition of Human Milk.

(RAJ, RADHAKRISHNAN AND RADHAKRISHNA RAO)

Essential amino acids content of 17 samples of milk of mothers belonging to low economic groups, determined by the microbiological assay technique, was reported last year. This work was further continued. In addition preliminary data, regarding the quantitative yield of milk was also collected from mothers in three local institutions.

During the period under report, 44 more samples of milk were analysed. In order to study the essential amino acid pattern at different stages of lactation, these samples are classified arbitrarily into the following five groups, depending upon the period of lactation : 1 to 5 days, 6 days to 3 months, 4 to 6 months, 7 to 12 months and over 12 months. The number of samples in each group are 5, 6, 14, 17 and 2 respectively.

The values for most of the amino acids in the samples of milk analysed are in agreement with those reported before. When these values are expressed on the basis of 100 ml. of milk, there is a considerable drop in the values immediately after the cessation of colostrum secretion. Following this period, the levels of the amino acids remain practically constant. On the other hand, when the amino acid values are expressed on the basis of 100 g. protein, there is a marked variation at different periods of lactation though the total nitrogen is practically constant after the initial period of 5 days.

In order to study the quantitative yield of milk in mothers belonging to low economic groups, test feeds were carried out in 45 cases. The child was weighed before and after each feed during 24 hours. The tests were carried out in three centres and the results are given in the table below :—

| Name of the Centre.                                                       | No. of Cases. | Age of Child | Total yield of milk per day in oz. |       |
|---------------------------------------------------------------------------|---------------|--------------|------------------------------------|-------|
|                                                                           |               | Range.       | Range.                             | Mean. |
| Nowrosjee Wadia Maternity Hospital, Parel, Bombay 12.                     | 25            | 1-17 days    | 7 to 25                            | 13.86 |
| The Bombay Mothers and Children Welfare Society, Delisle Road, Bombay 11. | 15            | 2-6 days     | 6 to 20                            | 12.77 |
| The Tata Mills, Parel, Bombay 12                                          | 5             | 2-10½ months | 12 to 22                           | 17.4  |

#### XXXVI. Influence of Fermented Foodstuffs on Fatty Infiltration of the Liver in Rats.

(WAGLE, PATEL AND RAO)

It has been observed last year (1953-54) that in rats fed high fat low protein diet (30 per cent. fat, 10 per cent. casein), "Iddlee" (fermented preparation) feeding at 50 per cent. and 100 per cent. replacement of dietary protein (casein) had beneficial effect on fatty infiltration in the liver. This was indicated both by biochemical and histological findings. The histological examination of the liver sections of the animals in the second experiment was then in progress. The observations on this material are given below.

Liver sections of animals fed on the unfermented preparation showed fatty infiltration but no fibrosis of the liver. In case of the "Iddlee" group, the liver sections showed normal lobular pattern with occasional fatty infiltration of the parenchymal cells here and there. Although the general histological picture was similar to that reported last year where 50 per cent. of the dietary protein was replaced by "Iddlee" the preventive effect was not so pronounced. This is in conformity with the biochemical findings.

During the year, it was intended to confirm the above results. For this purpose, another experiment was carried out to study the preventive effect of "Iddlee" on fatty infiltration. In these tests larger number of animals were used and 50 per cent. of the casein was replaced by "Iddlee" protein. The experiment was continued for 60 days. The other experimental details were reported before. The results are summarised in the table given below :—

| Group.                            | No. of rats. | No. of days. | Gain in wt. g. | g. per 100 g. of the body wt. |            |                |
|-----------------------------------|--------------|--------------|----------------|-------------------------------|------------|----------------|
|                                   |              |              |                | liver wt.                     | liver fat. | liver protein. |
| Control                           | 6            | 60           | 32.9           | 11.06                         | 3.3        | 1.14           |
| "Unfermented preparation"         | 12           | 60           | 43.0           | 8.57                          | 1.83       | 1.01           |
| "Iddlee" (Fermented preparation). | 12           | 60           | 58.0           | 6.55                          | 1.02       | 0.84           |

This experiment confirms our previous findings both biochemical and histological—that "Iddlee" affords greater protection to the liver than the "unfermented preparation". This finding is statistically significant. It is also evident that even in "Iddlee" group, a combination of protein (50 per cent., casein and 50 per cent. "iddlee" protein) is superior to either of the two alone.

Further work on similar lines in rats fed low fat low protein diet is in progress.

### XXXVII. Experimental Hepatic Necrosis.

(AMBEGAOKAR, PATEL, KRISHNAN AND RADHAKRISHNA RAO)

Acute massive necrosis of liver has now been produced regularly in rats fed yeast at 20 and 15 per cent. levels. In order to investigate the possible metabolic changes taking place in such livers, at the onset of the disease, some biochemical estimations were undertaken. To begin with water, nitrogen and alkaline phosphatase levels of livers—necrotic and normal—were determined.

One group of twelve rats was fed "yeast necrogenic diet" at 20 per cent. yeast level, while the control group of ten rats received diet containing casein at 10 per cent. level in place of yeast. The level of protein in both the diets was about equal. The rest of the supplements were similar to those used in our previous experiments. The initial average weight of the animals was about 114 g.

The estimations of moisture and nitrogen in samples of liver were carried out by the usual methods. Alkaline phosphatase was extracted from fresh liver samples by the method of Davis (1934) and the rest of the procedure was similar to that outlined in Hawk *et al* (1953) for serum phosphatase.

The rats maintained on both the "yeast necrogenic diet" as well as the control diet grew at suboptimal rate, the average increase in weight at the end of two months being 34 g. and 53 g. respectively. The rats on the test diet developed hepatic necrosis between the period 60-165 days. The time required for the production of liver necrosis in this group was longer than before as older animals were used. However, liver necrosis was uniformly found in all the animals fed on the test diet. It has been observed that the necrotic livers were heavier than the control, the average liver weight per 100 g. body weight being 5 g. in the former while it was 3 g. in the latter. This is evident from the water content values which are 78.4 per cent. and 69.5 per cent. in the necrotic and the non-necrotic livers, respectively. The estimations of nitrogen showed about 19 per cent. decrease in the necrotic livers as compared to the control. The alkaline phosphatase activity of the necrotic livers showed considerable increase in comparison to that of the livers from the control animals. The average phosphorous liberated in two hours by one gram of the necrotic liver was 5.5 mg., while it was 1.1 mg. in case of the non-necrotic liver.

Experiments to produce massive hepatic necrosis in mice have been planned and are in progress.

**List of Papers Published from the Haffkine Institute during the Year 1955.**

1. BHAGAVAN, N. V., NIMBKAR, Y. S. & RAO, S. S.—Antigenic Analysis of Pasteurella. *Current Science*. 24 : 85-86, 1955.
2. DUTTA, N. C., KRISHNAN, L. S. & RAO, M. V. R.—Dietetic Hepatic Lesions. I. Influence of Protein Level and Vitamin B<sub>2</sub> Complex Deficiency, on the Liver of Rats. (In Press).
3. DUTTA, N. K. & HABBU, M. K.—Experimental Cholera in Infant Rabbits ; A Method for Chemo-therapeutic Investigation. *Brit. J. Pharmacol. and Chemo.* 10 : 153-159, 1955.
4. HAZRA, A. K. & HABBU, M. K.—Combined Prophylactic of Typhoid Vaccine and Tetanus Toxoid. (In Press).
5. PATEL, S. M., KUMTA, U. S. & RAO, M. V. R.—Stability of Vitamin A in Aqueous Dispersions and in Oils. *J. Sc. Ind. Res.* 14c : 17-21, 1955.
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7. RAO, SHANTA, S. & RAO, S. S.—Action of Crystalline Trypsin on Egg Albumin. Part I. Action on Native Heat-denatured and Formalinised Crystalline Egg Albumin. *Ind. J. Med. Res.* 43 : 617-621, 1955.
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12. SETHI, K. & SOMAN, D. W.—Laboratory Diagnosis of Small-pox. *Ind. Med. Gazette*. 89 : 468-474, 1954.
13. SOMAN, D. W.—A Report on the Isolation of Two Strains of Influenza B. Virus in India. *Ind. Med. Gazette*. 89 : 358-363, 1954.



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1. OSCAR FELSENFELD, SOMAN, D. W., THORA WATERS & SACHIKO J. ISHIHARA (1951).—Studies on recently isolated cholera vibrios. Revaluation of culture methods. *J Bacteriology* Vol. 62. No. 2 Aug. p. 175.
2. OSCAR FELSENFELD, SOMAN, D. W., SACHIKO ISHIHARA, THORA WATERS & JENNETTE NORSEN (1951).—In vitro Sensitivity of recently isolated Cholera vibrios to ten antibiotics. *Proc. Soc. Exp. Biol. & Med.* Vol. 77. pp. 287-288.
3. OSCAR FELSENFELD, SOMAN, D. W., TAMA YOSHIMURA, THORA WATERS & SACHIKO J. ISHIHARA (1951).—Serologic Cross Reactivity of some entero bacteriaceae isolated in the United States with cholera vibrios. *Proc. Exp. Soc. Biol. & Med.* Vol. 77. pp. 284-286.
4. OSCAR FELSENFELD, SOMAN, D. W. (1952).—Treatment of Cholera with antibiotics. *Annals of the New York Academi of Sciences*, Vol. 55. Art 6. pp. 1050-1062, December 1952.

D. W. SOMAN,

Parel, Bombay, April 14, 1956.

Director, Haffkine Institute.

