



*Government of Bombay*

**REPORT OF THE HAFFKINE INSTITUTE  
FOR THE YEAR 1956**

by

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Printed in India by the Manager, Government Central Press, Bombay,  
Published by the Director, Government Printing, Publications and  
Stationery, Bombay State, Bombay.

Obtainable from the Government Publications Sales Depot, Institute of Science  
Building, Fort, Bombay (for purchasers in Bombay City); from the Government  
Book Depot, Charni Road Gardens, Bombay 4 (for orders from the mofussil) or  
through the High Commissioner for India, India House, Aldwych, London, W.C.2  
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# REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1956.

## PART I.

### GENERAL.

#### I. Staff.

##### *Director.*

Dr. D. W. Soman, M.D. (Bom.), B.Hy. (Bom.), D.P.H., R.C.P.S. (Eng.), D.T.M. & H. (Lond.).

##### *Assistant Directors.*

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. (Dunelm), 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 upto 4th July 1956.

Dr. M. V. Sant, M.D. (Bom.), F.C.P.S., B.M.S. (I), Assistant Director I/c Department of Clinical Pathology, from 5th July 1956 (F.N.).

##### *Other Sectional Officers.*

Dr. K. V. Kulkarni, M.B.B.S., B.M.S. II, Medical Officer I/c Blood Collecting Unit.

Dr. (Shrimati) Alamela Venkataraman, B.A., M.Sc., Ph.D., Senior Biochemist, Department of Biochemistry.

Shri V. D. Sardeshpande, B.Sc., Assistant Bacteriologist, I/c Media Department.

Shri P. K. Sholapurwala, B.Sc., LL.B., Business Manager.

Shri S. M. Nadkarni, M.Sc., Stores Officer (upto 11th June 1956).

Shri B. O. Shah, B.Com., Cost Accounts Officer (from 30th August 1956).

Shri W. H. Saraph, M.Sc., Personal Assistant to the Director.

Shri N. Lakshminarayan, L.M.E. (Mysore), L.E.E. (Mysore), Maintenance Engineer (from 16th June 1956).

Shri M. V. Kamat, B.A., LL.B., Librarian.

Shri D. A. Upadhye, B.A., Office Superintendent (from 2nd April 1956).

## II. General Review.

The general trend of activities of the Institute continued to be the same as in previous years, excepting that, during the latter part of the year, due to the reorganisation of States, the demands for our biologicals, etc. increased. 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 Institute's resources in funds and personnel. The Institute continued to supply its products to Approved Chemists under the Employees' State Insurance Scheme as in the past. During the year, three new products, viz., Vitamin A Forte, Nicotinamide and Magnesium Trisilicate Tablets were introduced for the Employees' State Insurance Scheme. The demand for plague vaccine was practically the same. The demand for T. A. B. Vaccine was slightly higher than that of last year. Special mention may be made of the demand for Cholera Vaccine, which increased by more than three times that of last year, because of the severe epidemic of cholera in the country. 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 samples sent by them under the Prohibition Scheme. A large number of such samples were analysed. The pressing demands for the biological products manufactured at this Institute is a distinct sign of their popularity and the confidence they enjoy at the hands of the public as well as the medical profession. Despite the pressure of routine work, research work was carried out by the senior members of the staff, which is detailed in Part II of this 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, Government of India, Indian Council of Medical Research, Council of Scientific and Industrial Research and Fellowships offered by Messrs. Unichem Laboratories.

The Institute played, as before, an important role in the dissemination of 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. The Library of the Haffkine Institute also serves as a reference library and is open to all research workers in the field of Biological and Medical Sciences. In addition to the members of the Institute staff, 1,798 readers from different Colleges and Institutions took advantage of the Library. During the year, contents of the entire Library were checked, rearranged and taken on New Accession Registers. The activity of the Critics Club also continued as before and three guest speakers gave a talk in the meetings in addition to the talks given by staff members. The visits of distinguished visitors has become almost a regular feature of the Institute. The list of such visitors is given in Part I of this Report, which includes Hon'ble Ministers, Foreign Scientists and Members of various Missions and Delegations. In addition, the Institute also offered special facilities for research to Dr. Gordon Ball and Mrs. Meridian.

Ball, Professors from University of California, U.S.A. who worked on haemoprotozoa of reptiles and leptospirosis respectively, for about three months.

Two new Officers, viz, the Cost Accounts Officer and the Maintenance Engineer, were appointed in the latter part of the year.

The total sales of the financial year amounted to about Rs. 25 lakhs, while the expenditure amounted to about Rs. 21 lakhs.

As will be seen from the report, the demands for plague, cholera and T. A. B. Vaccines from within and outside India increased, particularly those for Cholera Vaccine. A total of about 10·7 million millilitres of plague and cholera vaccines were issued during the year. Media were supplied to various departments of the Institute as before. Large quantities of casein hydrolysate medium for preparation of cholera vaccine and aerobic and anaerobic media for testing the sterility of vaccines and sera and parenteral solutions were prepared. The production and sale of parenteral solutions increased as compared to last year. This was due to the outbreak of cholera epidemic. During the year, a small room of this Department was airconditioned which greatly facilitated the work of this Section as well as satisfied the requirements of the Drugs Act. The Section of Public Health Chemistry 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. It also examined other samples, such as sewage, milk powder and ice.

The Department of Antitoxins and Sera continued to receive heavy demands for its products, particularly for lyophilised Antivenene. As a result of the overall increased demands, the department had to divert a good deal of its attention to production. The demands are still being received from foreign as well as local sources in progressively increasing quantities. It is feared that with the bigger New State created and having to meet all local demands of the State, it would be very difficult to spare material supplies for extra-provincial demands. To enable the department to meet the increased demands on its products, it is very essential to develop the Pimpri Farm into a production unit, expand the existing capacity at the Institute and also extend staff facilities.

The Department of Entomology continued to render assistance in connection with a number of enquiries regarding rat traps, rodenticides and rats in addition to identification of fleas, mosquitoes and a number of insects of medical importance. Work on statistical analysis of rat measurements and rat ecto-parasites was completed. Studies on pulicidal value of pyrethrum, breeding of *X. cheopis*, *X. astia* etc. were continued. Work on *Echis carinatus* was completed during the year. Studies on common poisonous snakes and sexual cycle in the snake, *Natrix piscator*, were continued.

The Department of Clinical Pathology continued to carry out, as in previous years, bacteriological, serological, biological and histopathological examinations etc. on clinical material received from Government Hospitals, private practitioners etc., All essential tests for the diagnosis of infectious diseases

were carried out free of charge. The Typhoid Scheme entered its ninth year and was very popular and useful. An overall increase was noticed in the number of clinical samples examined as compared to those of last year. There was a steady and increasing demand for diagnostic reagents, while there was a fall in the demands for stains. The number of histopathological specimens showed a slight increase. Special investigations were carried out on *in vitro* toxigenicity test of *C. diphtheria*, maintenance of Nicol's strain (*Treponema Pallidum*) in rabbits for V. D. Serology, Salmonellosis and Leptospirosis in animals and isolation of phages from *S. typhosa* etc.

The Department of Pharmacology continued to work under heavy pressure, there being 27 per cent. increase in the number of samples tested as compared with the figure of the last year. Thirty-seven per cent. of the samples tested did not comply with the required standard. However, a gradual improvement in the quality of the products is being observed. In addition to samples from Bombay State, samples were also received, as before, from different States of the Indian Union, such as, Delhi, U. P., M.P., Bihar and Himachal Pradesh. The number of samples tested on behalf of the commercial firms showed a slight decrease. Most of these samples were complex mixtures of vitamins. A large number of samples were tested for other departments of the Government of Bombay, viz., Prohibition and Excise and Police. Quite a large number of these samples were coloured alcohol substituted for genuine preparations. Out of the samples examined under the Drugs Act, 39 per cent. were not of standard quality. This shows a distinct improvement in the quality of drugs tested as compared to the figure of 45 per cent. of the last year. Investigations were also carried out on drugs which acted as leech-repellents, occurrence of reserpine in *Alstonia scholaris* and action of Albamycin in experimental cholera etc.

The manufacturing activity of the Department of Chemotherapy showed a steep rise. The total sale of its products increased considerably as compared to that of last year, in spite of the downward revision of prices of most of its products. During the year, three new products, viz., Vitamin A Forte, Nicotinamide and Magnesium Trisilicate Tablets were introduced. Preliminary experiments on the coating tablets have confirmed that by adopting this procedure, the self-life of the tablets could be extended. About 700 lbs. of pink sub-standard quinine powder was reprocessed and converted into B.P. Specifications which is being used for the preparation of Quinine Sulphate Tablets. The development of special procedure for the preparation of a very important intermediate, *para-nitro-acetophenone*, used in the manufacture of chloromycetin is a feature of the department's research activities. This process is being protected by filing an application for a patent, which should prove extremely useful.

The Department of Biochemistry continued to render 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 seamen for their Glucose Tolerance Test. Analysis of milk samples on behalf of the public has become a regular feature of the Department. Biochemical investigations were carried out in cases of cirrhosis and other conditions.

The Department of Virus Diseases including Rabies continued its activities in full swing. The production and supply of Antirabic Vaccine showed rise as compared to last year. The number of persons treated at the Haffkine Institute Treatment Centre also showed rise. Fifteen additional Antirabic Treatment Centres were opened. Diagnosis of rabies in animals was carried out as before. The number of specimens received for examination from Bombay and outstations increased as compared to that of last year. Four human brains were also received for the diagnosis of rabies. Diagnostic Laboratory Tests for primary atypical pneumonia, herpes, L. G. V., etc., were also carried out on materials received from different local hospitals. A special study of tissue culture techniques was undertaken to develop methods for diagnosis of new viral agents. The World Health Organisation Influenza Centre established in the Virus Section is doing excellent work. Four strains of influenza were isolated during the year. Field trials of hyperimmune rabies serum undertaken last year were further continued. As before, yellow fever inoculations were carried out by the Yellow Fever Diagnostic Unit housed at this Institute on behalf of the Government of India for air or sea passengers proceeding to or passing through Yellow Fever endemic areas. The Yellow Fever vaccine was regularly tested in the department for its potency.

During the year, the Blood Bank Department has maintained an upward trend in all its activities. The demand for dried human plasma increased as compared to the last year. With the establishment of the composite State, it is expected that the demand from State Hospitals may increase in the near future. However, the position regarding blood donors remained unsatisfactory as before, which has adversely affected the donor procurement programme. The demand for blood grouping sera has shown considerable increase. The Indian Council of Medical Research has sanctioned a scheme for the preparation of standard blood grouping sera as a part of the Second Five Year Plan. Due to the alterations carried out in connection with the Desivac Plant and the airconditioned room in which it is made to open, the risk of serial contaminations has been greatly minimised. A deep-freeze cabinet of 30 cubic feet. capacity was also installed, which has resulted in increasing the efficiency of Desivac Plant. As before, Rh investigations were carried out on behalf of hospitals and private practitioners.

On the whole, during the year under review, the Institute registered further progress not only in the field of manufacture, but also on research side and thus rendered valuable service to the public.

### III. Department of Vaccines.

During the year under review, the demand for Plague Vaccine was practically the same, while that for T.A.B. Vaccine was slightly higher than the last year. However, there was heavy demand for cholera vaccine during the year due to outbreak of epidemic in the country. Cholera Vaccine production had to be put up to a higher level during the months of July and August to meet the demands of 3.3 million millilitres during these two months.

After the re-organisation of New States, there was heavy demand for Cholera Vaccine from the newly merged areas of the Greater Bombay State. As large a quantity as 3.5 million millilitres of Cholera Vaccine had to be supplied

during the months of November and December. This shows the possibility of future increased demands for Cholera and Typhoid Vaccine with the merging of new areas into the Bombay State. It is gratifying to note that the heavy demand had been successfully met.

1·1 million millilitres of Plague Vaccine, 9·6 million millilitres of Cholera Vaccine and 3,00,411 millilitres of T.A.B. Vaccine were issued during the year as against 1·1 million millilitres of Plague, 2·7 million millilitres of Cholera and 2,74,099 millilitres of T.A.B. Vaccine during the year 1955.

The total quantity of Vaccine issued during the year is 1,11,23,693 millilitres. The demands for vaccines were received from all parts of the country and in respect of Plague Vaccine from Burma also.

The monthly distribution of Plague Vaccine is given below:—

#### A. PLAGUE VACCINE. •

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

| Months.   |    |    |    |    | Quantities in millilitres. |
|-----------|----|----|----|----|----------------------------|
| January   | .. | .. | .. | .. | 51,000                     |
| February  | .. | .. | .. | .. | 111,880                    |
| March     | .. | .. | .. | .. | 240,940                    |
| April     | .. | .. | .. | .. | 51,040                     |
| May       | .. | .. | .. | .. | 40,900                     |
| June      | .. | .. | .. | .. | 920                        |
| July      | .. | .. | .. | .. | 2,240                      |
| August    | .. | .. | .. | .. | 1,380                      |
| September | .. | .. | .. | .. | 259,540                    |
| October   | .. | .. | .. | .. | 150,920                    |
| November  | .. | .. | .. | .. | 193,700                    |
| December  | .. | .. | .. | .. | 52,940                     |
| Total ... |    |    |    |    | 1,157,402                  |



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                 |
| 1952-1956 | .. | .. | .. | .. | 9,370,581                  |
| Total     |    |    |    |    | 262,466,704                |

The Plague Vaccine is issued in India and 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    | ..  | .. | .. | ... | 31,380                     |
| Bhopal    | ..  | .. | .. | ..  | 22                         |
| Bihar     | ..  | .. | .. | ... | 31,800                     |
| Bengal    | ..  | .. | .. | ..  | 170,500                    |
| Assam     | ..  | .. | .. | ..  | 182,300                    |
| Delhi     | ..  | .. | .. | ..  | 320                        |
| Hyderabad | ... | .. | .. | ..  | 22,020                     |

| India.                    |    |    |    | Quantities in millilitres. |           |
|---------------------------|----|----|----|----------------------------|-----------|
| Madras                    | .. | .. | .. | 50,020                     |           |
| Madhya Pradesh            | .. | .. | .. | 158,500                    |           |
| Punjab                    | .. | .. | .. | 3,000                      |           |
| T. C. States              | .. | .. | .. | 4,620                      |           |
| U. P.                     | .. | .. | .. | 301,920                    |           |
| V. P.                     | .. | .. | .. | 1,000                      |           |
| Total                     |    |    |    | 957,402                    | 957,402   |
| <i>Foreign Countries—</i> |    |    |    |                            |           |
| Burma                     | .. | .. | .. | 2,00,000                   | 2,00,000  |
| Grand Total               |    |    |    | ..                         | 11,57,402 |

#### B. CHOLERA VACCINE.

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

| Months.   |    |    |    | Quantities in millilitres. |           |
|-----------|----|----|----|----------------------------|-----------|
| January   | .. | .. | .. | 96,754                     |           |
| February  | .. | .. | .. | 142,143                    |           |
| March     | .. | .. | .. | 746,790                    |           |
| April     | .. | .. | .. | 286,931                    |           |
| May       | .. | .. | .. | 134,958                    |           |
| June      | .. | .. | .. | 283,441                    |           |
| July      | .. | .. | .. | 1,295,253                  |           |
| August    | .. | .. | .. | 1,991,950                  |           |
| September | .. | .. | .. | 443,799                    |           |
| October   | .. | .. | .. | 725,961                    |           |
| November  | .. | .. | .. | 1,774,328                  |           |
| December  | .. | .. | .. | 1,743,572                  | 9,665,880 |

## 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   | ... | ... | ... | ...                        | 7,431   |
| February  | ... | ... | ... | ...                        | 6,990   |
| March     | ... | ... | ... | ...                        | 11,069  |
| April     | ... | ... | ... | ...                        | 17,371  |
| May       | ... | ... | ... | ...                        | 23,218  |
| June      | ... | ... | ... | ...                        | 49,889  |
| July      | ... | ... | ... | ...                        | 67,289  |
| August    | ... | ... | ... | ...                        | 35,869  |
| September | ... | ... | ... | ...                        | 28,505  |
| October   | ... | ... | ... | ...                        | 26,497  |
| November  | ... | ... | ... | ...                        | 14,013  |
| December  | ... | ... | ... | ...                        | 12,270  |
|           |     |     |     | Total ...                  | 300,411 |

## D. MEDIA SECTION.

The Media Section is carrying out the following functions:—

- (1) Production of media for the routine manufacture of vaccines.
- (2) Preparation of media for routine and research work carried out in the various departments of the Institute.
- (3) Manufacture of Parenteral Solutions.

During the year, 3,155 litres of Casein Hydrolysate Medium for Cholera Vaccine were prepared. For the preparation of Typhoid Vaccine, 2,828 Roux Bottles containing 200 ml. of agar in acid digest broth each were prepared. For testing the sterility of vaccines, sera and parenteral solutions, 45,000 bottles of each of Aerobic and Anaerobic Medium were prepared. The Parenteral Solutions Section showed greater activity than the previous years. The increased activity of the department was due to the prevalence of cholera epidemic in the Bombay State during the latter part of the year. The preparation of parenteral solutions was greatly facilitated by air conditioning a small room which also satisfied the requirements of the Drugs Act. The requirements from the various Hospitals in the State and also outside have been fully met with.

*Sale of Glucose Saline etc. during 1956.*

| Serial No. | Name of Product.                              | Number of bottles sold. |         |
|------------|-----------------------------------------------|-------------------------|---------|
|            |                                               | 1955                    | 1956    |
| 1          | Glucose Saline, 500 c.c. ...                  | 39,906                  | 52,55   |
| 2          | 5 per cent. Glucose Solution, 500 c.c. ...    | 17,693                  | 22,763  |
| 3          | Hypertonic Saline, 500 c.c. ...               | 1,120                   | 7,414   |
| 4          | Normal Saline, 500 c.c. ...                   | 4,317                   | 6,224   |
| 5          | Protein Hydrolysate, 400 c.c. ...             | 7,583                   | 5,258   |
| 6          | 25 per cent. Glucose Solution, 500 c.c. ...   | 2,352                   | 2,420   |
| 7          | 25 per cent. Glucose Solution, 100 c.c. ...   | 2,204                   | 1,910   |
| 8          | A.C.D. Solution (Anti-Coagulant Solution) ... | 1,314                   | 1,726   |
| 9          | Sodium Sulphate, 500 c.c. ...                 | 428                     | 707     |
| 10         | 50 per cent. Glucose Solution, 100 c.c. ...   | 796                     | 682     |
| 11         | Sodium Citrate, 500 c.c. ...                  | 578                     | 394     |
| 12         | Distilled Water, 500 c.c. ...                 | 6,069                   | 6,923   |
| Total ...  |                                               | 84,360                  | 108,973 |

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

This section mainly examined samples of water for ascertaining their fitness for drinking, sanitary and industrial purposes. It also examined other samples, such as sewage, milk powder, ice, etc.

These samples were received from Bombay State as well as from other States. The following table gives a summary of the various examinations carried out :—

| Sample examined.     |     | Nature of examination. |                 | Purpose of examination. |                                | No. of samples examined. |
|----------------------|-----|------------------------|-----------------|-------------------------|--------------------------------|--------------------------|
| Water                | ... | ...                    | Bacteriological | ...                     | Drinking ...                   | 140                      |
| Water                | ... | ...                    | Do.             | ...                     | Swimming Pool                  | 1                        |
| Water                | ... | ...                    | Chemical        | ...                     | Sanitary and Industrial        | 33                       |
| Industrial Effluents | ... | ...                    | Bacteriological | ...                     | Do.                            | 2                        |
| Do.                  | ... | ...                    | Chemical        | ...                     | Do.                            | 2                        |
| Ice                  | ... | ...                    | Bacteriological | ...                     | Fitness for human consumption. | 1                        |
| Skimmed Milk Powder  | ... | ...                    | Do.             | ...                     | Do.                            | 3                        |
| Total ...            |     |                        |                 |                         |                                | 182                      |

**IV. Department of Antitoxins and Sera.**

During the year under review, the department had to strain all available resources to meet the ever-increasing demands for various sera and toxoids from all over the country. As compared to the total production of sera in

1955 amounting to 14,08,370 ml., the corresponding production during the year under review was 14,75,400 ml. This increase over the peak production capacity is fully indicative of the very urgent need for the expansion of the department at an early date if the high quality and increasing demands are to be fulfilled satisfactorily. Since its beginning in 1942, the department has been constantly trying to do more and more by way of production. A farm at Pimpri was acquired on a permanent basis in 1953 to house horses, as a stand-by. This resulted, as was correctly anticipated, in appreciable increase in production, since the available space in the Institute was utilised to house only those horses which were under active immunization, and horses under rest were housed at Pimpri.

During the last few years, it is being felt that the Pimpri Farm could be developed with advantage to a production unit to meet the ever-increasing demands for our antitoxins and sera.

The lyophilised antivenin has been increasingly in demand, which is due to its ability to meet the special storage conditions prevailing in the country, as it requires no cold storage. It is felt that under the prevalent storage facilities, if all sera are issued in a lyophilised form to meet the special conditions of the whole country, as was originally intended, it would be doing a great service towards the alleviation of human suffering. This can be done if the production is further increased and more facilities by way of staff and space are provided. Otherwise, it would be necessary to make available adequate cold storage facilities to meet the present needs of the department.

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 176. Besides, these, 61 horses at Pimpri Stud Farm were undergoing preliminary immunization at the end of the year. The horses under immunization were grouped as follows :—

| Antitoxins. |                                            | Number of horses used. |     |
|-------------|--------------------------------------------|------------------------|-----|
| 1.          | Tetanus Antitoxin .. ..                    | ..                     | 64  |
| 2.          | Diphtheria Antitoxin .. ..                 | ..                     | 47  |
| 3.          | Antivenin .. ..                            | ..                     | 34  |
| 4.          | Gas Gangrene Antitoxin (Welchii) .. ..     | ..                     | 14  |
| 5.          | Gas Gangrene Antitoxin (Oedematiens) .. .. | ..                     | 2   |
| 6.          | Gas Gangrene Antitoxin (Septique) .. ..    | ..                     | 2   |
| 7.          | Anti-dysentery Serum .. ..                 | ..                     | 2   |
| 8.          | Anti-rabic .. ..                           | ..                     | 7   |
| 9.          | Anti-plague Serum .. ..                    | ..                     | 2   |
| 10.         | Normal Horse Serum .. ..                   | ..                     | 2   |
| Total ..    |                                            | ..                     | 176 |

2. *Preparation of Toxins and Toxoids.*—During the year, diphtheria, tetanus, welchii, oedematiens and septique toxins were produced. Part of the diphtheria and tetanus toxins were converted into toxoids for immunization of horses. A portion of these toxoids was also purified for human use.

| Types of Toxins and Toxoids.         |     |    |    | Quantities in millilitres. |
|--------------------------------------|-----|----|----|----------------------------|
| Tetanus Toxin                        | ..  | .. | .. | 448,200                    |
| Diphtheria Toxin                     | ..  | .. | .. | 653,000                    |
| Welchii Toxin                        | ..  | .. | .. | 55,000                     |
| Oedematiens Toxin                    | ..  | .. | .. | 12,500                     |
| Septique Toxin                       | ..  | .. | .. | 42,500                     |
| Tetanus Toxin converted to Toxoid    | ... | .. | .. | 231,200                    |
| Diphtheria Toxin converted to Toxoid | ..  | .. | .. | 346,700                    |

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

#### I. CONCENTRATED SERA.

|                            |    |    |    | Amount of concentrated serum in millilitres. |
|----------------------------|----|----|----|----------------------------------------------|
| (1) Tetanus Antitoxin      | .. | .. | .. | 678,700                                      |
| (2) Diphtheria Antitoxin   | .. | .. | .. | 313,400                                      |
| (3) Antivenin              | .. | .. | .. | 365,300                                      |
| (4) Gas Gangrene Antitoxin | .. | .. | .. | 48,000                                       |
| Total                      | .. | .. | .. | 1,405,400                                    |

#### II. NATURAL SERA.

|                    |    |    |    | Amount in millilitres. |
|--------------------|----|----|----|------------------------|
| Normal Horse Serum | .. | .. | .. | 70,700                 |

4. *Lyophilisation of Sera.*—Only Polyvalent Anti-snake-venom Serum was issued in a lyophilised form during the year. The amount of serum so issued was 346,810 ml.

33 boxes of Lyophilised Schick Test Toxin, each containing enough toxin for 25 tests, were issued.

5. *Supply.*—The following quantities of antitoxins, toxoids and sera were issued during the year :—

| Name of Sera and Toxoids.                            |    |    |    | Quantities in millilitres. |
|------------------------------------------------------|----|----|----|----------------------------|
| 1. <i>Tetanus Antitoxin.</i> —                       |    |    |    |                            |
| (a) Prophylactic 3,000 I. U. (1928) in 2 c.c.        | .. | .. | .. | 811,070                    |
| (b) Curative 10,000 I.U. (1928) in 5 c.c.            | .. | .. | .. | 31,520                     |
| (c) Curative 20,000 I.U. (1928) in 10 c.c.           | .. | .. | .. | 144,700                    |
| (d) Highly concentrated 20,000 I.U. (1928) in 4 c.c. | .. | .. | .. | 164,372                    |

| Name of Sera and Toxoids.                                                                                                                                  | Quantities in millilitres. |
|------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|
| 2. <i>Diphtheria Antitoxin</i> .—                                                                                                                          |                            |
| (a) Prophylactic 1,000 I.U. in 2 c.c. ..                                                                                                                   | 3,488                      |
| (b) Curative 5,000 I.U. in 5 c.c. ..                                                                                                                       | 6,195                      |
| (c) Curative 10,000 I.U. in 10 c.c. ..                                                                                                                     | 162,580                    |
| (d) Highly concentrated 10,000 I.U. in 5 c.c. ..                                                                                                           | 9,655                      |
| 3. <i>Gas-Gangrene Antitoxin</i> .—                                                                                                                        |                            |
| (a) 3,000 I.U. Welchii Antitoxin in 10 c.c. ..                                                                                                             | 114,910                    |
| (b) 9,000 I.U. Welchii, 4500 I.U. Septique and 9,000 I.U. Oedematiens Antitoxins, in a volume not exceeding 15 c.c. highly concentrated and purified .. .. | 11,055                     |
| 4. Anti-plague Serum 20 c.c. ... ..                                                                                                                        | Nil                        |
| 5. Lyophilised Polyvalent Anti-snake Venom Serum in 10 c.c. ... ..                                                                                         | 346,810                    |
| 6. <i>Anti-dysentery Serum</i> .—                                                                                                                          |                            |
| (a) Natural, 20 c.c. ... ..                                                                                                                                | Nil.                       |
| (b) Concentrated, 4000 I.U. in 5 c.c. ... ..                                                                                                               | 890                        |
| 7. <i>Tetanus Toxoid</i> .—                                                                                                                                |                            |
| (a) 10 c.c. ... ..                                                                                                                                         | 5,790                      |
| (b) 5 c.c. ... ..                                                                                                                                          | 1,405                      |
| (c) 1 c.c. ... ..                                                                                                                                          | 52,479                     |
| 8. <i>Diphtheria Toxoid, P. T. A. P.</i> .—                                                                                                                |                            |
| (a) 5 c.c. ... ..                                                                                                                                          | 17,050                     |
| (b) 1 c.c. ... ..                                                                                                                                          | 2,487                      |
| 9. Normal Horse Serum in 10 c.c. ... ..                                                                                                                    | 3,030                      |
| 10. Lyophilised Schick Test Toxin for 25 tests ... ..                                                                                                      | 33 boxes.                  |

Total 18,10,275 ml. of Antitoxins and Sera and 79,211 ml. of Toxoids.

In addition to the above, 28,710 ml. of natural horse serum was supplied to local hospitals.

6. *Other Activities of the Department*.—The department undertook the tests of samples of Antitoxins and other biologicals as well as sterility tests for surgical catguts submitted either directly or on behalf of the Drug Control Organisation. Three indigenous drugs said to be efficacious in snake bite cases, when tested in mice, were found to be ineffective in neutralizing snake venom.

## V. Department of Entomology.

During the year, this department dealt with a number of enquiries regarding rat-traps, rodenticides and rats. Identification of fleas, mosquitoes and a number of insects of medical importance was done on behalf of local bodies and Government agencies.

Work on the statistical analysis of rat measurements and rat-ectoparasites was completed. Conclusions drawn from these studies are incorporated in Part II of this report.

The studies on the pulicidal value of pyrethrum were continued. Pyrethrum flowers, after being extracted, were converted into smoke candles. Spreaders and wetting agents were tried to get a proper consistency in the performance of a suspension formulation out of pyrethrum extract. The pyrethrum powder, which has been tried in the laboratory, is being tested, on a comparative basis with other insecticides in the field, by a team of WHO workers in Uttar Pradesh.

Breeding of *X. cheopis* and *X. astia* was continued. Special attention was paid to vary the different food requirements of the larvae and to observe the effect on the emergence of the sex-ratio in adult fleas as compared to those reared on normal diets. Studies were also started to investigate the feeding and blocking mechanism of five kinds of common fleas, i.e. *X. cheopis*, *X. astia*, *C. canis*, *C. felis* and *E. gallinacea*.

Guidance was given to four post-graduate students in the department and as before demonstrations were given to visitors on Thursdays regarding extraction of snake venoms.

#### A. SNAKES.

Twenty-five specimens of snakes were referred to this department for identification. Of particular interest was *Callophis nigriscens* sent from Mahabaleshwar which is known to be very poisonous. The maximum number of snakes available for venom extraction during the year, was : Cobra 39, Krait 40, Russell's Viper 45 and Saw-scaled Viper 1,335. Seventy non-poisonous snakes were handled for experiments.

Studies were continued to find out the sexual cycle in the snake *Natrix piscator*. The morphological studies on common poisonous snakes were continued. The work on *Echis carinatus* has been completed.

Venoms were collected every month. Table No. 1 below gives an account of the total amount of venom produced at the Institute and of the amount supplied.

Table 1.

| Name of Snake.  |     |     |     | Venom produced.<br>(in gms.) | Venom supplied.<br>(in gms.) |
|-----------------|-----|-----|-----|------------------------------|------------------------------|
| Cobra           | ... | ... | ... | 64·4462                      | 64·0054                      |
| Russell's Viper | ... | ... | ... | 35·4449                      | 75·0754*                     |
| Krait           | ... | ... | ... | 5·0350                       | 4·9150                       |
| Echis           | ... | ... | ... | 12·7747                      | 5·0607                       |

\*The figure includes balance carried forward from last year.



## 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, 2,790 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 first classified according to species. Dead rats were dissected and examined for post-mortem signs and plague infection. In doubtful cases, diagnosis was confirmed by smear and cultural examination. In addition to this, a large number of live rats from the suburbs was also examined. 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.     |     |     | Number of rats used in experiments. | Number of rats died. | Mortality per cent. |
|----------------------|-----|-----|-------------------------------------|----------------------|---------------------|
| <i>R. rattus</i>     | ... | ... | 121                                 | 9                    | 7.5                 |
| <i>R. norvegicus</i> | ... | ... | 89                                  | 2                    | 2.2                 |
| <i>Gunomys kok</i>   | ... | ... | 89                                  | 67                   | 75.2                |

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

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

In all 11 dead rats and 36 smears from dead rats were received from different towns of the Bombay State. No plague infection was detected. During this year, 631 live rats were also received from the Health Officer of the Port of Bombay. Of these, 451 belonged to the species of *Rattus rattus rufescens*, 25 *Rattus norvegicus*, 2 *Gunomys kok* and 153 *Mus dubius*. None of them was found to be plague infected.

During the year under review, 9 rats were received from the City of Bombay through other agencies or private persons. None of them was found to be plague infected. 78 rats caught around suspected areas from the City of Bombay including the suburbs were used for determining the flea-index and also for the detection of infection in the fleas by flea-pool inoculation in mice. No plague infection could be detected either in rats or in fleas.

**Table 1.**

*Showing rats received at the Institute from the Bombay Municipality during 1956.*

| Month.        | Alive.    | Dead. | Total.    |
|---------------|-----------|-------|-----------|
| January ...   | 76,543    | 6     | 76,549    |
| February ...  | 88,791    | 1     | 88,792    |
| March ...     | 82,382    | 2     | 82,384    |
| April ...     | 80,503    | 1     | 80,504    |
| May ...       | 78,625    | 3     | 78,628    |
| June ...      | 83,452    | 3     | 83,455    |
| July ...      | 86,513    | 3     | 86,516    |
| August ...    | 85,760    | 5     | 85,765    |
| September ... | 82,675    | 4     | 82,679    |
| October ...   | 92,038    | 1     | 92,039    |
| November ...  | 86,875    | 3     | 86,878    |
| December ...  | 93,793    | 5     | 93,798    |
| Total ...     | 10,17,952 | 37    | 1,017,989 |

**Table 2.**

*Showing dead rats examined for plague during 1956.*

| Month.        | Dead rats examined. | Found plague infected. |
|---------------|---------------------|------------------------|
| January ...   | 6                   | Nil.                   |
| February ...  | 1                   | Nil.                   |
| March ...     | 2                   | Nil.                   |
| April ...     | ...                 | Nil.                   |
| May ...       | 2                   | Nil.                   |
| June ...      | 3                   | Nil.                   |
| July ...      | 2                   | Nil.                   |
| August ...    | 3                   | Nil.                   |
| September ... | 3                   | Nil.                   |
| October ...   | ...                 | Nil.                   |
| November ...  | ...                 | Nil.                   |
| December ...  | 5                   | Nil.                   |
| Total ...     | 27                  | Nil.                   |

In addition to dead rats, 21,241 live rats from the City including the suburbs and 7,741 live rats from the Bombay Port Trust area were examined during the year. The total number of live and dead rats examined is 28,739.

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*Showing the number and percentage of different species of rats  
trapped alive in the City of Bombay (including the Suburbs) during 1956.*

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Table

*Showing the number and percentage of different species of rats trapped alive*

| Species.                       | January.   |             | February. |             | March. |             |
|--------------------------------|------------|-------------|-----------|-------------|--------|-------------|
|                                | Number     | Per-centage | Number    | Per-centage | Number | Per-centage |
| <i>Rattus rattus rufescens</i> | ... 19,265 | 25·2        | 20,211    | 22·8        | 16,170 | 19·6        |
| <i>Rattus norvegicus</i>       | ... 12,211 | 16·0        | 13,885    | 15·6        | 12,764 | 15·5        |
| <i>Gunomys kok</i> ...         | ... 35,985 | 47·0        | 45,234    | 51·0        | 44,804 | 54·5        |
| <i>Bandicota malabarica</i>    | ... 546    | 0·7         | 571       | 0·6         | 369    | 0·4         |
| <i>Mus dubius</i> ...          | ... 3,150  | 4·1         | 3,196     | 3·6         | 2,481  | 3·0         |
| <i>Suncus caeruleus</i> ...    | ... 5,386  | 7·0         | 5,694     | 6·4         | 5,794  | 7·0         |
| Unidentifiable ...             | ... ..     | ...         | ... ..    | ...         | ... .. | ...         |
| Total                          | ... 76,543 | 100·0       | 88,791    | 100·0       | 82,382 | 100·0       |

  

| Species.                       | July.      |             | August. |             | September. |             |
|--------------------------------|------------|-------------|---------|-------------|------------|-------------|
|                                | Number     | Per-centage | Number  | Per-centage | Number     | Per-centage |
| <i>Rattus rattus rufescens</i> | ... 17,947 | 20·7        | 19,034  | 22·2        | 18,867     | 22·7        |
| <i>Rattus norvegicus</i>       | ... 13,394 | 15·5        | 13,707  | 16·0        | 13,592     | 16·4        |
| <i>Gunomys kok</i> ...         | ... 44,301 | 51·2        | 41,894  | 48·8        | 39,731     | 48·1        |
| <i>Bandicota malabarica</i>    | ... 501    | 0·6         | 475     | 0·6         | 396        | 0·5         |
| <i>Mus dubius</i> ...          | ... 2,662  | 3·1         | 3,188   | 3·7         | 3,466      | 4·2         |
| <i>Suncus caeruleus</i> ...    | ... 7,708  | 8·9         | 7,462   | 8·7         | 6,623      | 8·1         |
| Unidentifiable ...             | ... ..     | ...         | ... ..  | ...         | ... ..     | ...         |
| Total                          | ... 83,513 | 100·0       | 85,760  | 100·0       | 82,675     | 100·0       |

3.

*in the City of Bombay (including the Suburbs) during 1956.*

| April. |             | May.   |             | June.  |             |
|--------|-------------|--------|-------------|--------|-------------|
| Number | Per-centage | Number | Per-centage | Number | Per-centage |
| 14,867 | 18.5        | 14,867 | 18.9        | 15,098 | 17.9        |
| 12,978 | 16.1        | 12,720 | 16.1        | 13,355 | 16.0        |
| 44,062 | 54.7        | 42,560 | 54.1        | 45,301 | 54.3        |
| 324    | 0.4         | 324    | 0.4         | 403    | 0.5         |
| 2,561  | 3.2         | 2,182  | 2.8         | 2,235  | 2.8         |
| 5,711  | 7.1         | 5,972  | 7.7         | 7,060  | 8.5         |
| .....  | .....       | .....  | .....       | .....  | .....       |
| 80,503 | 100.0       | 78,625 | 100.0       | 83,452 | 100.0       |

  

| October. |             | November. |             | December. |             | Whole year. |             |
|----------|-------------|-----------|-------------|-----------|-------------|-------------|-------------|
| Number   | Per-centage | Number    | Per-centage | Number    | Per-centage | Number      | Per-centage |
| 27,019   | 29.4        | 23,528    | 27.1        | 25,146    | 26.8        | 2,32,019    | 22.8        |
| 13,537   | 14.7        | 13,435    | 15.5        | 15,614    | 16.7        | 1,61,192    | 15.9        |
| 38,433   | 41.8        | 37,834    | 43.5        | 40,752    | 43.4        | 5,00,891    | 49.2        |
| 472      | 0.5         | 351       | 0.4         | 407       | 0.4         | 5,139       | 0.5         |
| 4,173    | 4.5         | 4,156     | 4.8         | 4,214     | 4.5         | 37,664      | 3.7         |
| 8,404    | 9.1         | 7,571     | 8.7         | 7,231     | 7.7         | 80,616      | 7.9         |
| ...      | ...         | ...       | ...         | 431       | 0.5         | 431         | 0.0         |
| 92,038   | 100.0       | 86,875    | 100.0       | 93,795    | 100.0       | 10,17,952   | 100.0       |

Table

*Showing the number and percentage of different species of rats found dead in*

| Species.                       | January. |             | February. |             | March. |             |
|--------------------------------|----------|-------------|-----------|-------------|--------|-------------|
|                                | Number.  | Percentage. | Number.   | Percentage. | Number | Percentage. |
| <i>Rattus rattus rufescens</i> | .. 1     | 16.7        | .. ..     | .. ..       | .. ..  | .. ..       |
| <i>Rattus norvegicus</i> ..    | .. ..    | ..          | .. ..     | .. ..       | .. ..  | .. ..       |
| <i>Gunomys kok</i> ..          | .. 2     | 33.3        | 2         | 100.0       | 1      | 100.0       |
| <i>Bandicota malabarica</i>    | .. 3     | 50.0        | .. ..     | .. ..       | .. ..  | .. ..       |
| <i>Mus dubius</i> ..           | .. ..    | ..          | .. ..     | .. ..       | .. ..  | .. ..       |
| <i>Suncus Caeruleus</i> ..     | .. ..    | ..          | .. ..     | .. ..       | .. ..  | .. ..       |
| Putrid ..                      | .. ..    | ..          | .. ..     | .. ..       | .. ..  | .. ..       |
| Total ..                       | 6        | 100.0       | 2         | 100.0       | 1      | 100.0       |

  

| Species.                       | July.   |             | August. |             | September. |             |
|--------------------------------|---------|-------------|---------|-------------|------------|-------------|
|                                | Number. | Percentage. | Number. | Percentage. | Number.    | Percentage. |
| <i>Rattus rattus rufescens</i> | .. 1    | 33.3        | .. ..   | .. ..       | 2          | 50.0        |
| <i>Rattus norvegicus</i> ..    | .. ..   | ..          | .. ..   | .. ..       | .. ..      | .. ..       |
| <i>Gunomys kok</i> ..          | .. ..   | ..          | .. ..   | .. ..       | 1          | 25.0        |
| <i>Bandicota malabarica</i>    | .. 1    | 33.3        | 2       | 40.0        | .. ..      | .. ..       |
| <i>Mus dubius</i> ..           | .. ..   | ..          | 1       | 20.0        | .. ..      | .. ..       |
| <i>Suncus caeruleus</i> ..     | .. ..   | ..          | .. ..   | .. ..       | .. ..      | .. ..       |
| Putrid ...                     | ... 1   | 33.4        | 2       | 40.0        | 1          | 25.0        |
| Total ..                       | 3       | 100.0       | 5       | 100.0       | 4          | 100.0       |

4.

*the City of Bombay (including the Suburbs) during 1956.*

| April.  |             | May.    |             | June.   |             |
|---------|-------------|---------|-------------|---------|-------------|
| Number. | Percentage. | Number. | Percentage. | Number. | Percentage. |
| ....    | ....        | 1       | 33·3        | ....    | ....        |
| ....    | ....        | ....    | ....        | ....    | ....        |
| ....    | ....        | ....    | ....        | ....    | ....        |
| ....    | ....        | 1       | 33·3        | 2       | 66·7        |
| ....    | ....        | ....    | ....        | 1       | 33·3        |
| ....    | ....        | ....    | ....        | ....    | ....        |
| 1       | 100·0       | 1       | 33·4        | ....    | ....        |
| 1       | 100·0       | 3       | 100·0       | 3       | 100·0       |

| October. |             | November. |             | December. |             | Whole year. |             |
|----------|-------------|-----------|-------------|-----------|-------------|-------------|-------------|
| Number.  | Percentage. | Number.   | Percentage. | Number.   | Percentage. | Number.     | Percentage. |
| ..       | ..          | ..        | ..          | 1         | ....        | 5           | 13·5        |
| ..       | ..          | ..        | ..          | 2         | 40·0        | 2           | 5·4         |
| ..       | ..          | ..        | ..          | 3         | 60·0        | 9           | 24·3        |
| ..       | ..          | ..        | ..          | ..        | ....        | 9           | 24·3        |
| ..       | ..          | ..        | ..          | ..        | ....        | 2           | 5·4         |
| ..       | ..          | ..        | ..          | ..        | ....        | ..          | ....        |
| 1        | 100·0       | 3         | 100·0       | ..        | ....        | 10          | 27·1        |
| 1        | 100·0       | 3         | 100·0       | 5         | 100·0       | 37          | 100·0       |

Examination of rats from the

These rats were received along with those of the other wards of the Bombay  
in previous tables. A separate record of these rats giving the number and  
Public Health

Table

*Showing the number and percentage of different species of rats*

| Species                        | January. |                  | February. |                  | March. |                  |
|--------------------------------|----------|------------------|-----------|------------------|--------|------------------|
|                                | Number   | percent-<br>age. | Number    | Percent-<br>age. | Number | Percent-<br>age. |
| <i>Rattus rattus rufescens</i> | .. 263   | 48·5             | 341       | 49·5             | 317    | 51·7             |
| <i>Rattus norvegicus</i>       | .. 53    | 9·8              | 57        | 8·3              | 78     | 12·7             |
| <i>Gunomys kok</i>             | .. 115   | 21·2             | 227       | 32·9             | 154    | 25·1             |
| <i>Bandicota malabarica</i>    | .. 2     | 0·4              | 3         | 0·4              | 2      | 0·3              |
| <i>Mus dubius</i>              | .. 37    | 12·4             | 35        | 5·1              | 42     | 6·9              |
| <i>Suncus caeruleus</i>        | .. 42    | 7·7              | 26        | 3·8              | 20     | 3·3              |
| Unidentifiable                 | .. ..    | ....             | ..        | ....             | ..     | ....             |
| Total                          | .. 542   | 100·0            | 689       | 100·0            | 613    | 100·0            |

  

| Species.                       | July.  |                  | August. |                  | September. |                  |
|--------------------------------|--------|------------------|---------|------------------|------------|------------------|
|                                | Number | Percent-<br>age. | Number  | Percent-<br>age. | Number     | Percent-<br>age. |
| <i>Rattus rattus rufescens</i> | .. 282 | 43·7             | 186     | 38·6             | 216        | 44·7             |
| <i>Rattus norvegicus</i>       | .. 57  | 8·8              | 74      | 15·3             | 89         | 18·4             |
| <i>Ginomys kok</i>             | .. 237 | 36·7             | 149     | 30·9             | 99         | 20·4             |
| <i>Bandicota malabarica</i>    | .. 4   | 0·6              | 8       | 1·7              | 3          | 0·6              |
| <i>Mus dubius</i>              | .. 27  | 4·2              | 22      | 4·6              | 29         | 6·2              |
| <i>Suncus caeruleus</i>        | .. 39  | 6·0              | 43      | 8·9              | 47         | 9·7              |
| Unidentifiable                 | .. ..  | ....             | ..      | ....             | ..         | ....             |
| Total                          | .. 646 | 100·0            | 482     | 100·0            | 483        | 100·0            |



Bombay Port Trust Docks.

Municipality, and have been already accounted for in the monthly totals given percentage of the different species, is also maintained at the instance of the Commissioner.

5.

*caught alive from the Bombay Port Trust area in 1956.*

| April.  |             | May.    |             | June.   |             |
|---------|-------------|---------|-------------|---------|-------------|
| Number. | Percentage. | Number. | Percentage. | Number. | Percentage. |
| 200     | 37.3        | 179     | 34.8        | 222     | 36.9        |
| 124     | 22.9        | 109     | 21.2        | 111     | 18.5        |
| 147     | 27.2        | 156     | 30.3        | 219     | 36.4        |
| 3       | 0.5         | 4       | 0.7         | 3       | 0.5         |
| 30      | 5.5         | 40      | 7.8         | 23      | 3.8         |
| 36      | 6.6         | 27      | 5.2         | 24      | 3.9         |
| ....    | ....        | ....    | ....        | ....    | ....        |
| 540     | 100.0       | 515     | 100.0       | 602     | 100.0       |

| October. |             | November. |             | December. |             | Whole year. |             |
|----------|-------------|-----------|-------------|-----------|-------------|-------------|-------------|
| Number.  | Percentage. | Number.   | Percentage. | Number.   | Percentage. | Number.     | Percentage. |
| 487      | 50.7        | 615       | 56.2        | 371       | 46.4        | 3,679       | 46.2        |
| 162      | 16.9        | 113       | 10.3        | 110       | 13.8        | 1,137       | 14.3        |
| 225      | 23.4        | 213       | 19.5        | 159       | 19.9        | 2,100       | 26.4        |
| 6        | 0.6         | 6         | 0.5         | 3         | 0.4         | 47          | 0.6         |
| 51       | 5.3         | 83        | 7.6         | 56        | 7.0         | 505         | 6.3         |
| 30       | 3.1         | 64        | 5.9         | 48        | 6.0         | 446         | 5.6         |
| ...      | ....        | ....      | ....        | 53        | 6.5         | 53          | 0.6         |
| 961      | 100.0       | 1,094     | 100.0       | 800       | 100.0       | 7,967       | 100.0       |

Table 6.

*Showing the different localities in Bombay State from which smears and rats were received for examination during 1956.*

| Name of Town. |     |     |     | Number of smears. | Number of rats. |
|---------------|-----|-----|-----|-------------------|-----------------|
| Ahmednagar    | ... | ... | ... | 12                | ...             |
| Akola         | ... | ... | ... | 2                 | ...             |
| Kalyan        | ... | ... | ... | ...               | 2               |
| Kolaba        | ... | ... | ... | 7                 | ...             |
| Malad         | ... | ... | ... | ...               | 3               |
| Ratnagiri     | ..  | ... | ... | 7                 | 2               |
| Revdanda      | ..  | ... | ... | 2                 | ...             |
| North Satara  | ... | ... | ... | 4                 | ...             |
| Thana         | ... | ..  | ..  | 2                 | 4               |
| Total         |     |     |     | 36                | 11              |

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

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

The Department of Clinical Pathology and Diagnostic Reagents Section thereunder 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 medical and Science Graduates from outside for laboratory work; (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.*—This scheme was continued for the ninth year and became very popular and useful. 698 calls from 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 the necessary preventive measures at the earliest opportunity. From examination of 674 blood samples for culture and Widal test, 226, i.e., 33 per cent., were found to be positive for enteric infection.

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

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

(b) *Results of Blood Culture and Triple Widal Tests.*—Samples examined during 1954, 1955 and 1956 are tabulated below in order to show the type and extent of typhoid infection amongst cases of fevers of unknown origin.

| Year. | Total sam-<br>ples. | Hos-<br>pital<br>Sam-<br>ples. | Typhoid<br>Scheme<br>Sam-<br>ples. | Widal<br>—<br>Culture<br>+ | Widal<br>+<br>culture<br>— | Widal<br>+<br>Culture<br>+ | Total<br>Cul-<br>tures<br>done. | B.<br>typhosus<br>isola-<br>ted. | B.<br>para<br>A<br>isola-<br>ted. |
|-------|---------------------|--------------------------------|------------------------------------|----------------------------|----------------------------|----------------------------|---------------------------------|----------------------------------|-----------------------------------|
| 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                                |
| 1956  | .. 2,731            | 2,057                          | 674                                | 118                        | 626                        | 166                        | 2,697                           | 224                              | 60                                |

(ii) *Routine Examinations.*—The number of routine examinations carried out during the years 1954, 1955 and 1956 are shown in the following table for purposes of comparison. It will be observed that in the majority of items, there was an increase in the number of samples received during the year under review.

|                                      | 1954. | 1955. | 1956. |
|--------------------------------------|-------|-------|-------|
| I. <i>Serological Examinations</i> — |       |       |       |
| 1. Wassermann Tests                  | ...   | 3,664 | 3,341 |
| 2. Kahn Tests                        | ...   | 2,620 | 3,012 |
| 3. Triple Widal Tests                | ...   | 2,415 | 2,719 |
| 4. Agglutination Tests for—          |       |       |       |
| (a) Typhus                           | ...   | 2,415 | 2,634 |
| (b) Brucellosis                      | ...   | 2,422 | 2,630 |
| (c) Leptospirosis                    | ...   | 58    | 66    |
| (d) Paul-Bunnell Tests               | ...   | 9     | 12    |

|                                                                                          | 1954.       | 1955. | 1956.       |
|------------------------------------------------------------------------------------------|-------------|-------|-------------|
| <b>II. Bacteriological and Biological Tests—</b>                                         |             |       |             |
| 1. Blood cultures for enteric group ...                                                  | 3,476       | 2,780 | 2,697       |
| 2. Urine cultures for pathogenic organisms ...                                           | 78          | 112   | 117         |
| 3. Throat Swabs for Diphtheria                                                           | 405         | 556   | 819         |
| 4. Stools and rectal swabs for culture ...                                               | 65          | 6     | 106         |
| 5. Sputum and body fluids for AFB by smear culture and animal inoculation tests          | 870         | 1,111 | 1,472       |
| 6. Material from suspected plague cases ...                                              | <i>Nil.</i> | 1     | <i>Nil.</i> |
| 7. Material from Medico-legal Cases ...                                                  | 13          | 14    | <i>Nil.</i> |
| 8. Blood cultures, routine ...                                                           |             | 53    | 53          |
|                                                                                          | 1954.       | 1955. | 1956.       |
| <b>III. General Microscopic Examinations—</b>                                            |             |       |             |
| 1. Blood films for M.P. and Filariasis ...                                               | 27          | 47    | 256         |
| 2. Blood cell counts— ...                                                                | .....       | ..... | 4,534       |
| 3. Bone Marrow ...                                                                       | 8           | 15    | 14          |
| 4. Semen for fertility ...                                                               | 83          | 79    | 103         |
| 5. Material for smear examination (Presence of pathogenic organisms like G. C. etc.) ... | 46          | 44    | 61          |
| 6. Stool—Routine Tests ...                                                               | 1,062       | 973   | 1,064       |
| 7. Urine—Routine Tests ...                                                               | 1,203       | 914   | 860         |
| <b>IV. Pregnancy Tests</b> ...                                                           | 841         | 884   | 960         |
| <b>V. Rideal Walker Tests</b> ...                                                        | 53          | 59    | 61          |

(iii) *Autogenous Vaccines*.—Use of autogenous vaccines was 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, farunculosis, eczematoid dermatitis, etc., were recommended use of such vaccines. The number of courses issued during 1955 and 1956 were 46 and 57 respectively.

## B. DIAGNOSTIC REAGENTS.

(i) *Supply of Diagnostic Reagents*.—Adequate stocks of various reagents for diagnostic purposes were maintained for supply to laboratories all over the Bombay State and also outside. There is a steady and an increasing demand

for these reagents as will be seen from the annual supplies for the last three years shown in the table below :—

| Reagents—                                              | Quantities supplied in ml. |         |         |
|--------------------------------------------------------|----------------------------|---------|---------|
|                                                        | 1954.                      | 1955.   | 1956.   |
| (1) Killed suspensions for Widal and Weil-Felix Tests. | 160,215                    | 162,115 | 163,180 |
| (2) Blood Grouping Sera ...                            | 8,751                      | 10,638  | 12,038  |
| (3) High Titre Sera and Amboceptor.                    | 676                        | 768     | 727     |
| (4) Kahn Antigen ...                                   | 4,980                      | 7,000   | 5,820   |
| (5) W. R. Antigen ...                                  | 380                        | 420     | 590     |
| (6) Russell's Viper and Cobra Venom Solutions          | 2,545                      | 2,229   | 2,418   |

(ii) *Supply of Stains*.—The supply of various biological stains was also continued. A quantity of 208,350 ml. of the various stains was supplied this year as against the quantity of 478,030 ml. supplied during the last year.

(iii) *Collection of Standard Strains*.—A number of demands for standard strains of different micro-organisms from various colleges and industrial concerns were met with this year.

### C. HISTOPATHOLOGICAL SECTION.

*Pathological Specimens*.—During the year under review, 2,522 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, gynaecological conditions and some rare and interesting pathological specimens. In addition, thirty research specimens from experimental animals were processed. These were received mostly from different departments of this Institute. In all 2,552 specimens were subjected to histological examination. The total number of blocks cut were 4,085.

The classification of the specimens of tissues received from hospitals in the City of Bombay and outstations during the years 1954, 1955, and 1956 is tabulated below :—

| Classification—                                       | 1954. | 1955. | 1956. |
|-------------------------------------------------------|-------|-------|-------|
| (1) Number of samples found to be normal.             | 185   | 241   | 38    |
| (2) Number of samples unfit for examination.          | 140   | 104   | 137   |
| (3) Brain samples for Negri bodies.                   | 64    | 52    | 53    |
| (4) Samples of Miscellaneous pathological conditions. | 1,366 | 1,563 | 1,816 |
| (5) Samples of tuberculous infection.                 | 95    | 143   | 156   |
| (6) Non-malignant Neoplasms ...                       | 125   | 144   | 152   |
| (7) Malignant Tumours ...                             | 158   | 141   | 170   |
| Total ...                                             | 2,133 | 2,388 | 2,522 |

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

Interesting specimens received during the year were :—

(1) Two breast specimens (No. CP/H/1225 and CP/H/1464/56) in lactating women were diagnosed as “Plasma Cell Mastitis”.

(2) Specimen (CP/H/1969) of the uterus showing growth was diagnosed as “Syncytioma”.

(3) Specimen (CP/H/2360) of bone tumour was diagnosed as Ewing’s Sarcoma (Ewing’s Tumour).

(4) Specimen of lymph gland (CP/H/2255) was diagnosed as “Reticulum Cell Sarcoma.”

(5) Specimen of Ovarian Tumour (CP/H/2432) was diagnosed as “Dysgerminoma”.

#### D. SPECIAL INVESTIGATIONS

(i) In vitro toxigenicity test of *C. diphtheria* is being carried out on isolation of the organisms from throat swabs.

(ii) Maintenance of the special strain of *Treponema pallidum* (Nicol’s strain) in rabbits for *Treponema* Agglutination Tests.

(iii) A study of quantitative hormone correlation of Pregnancy Test in twin pregnancy and vesicular mole is being carried out.

#### VII. Department of Pharmacology.

The following table shows the total number of samples that were tested during the last three years :—

| Years.                | 1954. | 1955. | 1956. |
|-----------------------|-------|-------|-------|
| No. of Samples tested | 2,261 | 4,055 | 5,156 |

It will be observed that a steady progress has been maintained in the analytical work during the last three years. There was a rise in the number of tested samples in the year under review to the extent of 27 per cent. when compared with the figures of 1955.

Out of the 5,156 samples examined, 1915 (i.e., 37 per cent.) did not comply with the required standard. There was an improvement in the quality of the products in 1956 as compared with that of the preceding year, during which 42 per cent. of the samples were found to be not of standard quality.

One thousand three hundred and fifteen samples, out of 5,156, came under the category of “Biological and Special Products” and “Other Special Products”. In these groups, about 30 per cent. of the samples proved to be of non-standard quality.

The following table shows the different States from which the samples were received for testing :—

| Samples tested under the Drugs Act. |        |                   |                    |        |                           | Samples tested for—                            |                           |                 |
|-------------------------------------|--------|-------------------|--------------------|--------|---------------------------|------------------------------------------------|---------------------------|-----------------|
| Bom-<br>bay.                        | Delhi. | Uttar<br>Pradesh. | Madhya<br>Pradesh. | Bihar. | Hima-<br>chal<br>Pradesh. | Govern-<br>ment<br>Depart-<br>ments*<br>(Bom.) | Commer-<br>cial<br>firms. | Grand<br>Total. |
| 1,480                               | 173    | 138               | 69                 | 19     | 2                         | 2,982                                          | 293                       | 5,156           |

\*Include those of Haffkine Institute.

For the Prohibition and Excise Department and Police Department, 2,509 samples, were tested of which 2,399 were alcoholic preparations. One thousand and fifty-four of such drugs were declared to be of non-standard quality. Quite a large number of them were coloured alcohol substituted for genuine preparations.

Most of the samples received from Commercial firms were complex mixtures of vitamins. Facilities for testing these drugs are limited in India. Quite a considerable part of our time was devoted to these samples and, more often, microbiological methods had to be adopted for the analysis of the ingredients.

One thousand eight hundred and eighty-one samples were examined under the Drugs Act, out of which 39 per cent. were not of standard quality. In 1955, about 45 per cent. of the drugs had failed to comply with the required standard. However, there was a distinct improvement in the quality of drugs tested in 1956.

The reasons for failure of some samples to comply with the required standards are summarised below:—

*Examination of Drugs by Pharmacological Methods.*—Certain firms are regularly sending samples of Adrenaline, crude of liver extracts and digitalis for biological assay before they are put on the market. 25 samples of crude liver extracts were tested for the presence of histamine and histamine like substances. Twenty-seven samples of digitalis were biologically standardised. Forty-one samples of Tincture scillae were analysed and potency of 11 samples was less while 4 samples did not show the presence of glycosidal activity. In all, 55 samples of Adrenaline were tested. Only 3 samples received under the Drugs Act were found to possess less potency than the declared amount.

Of the 9 samples of pituitary (posterior lobe) tested, only one had a lower potency than the specified limit. The rat uterus method seemed to be quite convenient and accurate and, therefore, all the drugs were analysed by this method.

This department was called upon to express opinion on the leech-repellant activity of a chemical substance. As no standard procedure was available, a suitable laboratory method was developed for comparative study of drugs for their leech-repellant activity under different environmental conditions.

Several samples like, penicillin, streptomycin, protein hydrolysates, etc., were tested for the absence of undue toxicity. It was rather interesting to note that those samples in which lecithin was added as a suspending agent, special precautions were necessary while testing them for their intravenous toxicity. In order to reduce the particle size of lecithin, it was necessary to triturate thoroughly before the solution was given intravenously in the mice. Otherwise, higher mortality rate was observed.

Five hundred and twelve samples of parenteral solutions were examined for the absence of pyrogenic substances. Only four of them were rejected, as they contained pyrogens.

*Examination of Drugs by Chemical Methods.*—A large number of samples of calcium gluconate (injection) was found to contain boric acid which was not specifically allowed by Pharmacopoeias. This might have been added to stabilise the product. Out of 46 samples tested, 32 were declared non-standard for this reason. In addition, 9 samples failed on the ground that they contained suspended matter. One sample had pyrogenic substances.

Out of the 16 samples of liquid extract of ergot, 15 failed to meet the standard laid down by the B. P. C. for the content of total alkaloids.

Several tablet preparations were found not to comply with the pharmacopoeial requirement for the time of disintegration. All the 4 samples of carbarsone and 5 of penicillin tablets out of 7, failed on this account.

Another important reason for failure of some of the tablet preparations was deviation from the pharmacopoeial requirement for "uniformity of weight of tablets". Tablets of aspirin (1 out of 10), ephedrine hydrochloride (1 out of 10), soda mint (3 out of 5), and sulphanilamide (1 out of 6) could be cited as samples of this type.

Six samples of a mixture of acetylsalicylic acid, phenacetin and caffeine dispensed in loose packets were analysed. The variation in the contents in the individual packets in some cases was very wide and in a few, the actual weight of contents was less than the total weight, calculated from the labelled ingredients.

Five samples of anthelmintic mixtures containing santonin, calomel and sodium bicarbonate were examined. Three of them contained less amount of santonin and more of calomel and sodium bicarbonate and in another instance calomel was present although it was not mentioned on the label.

Out of 8 samples of solution of hydrogen peroxide, B. P., 5 did not comply with the required standard. The content of hydrogen peroxide was generally less and in few cases they did not comply with the B. P. test for the limit of alkalinity.



Out of the 11 samples of sandal wood oil, 7 were of sub-standard quality. They were adulterated with a fixed oil.

As many as 43 samples of tincture of iodine out of 47, were declared to be non-standard for various reasons, mostly because, the content of iodine or potassium iodine or alcohol was either more or less than the required amounts.

There were several instances where genuine ingredients were substituted or totally absent. Sixteen samples of "Gripe mixture" out of 53 examined, were found to be of non-standard quality for various reasons, mainly because, either they did not contain one or more labelled ingredients like sodium bicarbonate, potassium bromide or sucrose, or contained an unlabelled ingredient like saccharin.

One sample of sulphadiazine tablets out of 4, was found to contain boric acid instead of the proper drug. Amongst the 6 samples of sulphapyridine tablets, 3 were substituted by calcium carbonate. Out of 5, one sample of "sulphathiazole" tablets which failed, contained sulphanilamide.

One hundred and twenty samples of tincture cantharidini were tested, out of which only 15 were of standard quality. One hundred and five of them did not show the presence of cantharidin.

Most of the tinctures of tolutana did not contain balsam of tolu. Fifty-seven per cent. of "Tinct. Adhatoda" did not show the presence of alkaloid.

Tincture of quill contains haemolytic saponins. Most of the samples declared to be of non-standard quality, were lacking in the property of haemolysing red blood corpuscles. Out of the 36 samples of tinct. lycopodium examined, as many as 33 failed to show the presence of fixed oil. Fifty per cent. of the samples of *auristillae resorcinolis* did not contain resorcinol.

*Examination of Drugs by Physical, Biochemical and Microbiological Methods.*—As in the previous years, a large number of complex vitamin preparations were tested. Physical, biochemical and microbiological methods were used, depending on the nature of vitamins and in the way they were mixed up with other substances. Vitamin A, cyanocobalamine ( $B_{12}$ ) and ( $B_2$ ) when present in pure form were analysed spectrophotometrically. Chemical methods were generally used for vitamins A,  $B_1$ ,  $B_2$ , nicotinic acid and its amide when present in simple preparations like tablets, injectibles, etc. In those cases where physical and chemical methods were not applicable, microbiological methods of assay were applied for vitamin  $B_2$ , nicotinic acid and its amide, pantothenic acid and its calcium and sodium salts, folic acid and its sodium salt and  $B_{12}$ .

Forty-three preparations, which contained vitamin A, out of total 107, were declared to be of non-standard quality. Majority of these showed loss of potency. Thirty-three of these preparations were cod-liver oil shark-liver oil and vitamin A concentrate in pure form.

In all, 310 samples containing B<sub>1</sub> were tested. About 29 per cent of these failed to conform to the labelled amount. There was no improvement in the quality of this type of product when compared to the figure of 1955, when 30 per cent of the samples were found to be of non-standard quality.

About 33 per cent. of the preparations containing Vitamin C did not conform to the required standard. Like aneurine hydrochloride, it is a problem to keep ascorbic acid in a stable condition in oral preparations.

It is our experience that calcium pantothenate and folic acid deteriorate on storage. In eighty-two, out of 132 samples, pantothenate activity did not conform to the required amount. Similarly, in 61 per cent. of the preparations, folic acid, activity was found to be less than the labelled amount.

Out of 129 samples which contained vitamin B<sub>12</sub>, in 62, the amount of the vitamin did not comply with the stated amount. In most of them, the quantity of cyanocobalamine present was less.

All the 8 samples of insulin tested were of standard quality.

*Examination of Drugs by Bacteriological and Serological Methods.*—Out of the 134 samples of antibiotics tested, 13 were found to be not of standard quality. Amongst the samples failed, 5 were penicillin tablets, 3 penicillin ointment, 2. procaine penicillin G, 1 chloramphenicol palmitate, 1 streptomycin and 1 oxytetracyclin.

There were 36 samples of procaine penicillin G, 19 penicillin ointment, 7 penicillin G Sodium, 7 penicillin tablets, 5 penicillin lozenges, 12 penicillin and streptomycin, 12 streptomycin, 9 tetracyclin, 13 chloramphenicol capsules, 12 chloramphenicol palmitate syrup or suspension, 1 chloramphenicol dragees and 1 oxytetracyclin. In some cases chemical methods were followed for assay.

Six hundred and twenty-seven "sterility" tests were performed. Only 10 samples failed to pass the test. There was a considerable rise in the number of "Sterility" tests performed as compared with the figure of the preceding year which was 276.

Five samples of antitoxins (2 diphtheria and 3 tetanus) were tested, out of which only one (tetanus antitoxin) was declared to be not of standard quality.

*Surgical Dressings.*—Out of the 3 samples of absorbent bandage examined, none of them complied with the required standard. The thread per inch and the weight did not conform to B. P. C. specifications.

Nine samples of cotton wool were tested, but 5 of them were not of standard quality. They failed to pass tests for absorbency, water soluble extractives and ash.

Similarly, 3 out of 6 samples of absorbent gauze did not comply with the B. P. C. requirements for absorbency, water soluble extractives, etc.

None of the 3 samples of absorbent lint was of standard quality. Nap was raised from the weft yarns instead of warp yarns. Some of them did not comply with the weight and absorbency as specified in the B. P. C.

### VIII. Department of Chemotherapy.

A steep rise in the manufacturing activity of the department is the key-note for the year under review. The sales during 1956 were three times those of 1954. This is in spite of the fact that during 1956, the prices of most of the products were revised and considerably lowered.

The new products introduced during 1955, viz. Sulphadiazine, Sulphaguanidine, Quinine Sulphate and particularly the Multi-vitamin Tablets have shown very good response, and during the year under review three new products have been introduced viz. Vitamin A-Forte, Nicotinamide and Magnesium Trisilicate Tablets.

Preliminary experiments on the coating of tablets have confirmed that, by adopting this procedure, the shelf-life of the tablets could be extended. The staff is being trained in the art of coating and as soon as production-units of coating machines are made available to the Department, it would be possible to manufacture and supply coated tablets, particularly of those vitamins which are labile.

About 700 lbs. of pink sub-standard Quinine Powder, that had been in the custody of the Government of Bombay and which could not be disposed of conveniently, was reprocessed by the department and converted into B. P. Specifications and is being used for the preparation of Quinine Sulphate Tablets. An outstanding feature in the research activities was the development of a novel procedure for the preparation of *para*-nitro-acetophenone, a very important intermediate for the preparation of Chloromycetin. The known procedures for the preparation of *para*-nitro-acetophenone, besides being very expensive, also involve very careful and close supervision during production. The novel procedure developed in this department circumvents these difficulties by utilising cheaper raw materials as well as adopting a technique that is safe, convenient and elegant and does not involve the dangerous hazards and risks that accompany the customary procedures. This new process is being protected by filing an application for a patent, and this patent will prove extremely useful.

Other research programmes viz. establishment of economic, convenient and optimum conditions and data for the manufacture of synthetic chemotherapeutics, viz. Sulphadiazine, Sulphaguanidine, *para*-amino-salicylic acid, Di-amino-diphenyl sulphone etc. are being further pursued.

The complete assays and analysis of the raw materials and the final products manufactured by the department were also carried out. The number of such analyses totalled 750 approximately.

*Table indicating the Sales of the Various Products manufactured by the Department of Chemotherapy during the years 1954, 1955 and 1956.*

| Serial No.                         | Kind of Product.                       | Number of Tablets sold during the period. |                |               |
|------------------------------------|----------------------------------------|-------------------------------------------|----------------|---------------|
|                                    |                                        | 1954.                                     | 1955.          | 1956.         |
| 1                                  | Vitamin AD Tablets ..                  | 37,37,475                                 | 50,72,618      | 61,90,267     |
| 2                                  | Vitamin AD (Spl.) Tablets ..           | 88,000                                    | 1,25,000       | 1,36,000      |
| 3                                  | Vitamin A (Spl.) Tablets ..            | 1,000                                     | 2,000          | ....          |
| 4                                  | Vitamin A Forte Tablets ..             | ....                                      | ....           | 45,200        |
| 5                                  | Vitamin BC Tablets ..                  | 54,71,100                                 | 74,71,755      | 1,03,55,516   |
| 6                                  | Vitamin BC No. 3 Tablets ..            | 8,68,050                                  | 9,64,475       | 7,59,050      |
| 7                                  | Vitamin B1 Tablets ..                  | 2,06,950                                  | 4,61,760       | 7,58,617      |
| 8                                  | Vitamin B2 Tablets ..                  | 65,300                                    | 1,22,100       | 1,01,367      |
| 9                                  | Vitamin C Tablets ..                   | 3,33,450                                  | 5,67,560       | 8,72,952      |
| 10                                 | Acid Nicotinic Tablets ..              | 1,49,400                                  | 2,66,200       | 3,49,497      |
| 11                                 | Nicotinamide Tablets ..                | ....                                      | ....           | 2,450         |
| 12                                 | Multivitamin Tablets ..                | ....                                      | 7,21,980       | 41,15,406     |
| Total sale of Vitamin Tablets..    |                                        | 1,09,20,725                               | 1,57,75,448    | 2,36,86,322   |
| 13                                 | Sulphathiazole Tablets ..              | 19,99,550                                 | 23,55,825      | 23,56,550     |
| 14                                 | Sulphadiazine Tablets ..               | ....                                      | 18,41,500      | 35,71,125     |
| 15                                 | Sulphaguanidine Tablets ..             | ....                                      | 5,44,000       | 10,18,475     |
| Total Sale of Sulphas ..           |                                        | 19,99,550                                 | 47,41,325      | 69,46,150     |
| 16                                 | Quinine Sulphate Tablets ..            | ....                                      | 2,83,300       | 4,56,850      |
| 17                                 | Magnesium Trisilicate Tablets ..       | ....                                      | ....           | 17,000        |
| Total Sale of all kinds of Tablets |                                        | 1,29,20,275                               | 2,08,00,073    | 3,11,06,322   |
| 18                                 | Pemon .. ..                            | 6,000 lbs.                                | 7,754 lbs.     | 8,192 lbs.    |
| 19                                 | Sulphathiazole Injectible ..           | 155 × 12 boxes.                           | 212 × 12 boxes | 77 × 12 boxes |
| 20                                 | Solapstone Strong B.P.C. Solutions* .. | 1,34,250 c.c.                             | 1,27,300 ccs.  | 1,07,250 ccs. |

\*Free supply.

### IX. Department of Biochemistry.

The departmental activities have been in full swing during the year under review. The chemical investigations of clinical materials from hospitals and private practitioners are carried out by this department. The number of analyses carried out increased to 6,353 as against 5,543 in the year 1955. There was increased demand for many of the quantitative estimations of blood, such as, serum protein fractions, alkaline and acid phosphatases, calcium, phosphorus, urea, cholesterol, sugar, amylase etc., and it was often necessary to restrict the number of samples coming in. The accompanying table gives a comparative idea of the various tests carried out for the last five years :—

|                     |       | 1952. | 1953. | 1954. | 1955. | 1956. |
|---------------------|-------|-------|-------|-------|-------|-------|
| Blood               | .. .. | 2,637 | 3,752 | 3,468 | 3,264 | 3,991 |
| Urine               | .. .. | 149   | 280   | 241   | 234   | 298   |
| C. S. F. and Fluids | .. .. | 1,764 | 1,324 | 1,165 | 856   | 1,146 |
| Stools              | .. .. | 9     | 14    | 9     | 2     | 2     |
| Milk                | .. .. | 1,596 | 1,318 | 674   | 734   | 577   |
| Special Tests       | .. .. | 397   | 470   | 346   | 453   | 339   |
| Total               | .. .. | 6,552 | 7,158 | 5,903 | 5,543 | 6,353 |

In addition, due to lack of proper care in collecting and despatching, a number of samples had to be rejected as insufficient, haemolysed or unfit otherwise.

The Seamen's Medical Examination Board referred 29 patients for Glucose Tolerance Test. Due to pressure of work, it was not possible to take up the examination of Seamen as a routine.

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

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

The Department continued to (1) afford antirabic treatment to patients in Bombay City and Suburbs, (2) prepare antirabic vaccine for issue to antirabic treatment centres, (3) assist in the diagnosis of rabies in animals, (4) hold stocks and conduct Yellow Fever Inoculation 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) *Antirabic Treatment Centre at this Institute.*—During the year under report, 10,208 persons applied for antirabic treatment; of these 9,100 completed the full course of treatment and 711 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, is shown below in Table I.

Table I.

*Details of treatment carried out at the Treatment Centre of this Institute since 1952–1956.*

| Details.                                                            | 1952. | 1953. | 1954. | 1955. | 1956.  |
|---------------------------------------------------------------------|-------|-------|-------|-------|--------|
| Number of persons treated ..                                        | 7,779 | 8,714 | 9,051 | 9,581 | 10,208 |
| Advice Cases ..                                                     | 687   | 985   | 473   | 1,181 | 711    |
| Fully treated ..                                                    | 7,092 | 7,722 | 8,577 | 8,331 | 9,100  |
| Discontinued treatment ..                                           | Nil.  | 7     | 1     | 69    | 397    |
| Cases of hydrophobia among those treated at the Institute Centre .. | Nil.  | 1     | Nil.  | Nil.  | Nil.   |

(b) *Preparation and Supply of Antirabic Vaccine.*—Five per cent phenolised sheep brain vaccine (Semple) was prepared and issued to various Antirabic Centres recognised by the Institute for supply within and outside the Bombay State. Total number of such centres increased from 176 to 191 during the year out of which 84 were in Bombay State. The vaccine was also supplied to the Veterinary Surgeons within the State and outside for treatment of animals. 17,682 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 Antirabic Vaccine in millilitres month by month is shown in Table III.

Table II.

*Production of Carbolised Antirabic Vaccine and Canine Vaccine in ml. from 1952 to 1956.*

| Details.                      | 1952.     | 1953.     | 1954.     | 1955.     | 1956.     |
|-------------------------------|-----------|-----------|-----------|-----------|-----------|
| Anti-rabic Vaccine in ml. . . | 23,39,740 | 26,10,160 | 27,58,160 | 24,54,695 | 29,64,498 |
| Canine Vaccine in ml. ..      | 3,470     | 6,185     | 9,760     | 12,612    | 17,682    |

Table III.

*Production of Antirabic Vaccine and supplies in ml. during 1956,  
month by month.*

| Month.    |     |     |     | Number of ml.<br>vaccine<br>manufactured. | No. of ml.<br>vaccine<br>issued.                           |
|-----------|-----|-----|-----|-------------------------------------------|------------------------------------------------------------|
| January   | ... | ... | ... | 213,523                                   | 240,134                                                    |
| February  | ..  | ... | ... | 278,491                                   | 290,443                                                    |
| March     | ... | ... | ... | 326,389                                   | 270,918                                                    |
| April     | ..  | ..  | ..  | 275,672                                   | 272,166                                                    |
| May       | ..  | ... | ... | 265,283                                   | 245,027                                                    |
| June      | ... | ... | ... | 231,839                                   | 269,861                                                    |
| July      | ... | ... | ... | 228,913                                   | 229,619                                                    |
| August    | ... | ... | ... | 209,456                                   | 195,699                                                    |
| September | ... | ... | ... | 159,212                                   | 227,275                                                    |
| October   | ... | ... | ... | 332,019                                   | 215,475                                                    |
| November  | ... | ... | ... | 181,368                                   | 260,964                                                    |
| December  | ... | ... | ... | 262,333                                   | 250,440                                                    |
| Total     |     |     |     | 2,964,498                                 | 2,968,021<br>(including balance<br>from the last<br>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 Antirabic Vaccine and number of treatment centres.*

| Details.                                                        | 1952.  | 1953.  | 1954.  | 1955.  | 1956.  |
|-----------------------------------------------------------------|--------|--------|--------|--------|--------|
| Number of human treatments ..                                   | 17,604 | 19,230 | 21,555 | 27,340 | 28,270 |
| Number of Veterinary treatments ..                              | 827    | 804    | 804    | 1,244  | 1,297  |
| Reported cases of hydrophobia ..                                | 6      | 3      | 1      | Nil.   | 5*     |
| Number of sheep used for production<br>of anti-rabic vaccine .. | 1,337  | 1,546  | 1,618  | 1,609  | 1,909  |
| Number of treatment centres ..                                  | 145    | 153    | 163    | 176    | 191    |

\*Out of 5 cases that developed hydrophobia and died, 4 cases were due to rabid dog bite and one due to wolf-bite on the face. Three dog-bite cases started taking anti-rabic treatment very late and probably they were in the incubation stage. In the fourth case, only 3 out of 14 injections of anti-rabic vaccine were taken. The wolf-bite cases are usually so severe and with such a short incubation period, that even immediate treatment with anti-rabic vaccine often proves a failure.

A total of 29,64,498 ml. of Antirabic Vaccine was manufactured at this Institute and 29,68,021 ml. was supplied to various Antirabic Centres (including the one at this Institute) during the year. The total number of treatments issued during the year was 28,270 according to new classification :—

|                    |     |     |     |        |
|--------------------|-----|-----|-----|--------|
| Class I            | ... | ... | ... | 2,505  |
| Class II (a) Child | ... | ... | ... | 1,930  |
| Class II (a) Adult | }   | ... | ... | 13,740 |
| Class II (b) Child |     |     |     |        |
| Class II (b) Adult | ... | ... | ..  | 10,095 |

Fifteen additional centres for Antirabic treatment were opened during the year, making the total to 191. They are shown along with dates of their sanction. In addition, there are 17 Railway Centres, 18 Military Centres and 72 Centres in States other than Bombay State.

| Serial No. | Name of Antirabic Centre.                                                                           | Date of sanction.    |
|------------|-----------------------------------------------------------------------------------------------------|----------------------|
| 1          | Assistant Surgeon, Main Hospital, Balaghat (Madhya Pradesh).                                        | 23rd January 1956.   |
| 2          | Medical Officer, Cottage Hospital, Dahanu (Thana).                                                  | 15th March 1956.     |
| 3          | Medical Superintendent, St. Luke's Hospital and Hillside Sanatorium, Vengurla (district Ratnagiri). | 5th July 1956.       |
| 4          | Assistant Surgeon, Main Hospital, Durg (Madhya Pradesh).                                            | 13th April 1956.     |
| 5          | Medical Officer, Civil Hospital, Khargone (Madhya Bharat).                                          | 13th April 1956.     |
| 6          | Medical Officer, Gandhidham Hospital, Adipur (Kutch).                                               | 4th June 1956.       |
| 7          | Assistant Surgeon, Main Hospital, Tumsar (Madhya Pradesh).                                          | 2nd May 1956.        |
| 8          | Assistant Surgeon, Katni Hospital, Katni (district Jubbulpur, Madhya Pradesh).                      | 10th August 1956.    |
| 9          | Assistant Surgeon, K. T. S. Hospital, Gondia, (district Bhandara, Madhya Pradesh).                  | 20th August 1956.    |
| 10         | Assistant Surgeon, K. G. S. J. Hospital, Khairagarh (district Durg, Madhya Pradesh).                | 10th September 1956. |
| 11         | Medical Officer, Government Dispensary, Bantwa.                                                     | 19th September 1956. |
| 12         | Medical Officer, Government Dispensary, Kutiyana.                                                   | 19th September 1956. |
| 13         | Medical Officer, Government Hospital, Santrapur (Panchmahals).                                      | 11th October 1956.   |
| 14         | Medical Officer, Government Dispensary, Lunawada (Panchmahals).                                     | 11th October 1956.   |
| 15         | Medical Officer, Government Hospital, Jambughoda.                                                   | 11th October 1956.   |



(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 histopathological examination. When received in glycerine the diagnosis is usually made by examination of impression smears and by histological methods if necessary. Where no negri-bodies could be demonstrated by either of these methods they are subjected to a biological test in mice. Out of 91 brain specimens received, 64 were sent in glycerine and 20 in formalin. In addition, one saliva sample was also received for testing the presence of rabies virus. Besides microscopic and histological examination, the number of biological tests carried out in mice to diagnose rabies was 37. The number of brain specimens received for diagnosis from Bombay City and outstations during the year and the classification of different animals with the results of their brain examination for rabies is given below in Tables V and VI.

**Table V.**

*Number of brain specimens received for diagnosis of rabies from Bombay City and Outstations during the year 1956.*

|             |    |    | Total. | Positive. | Negative. | Unfit for examination. |
|-------------|----|----|--------|-----------|-----------|------------------------|
| Bombay City | .. | .. | 50     | 33        | 16        | 1                      |
| Outstations | .. | .. | 41     | 17        | 18        | 6                      |
| Grand Total | .. | .. | 91     | 50        | 34        | 7                      |

**Table VI.**

*Statement showing classification of different animals with the results of their brain examination for rabies during 1956.*

| Animals. |    |    | Total. | Positive. | Negative. | Unfit for examination |
|----------|----|----|--------|-----------|-----------|-----------------------|
| Dog      | .. | .. | 76     | 44        | 27        | 5                     |
| Human    | .. | .. | 4      | 3         | 1         | ....                  |
| Bull     | .. | .. | 2      | 1         | ....      | 1                     |
| Buffalo  | .. | .. | 2      | 1         | 1         | ....                  |
| Cow      | .. | .. | 2      | ....      | 1         | 1                     |
| Cat      | .. | .. | 3      | ....      | 3         | ....                  |
| Por      | .. | .. | 1      | 1         | ....      | ....                  |
| Mongoose | .. | .. | 1      | ....      | 1         | ....                  |
| Total    | .. | .. | 91     | 50        | 34        | 7                     |

As shown in Table VI, 4 human brains were received for diagnosis of rabies. Out of these, one was received from Topiwala National Medical College, Bombay, one from Grant Medical College, Bombay, and one from Police Surgeon, Northcote Police Hospital, Bombay. In the first case, negri bodies were not seen but virus was isolated in mice. In the other two cases, negri bodies were seen on direct smear examination. Among the 3 cases, the last case had a history of cat-bite. One more brain was also received from Police Surgeon, Northcote Police Hospital, Bombay, which was found to be negative by microscopic examination as well as by mouse inoculation.

(d) *Other problems carried out in the Department.*—Besides the routine work of the department, studies were continued on Influenza and Rabies Virus besides carrying out diagnostic laboratory tests for primary atypical pneumonia, mumps, herpes, L. G. V. and typhus with materials received from different local hospitals. A special study of tissue culture techniques was undertaken to develop methods for diagnosis of new viral agents.

(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 were carried out by the Yellow Fever Diagnostic Unit housed at this Institute. Several batches of the Rockefeller Foundation Yellow Fever Vaccine and one batch of the Burroughs Wellcome Yellow Fever Vaccine were tested for potency. Total number of potency tests performed was 9.

Yellow Fever Inoculation figures during 1956 are shown in Table VII along with corresponding figures for 1954 and 1955. The persons inoculated were air or sea passengers from Bombay proceeding to or passing through yellow fever endemic areas.

Table VII.

| Months.   | Number of persons inoculated |       |       |
|-----------|------------------------------|-------|-------|
|           | 1954.                        | 1955. | 1956. |
| January   | 675                          | 404   | 534   |
| February  | 506                          | 638   | 742   |
| March     | 685                          | 825   | 675   |
| April     | 556                          | 776   | 567   |
| May       | 533                          | 559   | 783   |
| June      | 498                          | 799   | 538   |
| July      | 721                          | 608   | 411   |
| August    | 450                          | 605   | 644   |
| September | 439                          | 645   | 401   |
| October   | 470                          | 601   | 1,069 |
| November  | 716                          | 666   | 762   |
| December  | 597                          | 959   | 733   |
|           | 6,846                        | 8,085 | 7,859 |

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.  | 1956.  |
| 1. The Chief Medical Officer, Porbunder...                            | 300                                    | 240    | 550    |
| 2. The Port Health Officer, Jamnagar ...                              | 400                                    | 400    | 500    |
| 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  | 3,000  |
| 6. The King Institute, Guindy, Madras...                              | 1,000                                  | 950    | 3,150  |
| 7. The All India Institute of Hygiene and Public Health, Calcutta ... | 6,900                                  | 2,525  | 4,550  |
| 8. The Air Port Health Officer, Santacruz.                            | 1,100                                  | 720    | 1,495  |
| 9. The Port Health Officer, Visakapatam.                              | ...                                    | 1,375  | 450    |
| 10. The Port Health Officer, Cochin ...                               | ...                                    | ...    | 650    |
| Total ...                                                             | 16,000                                 | 13,110 | 14,345 |

(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 11 years cold storage at 0-4°C. Out of the 7859 inoculations carried out during the year, no cases of post-vaccinal reactions were reported.

## XI. Blood Bank Department.

During the year under report the department has maintained the upward trend in all its activities.

The demand for dried human plasma was greater than that of the last year, especially from outside the State, and it has been possible to meet this demand. It is expected that with the establishment of the Composite State, the demand from the State Hospitals may also increase in the near future. However, the position regarding the blood donors remains unsatisfactory as before. The number of blood donors from amongst the prisoners has fallen down considerably due to the recent reduction in the concessions allowed to them for donating blood. This has adversely affected the donor procurement programme from this source.

The demand for blood grouping sera has been on the increase and it is becoming difficult to meet it from normal source. A scheme for preparation of standard blood grouping sera is being started on behalf of the Indian Council of Medical Research as a part of the Second Five-Year Plan and will start functioning soon.

The lyophilisation of antivenine continued on a greater scale, as the demand for this product is still increasing. With the help of the desivac plant, it has been possible to meet this demand. This plant was partly dismantled, refabricated, remodelled and reinstalled, so that the loading and unloading operations could be carried out under sterile conditions. Now the plasma bottles and ampoules are not exposed to the outside atmosphere and the risk of aerial contamination is minimised. During the year, this room was airconditioned and sterile conditions are maintained. A deep-freeze cabinet of 30 cubic feet capacity was also installed in this room to store frozen ampoules under sterile conditions. This has resulted in the plant rendering greater efficiency. The Desivac Plant is now being utilised more frequently than before and it should be possible to lyophilise more of our biological products now.

5056 donors were screened by  $\text{CuSO}_4$  specific gravity method for their fitness to give blood, out of which 1724 were rejected, thus giving a rejection rate of 30 per cent. Routine ABO grouping of all samples of the blood collected was carried out. Kahn tests were performed on their blood samples.

Ri investigations were carried out on 596 cases referred for investigations of isoimmunisation with suspicious obstetrical history.

Ri grouping tests were also carried out on all the 1241 samples of blood of voluntary blood donors of the St. George's Hospital Voluntary Blood Transfusion Service.

25,185 ampoules of Antivenine (10 c.c. each) were lyophilised during the year for the Serum Department of the Institute.

The usual routine activity of collection of blood and lyophilisation of plasma and antivenine was carried on. The following tables show the work carried out during the year under report.

**Table No. I.**  
*Showing the number of blood donors.*

| Centre.            | Type of Donors. | Number of donors bled. | Total Number of donors bled. |
|--------------------|-----------------|------------------------|------------------------------|
| Haffkine Institute | .. Paid         | .. 2,402               |                              |
|                    | Voluntary       | .. 7                   |                              |
|                    | Jail            | .. 221                 |                              |
|                    |                 |                        | <hr/> 2,630                  |
| Red Cross ..       | .. Paid         | .. 930                 |                              |
|                    | Voluntary       | .. Nil.                |                              |
|                    |                 |                        | <hr/> 930                    |
| Other Hospitals .. | ..              | 300                    |                              |
|                    |                 |                        | <hr/> 300                    |
|                    | Grand Total...  |                        | 3,860                        |

**Table No. II.***Showing the Total Yield of Blood Plasma.*

| Product.      |    |    |    | Quantity in litres. | Bottles. |
|---------------|----|----|----|---------------------|----------|
| Blood         | .. | .. | .. | 1,058·930           |          |
| Liquid Plasma | .. | .. | .. | 669·000             |          |
| Dried Plasma  | .. | .. | .. | 594·250             | 2,377    |

**Table No. III.***Showing the distribution of Plasma.*

| Nature. |    |    |    | Quantity in litres. | Bottles. |
|---------|----|----|----|---------------------|----------|
| Sold    | .. | .. | .. | 538·000             | 2,152    |
| Free    | .. | .. | .. | 124·750             | 499      |
|         |    |    |    | 662·750             | 2,651    |

**Table No. IV.***Showing the results of Kahn Tests carried out during the year.*

|                       |    |    |                         |
|-----------------------|----|----|-------------------------|
| No. of samples tested | .. | .. | 2,657                   |
| No. of Negative Cases | .. | .. | 1,938 (72·94 per cent.) |
| No. of positive cases | .. | .. | 719 (27·06 per cent.)   |

**Table No. V.**

*Showing the distribution of blood groups and the corresponding percentages.*

| Group. |     |     | Number belonging<br>to the Group. | Percentage. |
|--------|-----|-----|-----------------------------------|-------------|
| AB     | ... | ... | 171                               | 7.14        |
| B      | ... | ... | 883                               | 36.86       |
| A      | ... | ... | 584                               | 24.40       |
| O      | ... | ... | 758                               | 31.60       |
| Total  |     |     | 2,396                             |             |

**Table No. VI.**

*Showing the results of Rh examinations.*

|                                |     |     |                      |
|--------------------------------|-----|-----|----------------------|
| No. of individuals tested      | ... | ... | 596                  |
| No. of Rh Negative individuals | ... | ... | 36 (6.04 per cent.). |

**Table No. VII.**

*Showing Voluntary Blood Donors from St. George's Hospital.*

|                         |     |     |                     |
|-------------------------|-----|-----|---------------------|
| No. of samples examined | ... | ... | 1,241               |
| No. of Rh negatives     | ... | ... | 50 (4.0 per cent.). |

## **XII. Training of Health Personnel.**

The Institute plays an important role in the 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. Following is the list of persons to whom training was given in various departments during the year.

| Name.                                                                                          | Address.                                                      | Department in which trained.     | Duration of training.                      |
|------------------------------------------------------------------------------------------------|---------------------------------------------------------------|----------------------------------|--------------------------------------------|
| 1. Shri V. V. Badigar, M.Sc.                                                                   | From Karnatak University, Dharwar.                            | Clinical Pathology.              | 1st July 1955 to 30th June 1956.           |
| 2. Shri P. N. Desai.                                                                           | From Borough Municipality, Surat.                             | Do. ...                          | 3rd March 1956 to 2nd June 1956.           |
| 3. Shri S. N. Kelkar.                                                                          | From J. S. S. College, Dharwar.                               | Pharmacology ...                 | 5th May 1956 to 17th May 1956.             |
| 4. Dr. B. B. Singh, D. A.                                                                      | From Nepal Government under Colombo Plan 1956.                | Blood Bank ...                   | 25th May 1956 to 14th June 1956.           |
| 5. Dr. H. R. Sen, M.B.B.S., L.M. (Rot.), D.G.O. (Dublin), L.R.C.S. (Edin.), M.V.A.S. (Vienna). | Medical Officer, I/c. Municipal Hospital, Amalnear, Khandesh. | Virus Diseases including Rabies. | 11th June 1956 to 18th June 1956.          |
| 6. Shri C. K. Kamath, B.Sc., M.Pharm.                                                          | Chemist, M/s. Unichem Laboratories.                           | Chemotherapy ...                 | 3rd July 1956 onwards.                     |
| 7. Shri J. M. Vachharajani, M.Sc.                                                              | M/s. International Franchises Private Ltd.                    | Pharmacology ...                 | 2nd August 1956 to 1st December 1956.      |
| 8. Dr. N. K. Keshav Murthy, M.B. B.S.                                                          | Medical Officer, Umaria Collieries, V. P.                     | Virus Diseases including Rabies. | 23rd August 1956 to 31st August 1956.      |
| 9. Shri C. S. Bhambure, M.Sc.                                                                  | From Village Industries Board, Poona 2.                       | Clinical Pathology.              | 4th September 1956 to 29th September 1956. |
| 10. Shri B. Choudhary.                                                                         | From D. M.'s Head Office, Central Railway, Bombay.            | Do. ...                          | 15th October 1956 to 14th January 1957.    |
| 11. Dr. S. C. Sinha, B.M.S. Cl. III.                                                           | Medical Officer, I/c. Ophthalmic Ward, Civil Hospital, Thana. | Blood Bank ...                   | 15th October 1956 to 29th October 1956.    |
| 12. Shri M. V. Soneji, M.B.B.S., B.M.S. Cl. II.                                                | Medical Officer, I/c. Dispensary-cum Maternity Home, Khopoli. | Do. ...                          | 17th November 1956 to 1st December 1956.   |
| 13. Shri A. D. Deodhar, M.Sc.                                                                  | Chemist, M/s. 'Pharma Search Laboratories, Bombay 7.          | Pharmacology ...                 | 1st December 1956 onwards.                 |
| 14. Shri L. S. Karabelkar.                                                                     | Office of the Metallurgist, Western Railway, Ajmer.           | Vaccine ...                      | 1st December 1956 to 31st December 1956.   |

## XIII. Critics Club.

Under the auspices of the Critics Club, 24 scientific meetings were held where the staff of the different departments of this Institute took part. There were four lectures by guest speakers. The list is given below :—

| Speaker.                                                                               | Subject.                                                                                        |
|----------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| 1. Dr. W. R. Aykroyd, Director, Nutrition Division, F.A.O., Rome.                      | International work in the field of Nutrition.                                                   |
| 2. Dr. G. H. Ball, Prof. of Zoology, University of California, Los Angeles.            | Experiments on the cultivation of the Mosquito phase of bird malaria.                           |
| 3. Dr. G. H. Ball, Prof. of Zoology, University of California, Los Angeles.            | A blood inhibiting protozoan from the water snake <i>Coleuber Karolini</i> .                    |
| 4. Dr. (Mrs) M. R. Ball, Prof. of Bacteriology, University of California, Los Angeles. | Student life in American Universities.                                                          |
| 5. Dr. P. J. Deoras and Shri M. S. Gokhale.                                            | Collection of fleas from Bombay Rats.                                                           |
| 6. Kumari K. C. Patel ...                                                              | Comparative toxigenicity test on <i>C. diphtheria</i> strains.                                  |
| 7. Dr. (Smt.) A. Venkataraman.                                                         | Metabolic Effects of Insulin.                                                                   |
| 8. Shri M. J. Sardesai ...                                                             | Approach to Chemotherapy through antimetabolites with special reference to benzimidazoles.      |
| 9. Dr. K. V. Kulkarni ...                                                              | Immuno-haematology.                                                                             |
| 10. Shri A. S. Narasimham ...                                                          | Bacterial resistance to antibiotics.                                                            |
| 11. Shri Y. S. Nimbkar ...                                                             | Bacteriological examination of swimming pool water.                                             |
| 12. Shri V. P. Kulkarni ...                                                            | Estimation of Vitamin B-12.                                                                     |
| 13. Shri V. Y. More ...                                                                | Effect of lipid extraction on horse antitoxic pseudoglobulin.                                   |
| 14. Dr. V. N. Jai, Dr. S. M. Patel and Kumari S. S. Corley.                            | Investigations on the high incidence of urinary stone in Mehasana District.                     |
| 15. Dr. G. T. Tonpi ...                                                                | Respiratory apparatus in insects in relation to the insecticides.                               |
| 16. Kumari M. Savitri ...                                                              | Vi phage typing of <i>S. typhosa</i> and its significance in epidemiology.                      |
| 17. Shri R. N. Joshi ...                                                               | Physiology of calcium metabolism.                                                               |
| 18. Dr. C. V. Deliwala ...                                                             | Synthesis of Vitamin A.                                                                         |
| 19. Shri C. J. Karkhanis ...                                                           | Recent Advances in blood and blood products in USSR.                                            |
| 20. Shri L. S. Krishnan ...                                                            | Plasma cell mastitis in lactating <del>beast</del> <sup>beast</sup> .<br>A review of two cases. |
| 21. Shri M. K. Habbu ...                                                               | Plague Toxin.                                                                                   |



| Speaker.                 | Subject.                                                     |
|--------------------------|--------------------------------------------------------------|
| 22. Shri M. S. Shastry   | ... Indian Rauwolfias and their place in therapy.            |
| 23. Shri M. S. Gokhale   | ... Collection of rat fleas and some studies on Bombay Rats. |
| 24. Shri S. R. Agnihotri | ... Diphtheria-tetanus-Pertusi, combined immunization.       |

The scientific staff of the Institute took part in two meetings of the Teaching Pathologists held on 31st March 1956, and 27th October 1956. The following papers were presented :—

- (1) Shri Y. I. Shethna ... Laboratory Diagnosis of Rabies with special reference to the use of complement fixation test.
- (2) Kum. K. A. Nadkarni ... Analysis of human and bovine lens.
- (3) Dr. (Smt.) A. Venkataraman. A discussion on the fractionation and estimation of serum proteins for clinical work.
- (4) Dr. (Mrs.) M. R. Ball ... Laboratory Diagnosis of Leptospirosis.
- (5) Shri R. S. Karbhari ... Effect of heat on Typhoid Antigens.
- (6) Kum. K. C. Patel ... Toxigenicity test for *C. diphtheria* by three different methods.

#### B. LIST OF DISTINGUISHED VISITORS VISITING THE INSTITUTE DURING 1956.

- (1) Dr. Herrlich, Specialist in Tropical Diseases, Germany. 14th January 1956.
- (2) Dr. Franz Weiss, University of Caracas (Venezuela). 14th January 1956.
- (3) His Excellency Dr. Hare Krushna Mehtab, Governor of Bombay. 24th January 1956.
- (4) Prof. & Mrs. J. L. Melnick, Yale University, School of Medicine, New Haven, Conn. 10th February 1956.
- (5) Dr. W. R. Aykroyd, Director, Nutrition Division, F.A.O., Rome. 11th February 1956.
- (6) Dr. C. G. King, Scientific Director, Nutrition Foundation, Inc., New York. 11th February 1956.
- (7) Prof. & Mrs. Pi Suner, Head of the Institute of Physiology, University of Caracas (Venezuela). 15th February 1956.
- (8) Dr. R. M. Taylor, Field Director, Int. Health Dn., Rockefeller Foundation, N. Y. 24th February 1956.
- (9) Soviet Youth Delegation ... 2nd March 1956.
- (10) Russian Delegation of Pharmaceutical & Drug Industry. 14th March 1956.

- (11) Chief Minister, Assam ... 4th June 1956.
- (12) Prof. Wittenburgh, Professor of Parasitology, Hebru University, Jerusalem. 7th June 1956.
- (13) Shri Morarji Desai, Chief Minister, Bombay State. 7th July 1956.
- (14) His Excellency Benjamin Munez, Costa Rican Ambassador to United Nations. 10th July 1956.
- (15) International Atomic Energy Delegates ... 29th July 1956.
- (16) An Ethiopian Delegation ... 10th August 1956.
- (17) Shri Shantilal Shah, Health Minister, Bombay State. 15th September 1956.
- (18) Chinese Agricultural Science Study Mission 26th October 1956.
- (19) Shri M. S. Kannamwar, Health Minister, Bombay State. 9th November 1956.
- (20) Cambodian Delegation ... 13th November 1956.
- (21) Shri S. G. S. N. Kazi, Deputy Health Minister, Bombay State. 30th November 1956.
- (22) Dr. & Mrs. M. M. Wintrobe, Prof. of Medicine, University of Utah, School of Medicine, Salt Lake City, Utah, U. S. A. 3rd December 1956.
- (23) His Excellency Shri Tanka Prasad Acharya, Prime Minister of Nepal. 11th December 1956.
- (24) Chinese Women's Delegation ... 29th December 1956.

#### 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 1956 :—

- 1. Enquiry of the Pulicidal value of Pyrethrum. Under the Director, Haffkine Institute, Bombay, upto 31st March 1956.
- 2. Enquiry on the Role of Nutritional Factors in Hepatic Cirrhosis. Under Dr. M. V. Radhakrishna Rao, Assistant Director, I/c. Department of Nutrition, Haffkine Institute, Bombay, upto 31st March 1956.
- 3. Enquiry on the study of Indian Poisonous Snakes. Under Dr. P. J. Deoras, Assistant Director, Department of Entomology, Haffkine Institute.

In addition to abovementioned Scientific Enquiries conducted here, the following Research Fellowships and Scholarships were attached to this Institute during the year under review. Particulars of sponsors, the subject of Fellowship and the names of candidates working under them are as under :—

| Serial No. | Sponsors.                                                 | Subject of Fellowship                                                                                  | Names of Candidates.                                                                                   |
|------------|-----------------------------------------------------------|--------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|
| 1          | Unichem Laboratory, Bombay.                               | Research on Indigenous Drugs.                                                                          | Shri M. Shrinivas Shastri, M.Sc.                                                                       |
| 2          | Do. do. ...                                               |                                                                                                        | Vacant.                                                                                                |
| 3          | Council of Scientific and Industrial Research, New Delhi. | Isolation and study of the biologically important constituents of venoms of Cobra and Russell's Viper. | Dr. (Smt.) Shanta S. Rao, M.Sc., Ph.D.; upto 30th September 1956.                                      |
| 4          | Government of India Research Scholarship.                 | Study of Tissue Culture Techniques.                                                                    | Kumari M. Savitri, B.Sc.; from 28th August 1956.                                                       |
| 5          | Do. ...                                                   |                                                                                                        | Vacant.                                                                                                |
| 6          | Government of Bombay Research Scholarship.                | Study of Influenza Viruses.                                                                            | Kumari Rayna A. Mendes, B.Sc.; from 30th April 1956.                                                   |
| 7          | Government of Bombay Research Scholarship.                | Salmonella Typing ...                                                                                  | Kumari M. C. Patel, B.Sc.; from 30th August 1956.                                                      |
| 8          | Indian Council of Medical Research, New Delhi.            | Higher training in Bacteriology, under Dr. D. W. Soman.                                                | Dr. M. V. Bapat, B.Sc., M.B.B.S., Jr. Lecturer, B. J. Medical College, Poona; from 12th November 1956. |

Facilities were also offered to Science Graduates, on payment of the prescribed fees, to take their post-graduate degrees by research. During the year, the following candidates were given such facilities :—

1. Shri Yogendra I. Sethna, B.Sc. (Microbiology).
2. Kumari M. S. Savant, B.Sc. (Zoology).
3. Kumari K. C. Patel, B.Sc. (Microbiology).
4. Shrimati Susy Cherian, B.Sc. (Microbiology).
5. Shri A. K. Joshee, M.Sc. (Zoology).
6. Kumari K. K. Patel, B.Sc. (Zoology).
7. Kumari L. S. Kadaba, B.Sc. (Biochemistry).
8. Kumari P. K. Khandwala, B.Sc. (Biochemistry).
9. Kumari I. R. Ovalekar, B.Sc. (Microbiology).

Following is the list of Post-graduate students who obtained their M.Sc. Degrees (by research) of the Bombay University during the year .—

| Name.                | Subject.     | Guide.                          |
|----------------------|--------------|---------------------------------|
| Shri S. R. Wagle ... | Biochemistry | ... Dr. M. V. Radhakrishna Rao. |
| Shri V. Y. More ...  | Chemistry    | ... Dr. S. S. Rao.              |
| Kum. R. D. Mehta ... | Microbiology | ... Dr. P. M. Wagle.            |

## XV. Income and Expenditure for 1955-56.

| Income.                                 |                  | Expenditure.                           |                  |
|-----------------------------------------|------------------|----------------------------------------|------------------|
| <b>XXVII-Medical Bact. Laboratories</b> | <b>Rs.</b>       | <b>38-Medical—</b>                     | <b>Rs.</b>       |
| Sale proceeds of Sera and Vaccines.     | 24,73,637        | <i>I. Bacteriological Laboratories</i> |                  |
|                                         |                  | <i>I.1 Haffkine Institute.</i>         |                  |
| Other Miscellaneous Receipts.           | 24,178           | Pay of Officers                        | ... 1,96,907     |
|                                         |                  | Pay of Establishment                   | ... 4,61,338     |
|                                         |                  | Allowances                             | ... 3,52,280     |
|                                         |                  | Contingencies                          | ... 10,60,110    |
|                                         |                  | Research Scholarships                  | ... 2,870        |
|                                         |                  | <i>I.2 Pasteur Institute.</i>          |                  |
|                                         |                  | Pay of Officers                        | ... 5,190        |
|                                         |                  | Allowances                             | ... 875          |
| <b>Total</b>                            | <b>24,97,815</b> | <b>Total</b>                           | <b>20,79,570</b> |

## PART II

## RESEARCH WORK

## I. Biological Assay of Antibiotics.

(Habbu and Soman).

*Assay of Penicillin and Streptomycin Samples.*

During the year under report 52 samples of penicillin including crystalline penicillin, procaine penicillin, penicillin ointments and tablets were assayed by the dilution method against *S. aureus* as test organism. Out of 52 samples assayed 46 were up to the standard. Out of 6 sub-standard samples, 5 were either tablets or ointments and one was powder.

19 samples of streptomycin were also assayed by the dilution method against *Kl. pneumonia* as test organism. All samples were found to be up to the standard.

## II. Studies on Influenza

(Soman, Smt. Cherian and Nimbkar).

During the year 1956-57 about 25 throat-washings were collected by the Influenza Centre at the Institute from suspected cases and were subjected to the isolation procedure in chick embryos. Serological examination of the acute and convalescent phase serum samples of the patients was carried out wherever possible.

Four strains of influenza, three belonging to the type-A and one to the type-B were isolated and identified by H. A. Inhibition test. They were subsequently confirmed by the World Influenza Centre by H. A. Inhibition tests. Even before the final identification of these four strains, it was possible to type two of them by C. F. technique using soluble antigens of the infected membranes of the primary passage and the specific type sera prepared in g. pigs. Thus this method of diagnosis is very rapid and simple and will be adopted as a routine in the isolation procedures of the influenza trials in future. The following table gives the details of the strains isolated and the serology of the patients' sera.

| Serial No. | Date of Isolation. | Patient's Name.   | Nomenclature. | H. A. I. titre of patients sera. |
|------------|--------------------|-------------------|---------------|----------------------------------|
| 1          | 15th March 1956    | Dr. Gordon Ball   | A/India/34/56 | 16/128                           |
| 2          | 19th March 1956    | Dr. Deoras        | A/India/35/56 | 8/32.                            |
| 3          | 31st July 1956     | Shri Sholapurwala | B/India/36/56 | 16/64.                           |
| 4          | 21st October 1956  | Shri Shethna      | A/India/37/56 | 16/256.                          |

The comparative study of three type-A strains showed that all these strains were similar to the 'Dutch 56' strain which in turn resembled A/England/1/53 though distinct from it.

The strains A/India/34/56 and A/India/35/56 were the first of this type to be isolated in India though they had been isolated in Holland a little earlier. Hence it is likely that this type might have originated in Holland and then spread to India. Dr. Gordon Ball from whom the strain A/India/34/56 was isolated was staying in Hotel Ambassador and was working at the Institute during the period of February to June 1956, and it is very likely that the infection reached this hotel from continental tourists passing through the same hotel. This indicates a probable mode of spread of the virus. The strain A/India/35/56 was isolated from Dr. Deoras who was closely associated with Dr. Ball in his work at the Institute.

The strain B/India/36/56 gave very irregular and erratic growth in chick embryos like the other two type-B strains isolated some years ago in this laboratory, but after some perseverance the strain was finally established and it was found to be antigenically similar to B/England/54 virus. Similar difficulties were met with by the World Influenza Centre, London in the cultivation of this strain in chick embryos.

### III. Studies on Normal Serum Inhibitors.

(Soman and Nimbkar).

The results of the inactivation of the normal rabbit serum inhibitors inhibiting the haemagglutination of the influenza virus with trypsin were reported last year. As the method was found to be very convenient in Haemagglutination Inhibition Test, a more detailed study of the problem was undertaken.

Firstly, the sera of common laboratory animals such as rabbits, g.pigs, fowls, hamsters and horses were studied and a quantitative estimate of the inhibitors present in normal sera of these animals was obtained using A/India/31/55 and B/India/33/52 strains of the virus. It was observed that only rabbit and g.pig sera showed the presence of appreciable quantity of inhibitors while fowl, hamster and horse sera were poor in inhibitor contents. It was also observed that the inhibition titre of the rabbit serum was unaffected by heating at 56° C for  $\frac{1}{2}$  hour while the inhibitors present in g.pig serum could be inactivated under the same conditions.

Activity of the inhibitors in pooled rabbit serum against different standard as well as locally isolated strains was determined. It was observed that the standard strains PR8, FMI, Lee and locally isolated strain A/India/31/55 and B/India/33/52 were equally susceptible to normal rabbit serum inhibitors. However, a strain A/USA/47 received as a standard of an antigen from W. H. O. for H. A.-I tests was much less susceptible to these inhibitors.

A comparative study of inactivation of crude cholera filtrate and trypsin was also made. For this purpose N. rabbit serum was mixed with crude cholera filtrate in the proportion 1 : 4 and kept overnight at 37° C. After about 16 hours incubation the mixture was inactivated by heating at 56° C for them and the inhibitory activity was determined as usual. A sample of the same serum was treated with 32 mg/ml. of B. D. H. trypsin by the method of Sampaio and Issac and inhibition titre of the mixture was determined. It was observed that both crude cholera filtrate and trypsin were equally effective in destroying the inhibitors in the normal rabbit serum.

As the inactivation with trypsin is a quicker method its use in H. A.-I Test is of definite advantage. Hence the optimum quantities of B. D. H. trypsin that could be used for inactivation of normal inhibitors in the immune serum without any loss in its antibody content was determined. It was observed that immune rabbit serum treated with 4 mg/ml of trypsin inactivates the inhibitors without affecting the antibody content while quantities of 8 mg/ml or over destroy the antibodies also.

#### IV. Comparative Study of Influenza Type-B Strains by Serological Methods.

(Soman, Smt. Cherian & Nimbkar).

Since 1950, 15 influenza strains isolated at this Institute included 3 strains of influenza type-B. A comparative study of these strains is being made with some of the earlier type-B strains obtained from National Institute of Health, Bethesda. It was proposed to carry out their antigenic analysis by hemagglutination inhibition test as well as by complement fixation test.

Pooled infected allantoic fluids were used for the hemagglutination tests as well as for the immunization of animals. Rabbits and fowls were injected intravenously with 5 ml of infected allantoic fluid followed by 10 ml. of the same intraperitoneally at the same time. G. pigs received 0.5 ml intranasally and 2 ml. intraperitoneally. Two weeks later, all the animals were bled from the heart. All these sera were tested for their specific antibody content and stored at 20° C.

Normal fowl sera and normal g.pig sera did not show the presence of appreciable quantity of serum inhibitors by hemagglutination-inhibition test. In view of the fact that even normal rabbit sera always contained an appreciable amount of serum inhibitor immune rabbit sera were treated either by cholera filtrate or by trypsin to destroy it as per technique of Sampaio and Isaacs, with the difference that 4 mgs/ml of Difco trypsin was used instead of 8 mg/ml of the Armour as recommended by them. The normal serum inhibitors in g.pig and fowl sera being practically absent or very low, the immune sera were not required to be treated with trypsin or cholera filtrate. The hemagglutination-inhibition tests were performed according to the standard procedure. Cross hemagglutination-inhibition tests using rabbit, g.pig and fowl antisera indicated that the type-B strains isolated in different years were dissimilar in their antigenic composition.

Complement fixation tests were carried out according to the standard method. Soluble antigens of 6 type-B strains were prepared from infected chorioallantoic membranes. Only the immune g.pig sera were used in these tests. Further work in this connection is in progress.

#### V. Further Observations on the Complement-Fixation Test in the Diagnosis of Rabies.

(Soman and Shethna).

The study of the complement-fixation test in the diagnosis of rabies was continued throughout the year 1956. A total of 101 brain specimens were tested by smear examination, mice inoculation and complement-fixation test, together with the study of the important factors likely to affect the sensitivity of the test.

The results of complement-fixation test agreed with the smear examination and mice inoculation except in 4 cases. Brains from 2 cases were received in a putrified condition which mainly explained why smear examination could not be performed and why complement-fixation test was negative in spite of the positive virus isolation in mice.

In preliminary studies of this test it was thought that some parts of the brain might be more antigenic than others or might be altogether nonantigenic. The experiment on the antigenic content of various parts of the brain was therefore carried out 15 brain specimens of which 40% suspension was obtained, were tested by complement-fixation test for the antigen content of Ammon's horn, Cerebral cortex, Cerebellum and Medulla oblongata. Complement-fixing antigen could only be demonstrated in the Ammon's horn, Cerebellum and Medulla oblongata while in the Cerebral cortex it was not always so.

Further, various concentrations of antigen, such as 40 per cent, 20 per cent., 10 per cent. and 5 per cent. were tried and it was observed that 40 per cent. antigen was more reliable than the other concentrations.

Similarly, for the elution of virus from the brain cells the effect of heat on the preparation of the antigen was studied. It was observed that the virus could easily be eluted at temperatures over 30° C for one hour and therefore it was unnecessary to heat the suspension. However, since the daily temperature varied within wide limits it was thought that the suspension be heated at 37° C for one hour.

The remaining two discrepancies perhaps could be explained by the fact that the virus content was too low, as determined by its LD 50 titre in mice. In one case only 2 out of 5 mice were paralysed and in the other only 1 out of 6; but all the paralysed mice showed Negri bodies on post-mortem. Thus it appeared that the mouse inoculation test was more sensitive than the complement-fixation test.

43 salivary glands were tested by mice inoculation and complement-fixation test. In 5 cases the results of C. F. test were not in agreement with those of mice inoculation. The cause of the discrepancy has been attributed to the presence of mucin in the salivary gland, by the Japanese workers, which inhibits the complement-fixing reaction.

The results have shown that the complement-fixation test has got a definite place in the diagnosis of rabies in animals, especially for the Negri-negative specimens and salivary glands, although the last word could be obtained by mice inoculation.

## VI. Biological Test for the Diagnosis of Rabies.

(Soman and Khemani).

The biological test using white mice for the diagnosis of rabies was continued during this year also which, as previously reported, was mainly carried out with Negri negative specimens. Of the 91 specimens received during the year, 64 were in glycerine and the remaining in normal saline. Of these 64 specimens, biological tests were carried out on 38 specimens only.

The results of biological tests for the last 5 years are summarised in the following table :—

Table.

| Year.       | Total. | Negri<br>negative.<br>Mouse<br>negative. | Negri<br>positive.<br>Mouse<br>positive. | Negri<br>negative.<br>Mouse<br>positive. |
|-------------|--------|------------------------------------------|------------------------------------------|------------------------------------------|
| 1952 .. ..  | 28     | 15                                       | 10                                       | 3                                        |
| 1953 .. ..  | 28     | 17                                       | 4                                        | 7                                        |
| 1954 .. ..  | 28     | 17                                       | 3                                        | 8                                        |
| 1955 .. ..  | 24     | 14                                       | 6                                        | 4                                        |
| 1956 .. ..  | 38     | 20                                       | 9                                        | 9                                        |
| Total .. .. | 146    | 83                                       | 32                                       | 31<br>(21.2%)                            |



As will be seen from the table that 56·8 per cent. of these specimens were negative for Rabies, 21·9 per cent. were positive for Rabies. while in 21·2 per cent. though Negri negative, rabies virus could be demonstrated by mice inoculation. In view of these important findings the biological test in mice has to be accepted as a routine method for diagnosis of rabies at this Institute.

## VII. Studies of Street Virus of Rabies.

(Soman and Khemani)

Comparative studies for the presence of virus in brain and salivary glands were continued during this year also. In all 39 specimens were tested during the year under report. The results are summarised in the table along with those of previous four years. In all cases the diagnosis of rabies in animal was established by finding out negri bodies or by isolation of virus.

Table.

| Year.      | Total. | Brain pos.<br>Sal. gland<br>pos. | Brain pos.<br>Sal. gland<br>neg. | Brain neg.<br>Sal. gland<br>neg. | Brain neg.<br>Sal. gland<br>pos. |
|------------|--------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| 1953 .. .. | 29     | 8                                | 8                                | 13                               | 0                                |
| 1954 .. .. | 28     | 16                               | 4                                | 8                                | 0                                |
| 1955 .. .. | 25     | 9                                | 8                                | 8                                | 0                                |
| 1956 .. .. | 39     | 20                               | 7                                | 12                               | 0                                |
| Total ..   | 121    | 53                               | 27<br>(22·9%)                    | 41                               | 0                                |

Thus, out of 121 brain specimens and salivary glands tested, in 43·4 per cent. the virus could be demonstrated in both Brain and Salivary glands, in 33·6 per cent. the virus was shown to be absent in both brain specimens and salivary glands, but in 22·9 per cent. the virus was present only in the brain, and not in salivary glands, which shows that all rabid animals may not transmit rabies virus. Besides confirming the epidemiological significance of these observations on transmission of rabies in nature, they indicate that the fact has to be considered in the correct appraisal of the value of prophylactic treatment of rabies by antirabic vaccine and serum.

## VIII. The value of Hyperimmune Rabies Serum in Rabies Prophylaxis.

(Soman and Khemani).

As already reported (Annual Report 1955) hyperimmune rabies serum of high antibody content prepared at this Institute was supplied to five antirabic treatment centres for the treatment of severely bitten patients in conjunction with antirabic vaccine. In all 22 cases have been treated with the serum in the past two years, 2 of the treated cases were jackal-bites and the rest were dog-bites.

In all cases except one, the serum was administered within 72 hours after the bite, the period in which it is likely to be most useful. The bites were usually severe according to the Medical Officer administering the treatment and in many cases there was enough circumstantial evidence to show that the biting animal was rabid. In 2 cases, however the evidence of rabies in the biting animal was confirmed by the subsequent development of rabies in untreated animals. The value of hyperimmune rabies serum in the treatment of rabies has already been established as reported by W. H. O. in their trials in Iran and it is, therefore, very essential that the period of field trials for our serum should be further extended.

### **IX. Study on the Protective Value of Antirabic Vaccine on Rhesus Monkeys**

(Soman and Kum. Ver.)

Habel's test has been accepted as the standard method for estimation of potency of killed virus antirabic vaccine by all the laboratories the world over. The test is carried out by immunising white mice of known age and weight with the antirabic vaccine and challenging them afterwards intracerebrally with fixed virus.

The above test was conducted to study the Protective Value of the vaccine in bigger animals like monkeys and challenging them with street virus intramuscularly. The challenge virus was street virus obtained from dog brain and passaged in mice.

Six rhesus monkeys were used in the experiment, out of which 3 were immunized with antirabic vaccine 5 ml daily for 14 days and the remaining 3 were used as "controls."

All these monkeys were challenged after one month with 1 ml. of 10 per cent. mouse brain suspension of street virus in the neck muscle.

The untreated monkeys, i.e., "controls" died of rabies with the incubation periods of 16, 26, 36 days respectively, while the immunized animals survived for 3 months after the challenge. To ascertain if the immunity still persisted in these monkeys, they were rechallenged 2 days after, with 1 ml of 10 per cent. g. pig brain suspension of street virus (LD<sub>50</sub>, 10-4.5 in mice) in the neck muscle. As a result of protection conferred by the vaccine all the three monkeys have survived to this date, i.e., 234 days in spite of them being twice challenged. Further work is in progress.

### **X. Study of Tissue Culture Techniques.**

(Soman, Nimbkar, Kum. Savitri and Kum. Mendes)

The isolation and propagation of viruses in this Institute is being done at present by egg and animal inoculation. As tissue culture is in many ways a more specialised and advanced method than the above two, it was decided to develop the tissue culture technique.

To begin with many difficulties were encountered. Many of the required materials like pyrex glassware, instruments, chemicals, such as trypsin, lactalbumin had to be imported. The method of washing the glassware etc. required careful study; accuracy in preparing and autoclaving the solutions, used in the preparation of the medium was absolutely necessary, as slight variations hampered the growth of the cells. Besides many adjustments had to be made in the procedures to obtain good growth of cells.

With these preliminary arrangements and adjustments it has been possible to obtain monolayer cultures of chick embryo, chick embryo lungs and rabbit kidneys, and plasma clot cultures of the above mentioned tissues and human tonsillar tissue.

The tissues were first trypsinised and then centrifuged. The deposit containing cells was diluted with the medium (Hank's), a cell count was taken and then a suspension was made containing the required amount of cells which was tubed in 0.5 ml. amounts. In the case of chick embryo cultures, a sheet of fibroblast cells was observed on the third day and the sheet remained intact for about 10-12 days after which degeneration of cells was observed. As far as the rabbit kidney and chick embryo lung cultures were concerned, a sheet of epithelial cells was observed between the sixth and eighth day which continued up to the fifteenth day. After that there was rounding up and gradual falling of cells.

In the plasma clot technique, using heparinised plasma with chick embryo extract and then incorporating tiny bits of the tonsillar tissue, good growth of both types of cells, i.e. the fibroblasts and epithelial cells was obtained, while using the whole chick embryo and chick embryo lungs only fibroblasts and epithelial cells were seen respectively. The growth appeared about the second or third day and continued for 20-30 days.

Attempts were made to propagate influenza and Rabies viruses in Tissue Culture but the initial success obtained was not maintained in successive passages. Further work is necessary and is being continued.

## **XI. Heat Stability and Complexity of Vi Antigen of Salmonella Typhi.**

(Karbhari, Rao and Hazra.)

It was reported last year that the extracts of suspensions of 24 hour's growth on nutrient agar of Ty<sub>2</sub> and Vi<sub>1</sub> strains of *S. typhi* prepared by heating to 100° C for one hour were found to contain a large amount of Vi antigen when tested by precipitation test against pure Vi serum. It was, therefore, thought necessary to see whether the Vi antigen in the heated extracts still retained its agglutinogenic property. Extracts of Ty<sub>2</sub> and Vi<sub>1</sub> strains prepared by repeated freezing and thawing the same suspensions between 20° C and room temperature were compared with the heated extracts for their agglutinogenic property. The four extracts were used for immunizing rabbits. Two rabbits

were used for each extract and the sera were tested for Vi agglutinin titres. The results are tabulated below :

| Strain<br>of<br>Extract. | Immunization with extracts prepared<br>by freezing and thawing. |                                  | Immunization with extracts prepared<br>by boiling. |                                  |
|--------------------------|-----------------------------------------------------------------|----------------------------------|----------------------------------------------------|----------------------------------|
|                          | Rabbit<br>No.                                                   | Vi agglutinin<br>titre of serum. | Rabbit<br>No.                                      | Vi agglutinin<br>titre of serum. |
| Ty <sub>2</sub> ..       | 1                                                               | 4,800                            | 1                                                  | 100                              |
|                          | 2                                                               | 3,200                            | 2                                                  | 100                              |
| Vi <sub>1</sub> ..       | 3                                                               | 4,800                            | 3                                                  | 50                               |
|                          | 4                                                               | 6,400                            | 4                                                  | 100                              |

The results show that the Vi antigen in the extracts prepared by boiling suspensions has undergone some change as shown by impairment of its agglutination property.

It is known that the Vi antigen is not a single antigen but consists of a number of components. No attempt has yet been made to determine the number of these components. It was, therefore, thought interesting to determine the number of these components and to study the effect of heat on them by Ouchterlony's horizontal gel diffusion precipitin technique.

The results of the test revealed that the Vi antigen of *S. typhi* consists of a number of components of which the strain Vi<sub>1</sub> contains atleast 10 components and the strain Ty<sub>2</sub> atleast 9 components. In the case of both the strains, two of these components are in large amounts and the rest in smaller amounts. The major components are not affected by heating even to 100° C for one hour. But four of the minor components are completely destroyed at this stage. The major components which are not affected by heat may be responsible for the precipitation reaction which remains almost unaffected even when the antigen is subjected to 100°C for one hour.

## **XII. The Haemolytic Property of *P. Pestis* and Allied Organisms.**

(Kumari Mehta, Nimbkar & Wagle.)

The haemolytic property of *P. pestis* was first reported by Rayband and Pellisier in 10-14 day old cultures of *P. pestis* in 1902. Since then many contradictory findings have been reported by different investigators. In our laboratory it was observed during the routine testing of *P. pestis* cultures for the production of Anti-Plague Vaccine that clear zones of haemolysis sometimes appeared around individual colonies of *P. pestis* cultivated on rabbit blood agar slopes with acid digest agar as basal medium. However, this was neither a regular phenomenon nor could it be easily reproduced. It was also considered probable that the haemolytic property of the organism may be related to the antigenic make-up of organism which may ultimately affect the production of a potent Anti-Plague Vaccine. In view of these facts a detailed study of the problem was undertaken.

The irregularity of production of zones of haemolysis on blood agar indicated that the organism might produce haemolytic zones regularly if it is cultivated under favourable conditions. Hence, it was decided to study the effect of the following factors on haemolytic activity.

1. Composition of the medium.
2. pH of the medium.
3. Temperature of incubation of cultures.

Seven different media and six modifications of nutrient agar were used as basal media in the preparation of blood agar slopes. Of these only five, viz. sulphite agar, nutrient agar, nutrient agar with calcium and magnesium, nutrient agar with egg yolk, calcium and magnesium, and nutrient agar with broth which was previously treated with charcoal proved to be suitable. However, the nutrient agar with egg yolk gave the best results.

pH range of 6.8 to 7.2 was found to be suitable for the production of haemolytic zones round individual colonies.

It was observed that only at 37°C clear haemolytic zones developed around *P. pestis* colonies. At temperature of 28°C the production of zones was not well marked. The temperature of 32°C gave intermediate results.

Resistance of erythrocytes of four different animal species, viz. g. pig, rabbit, sheep and horse to the haemolytic activity was tested. G. pig cells were found to be the most susceptible, while those of sheep most resistant.

Thirteen different strains of *P. pestis* consisting of both virulent and avirulent type and two strains of *P. pseudotuberculosis* were tested for haemolytic activity under optimum conditions. All of them produced haemolytic zones on blood agar. Thus, no correlation was observed between the virulence and the haemolytic activity of the organism. It was also observed that haemolytic activity could not be used for differentiation of *P. pestis* and *P. pseudotuberculosis*. From the study of the cultures in liquid medium no soluble haemolysin could be detected. However, a distilled water extract of the organism containing 0.85 per cent sodium chloride haemolysed the red blood cells indicating the possibility of the presence of an intracellular haemolysin.

### XIII. Common Antigens in the Human, Horse and Bovine Sera.

(Kulkarni.)

Anti-horse serum was prepared by immunizing rabbits with normal horse serum. Using Ouchterloney's horizontal gel diffusion precipitin test, the rabbit anti-horse serum was put up against normal horse serum, human serum and bovine serum. Human serum was found to contain 4 common antigens with horse serum, while bovine serum showed 3 common antigens with horse serum. Two components were common to all the three sera.

#### XIV. Application of Gel-diffusion Technique for Assaying the Antivenin Sera.

(Kulkarni and Rao.)

Pope, Oudin and Oakley and Fulthrope have shown that position of band obtained in Oudin's gel diffusion precipitin test depends upon concentration of antigen and antibody when area of cross-section of the tubes and other factors are kept constant.

An attempt was made making use of the above facts to find the relative position of band by using the following three methods: (1) Keeping the antigen constant in top layer and varying antibody in bottom layer. (2) Keeping antibody constant in bottom layer and varying antigen. (3) Antibody constant in bottom layer and mixture of antigen and antibody in top layer keeping antibody constant and varying the antigen.

A straight line relationship was always obtained when the position of any one major band was plotted against the logarithm of the concentration of the venom or antivenin whichever is varied in the experiment.

This relationship obtained was used for standardization of antivenin in comparison with a reference standard using the above three methods.

It was observed that the results obtained by these methods agreed fairly well with results obtained by animal tests. Among the three methods the first two gave better results than the third.

#### XV. Observations on the Flea Parasites of Bombay Rats.

(Deoras and Gokhale.)

Statistical analysis of the data of fleas collected from *Rattus rattus*, *Rattus norvegicus* and *Bandicoota bengalensis* of during 1954 and 1955 has shown the following:—

(a) *Rattus rattus* carried more fleas than *B. bengalensis* and the latter has more fleas than *R. norvegicus*.

(b) *Rattus rattus* carried *X. cheopis*, significantly more than *X. astia*, and *B. bengalensis* carried *X. astia* significantly more than *X. cheopis*. *R. norvegicus* carries *X. cheopis* more than *X. astia*.

(c) There was a difference in the collection of fleas in different seasons. The number was highest during March to June.

(d) The number of female fleas was higher during the first half of the year.

(e) The number of males of *X. cheopis* was significantly more in the collections than females. In the case of *X. astia* the number of females was significantly more than the males.

The findings of these studies were compared to those of previous workers with particular reference to the percentage of different fleas caught in the collection and their sex-ratio. From these it is seen that they are more or less on the same pattern, though the methods of collection were different.

## XVI. Some Biometrical Studies on Bombay Rats.

(Deoras and Gokhale)

The six common specimen of rats which are regularly brought to this Institute by the Bombay Municipality were studied with an idea of getting characters that may help in classifying them on a general basis. The rats have so far being classified as *Rattus rattus*, *Rattus norvegicus*, *Bandicoota bengalensis*, *Bandicoota malabarica*, *Mus musculus* and *Suncus caeruleus*.

The characters studied on a statistical basis were the length of the whole body, length of tail, length of head, length of ear, number of mammary glands, number of different vibrissae, tail rings, density of fur, and the type of pads on the legs. Total number of rats examined were: *Rattus rattus* 1829, *Rattus norvegicus* 1117, *Bandicoota Bengalensis* 1360, *Bandicoota malabarica* 42, *Mus musculus* 40, and *Suncus caeruleus* 177.

A tentative table based on these characters is available for ready reference.

## XVII. Studies on Salmonellosis.

(Sant, Narasimham and Kum Patel).

The importance of salmonella infection as a major public health problem has been recognized. Arrangements have now been made to study the nature of the Salmonella types and 69 strains of different types of Salmonella were obtained from abroad through the kind courtesy of Dr. Meridian Ball and the preparations of the various antisera for diagnostic work are under-way.

Besides providing valuable information on the subject, it is aimed to incorporate in this work the services of Reference Laboratory for Salmonellosis.

## XVIII. Plasma Cell Mastitis in Lactating Breast.

(Sant).

Plasma cell mastitis is not a very common disease and the condition occurring in a lactating breast is rather rare. So far, very few cases have been reported in the literature. A detailed report of the two cases of plasma cell mastitis in lactating breast specimens of young women has been submitted for publication. It is possible to diagnose the condition clinically in early stages presenting the typical signs and symptoms. For diagnosis in the later stages a biopsy is advocated to rule out cancer when a history with a hard mass is presented. Frozen section method can also be tried. It is not a precancerous condition and local recurrence in the same or opposite breast although possible, malignant change does not supervene in later period.

## XIX. Salmonellosis in Dogs.

(Meridian Ball, Shaikh and Soman).

While investigating the problem of Leptospirosis in dogs, it was thought desirable to look into the problem of Salmonellosis in them side by side. With this idea in view, arrangements were made with the Municipal Dog Destruction Department at Mahalaxmi siding station to collect blood samples and rectal swabs of pariah dogs irrespective of their age and sex.

One hundred and fifty nine rectal swabs were collected and were inoculated into tetrathionate-broth containing Brilliant Green and Sulphadiazine and plated on E.M.B., B.S.A., Mckonkey and D.E.C. media. *Salmonella* organisms could be isolated from 10 swabs and the cultures were finally indentified as *Salmonella typhimurium* 5 strains, *S. readins* 2 strains, *S. anatum* 1 strain, *S. virchow* 1 strain, *S. bethesda* bellerup 1 strain.

## XX. Studies on Treponema Agglutination Test.

(Soman and Sant).

The Treponema agglutination test is a very useful biological test to confirm the serologically doubtful, false positive or false negative cases. It is one of the recent and latest laboratory procedure for the diagnosis of syphilis. For this, a special strain (Nichols) of *Treponema pallida* is required for the preparation of the spirochetal antigen. This strain has to be maintained in rabbit's testis by several animal passages. At present, this Nichols strain of *Treponema pallida* is being maintained in rabbit's testis for the preparation of the antigen. Further work is in Progress

## XXI. Studies on Leptospirosis.

(Meridian Ball, Shaikh and Soman).

Previous studies on Leptospirosis in Bombay City either referred to the serological evidence of Leptospirosis in jaundice cases or in Bombay rats and agglutination-lysis tests were carried out by using a single strain of *L. icterohaemorrhagiae*.

With the present knowledge of various serotypes of *Leptospira*, it was thought desirable to investigate into these types responsible for infection in human cases as well as in animals.

Twenty-four serotypes of *Leptospira* were obtained from U.S.A. during this year through the kind courtesy of Dr. Meridian Ball who worked in this Institute for four months on this subject. Since she left, these strains have been maintained in Vervoort's medium by regular subculture.

During this year, 65 blood samples from cases of jaundice were received from different hospitals in Bombay City. (1) Blood clots from 12 were inoculated into g. pigs intraperitoneally. All were, however, found to be negative. (2) All sera were tested for agglutination by tube method against 10 serotypes of *Leptospira* in different dilutions varying from 100 to 10,000 and when the tests were positive, they were further confirmed by agglutination-lysis test by using live suspensions.

Following strains were used for tube agglutination test using formalised cultures :—

1. *L. icterohaemorrhagiae*.
2. *L. canicola*.
3. *L. bataviae*.
4. *L. pomana*.



5. *L. autumnalis*.
6. *L. balum*.
7. *L. hebdomadis*.
8. *L. grippotyphosa*.
9. *L. sejroe*.
10. *L. pyrogenus salinem*.

Only 13 sera showed positive agglutination ; 2 of these 13 cases were subjected to a repeat test on a second sample taken after 10 days and both showed distinct rise in titre.

**Table 1.**

*Results of agglutination tests on 13 positive sera, classified as per range of titres.*

| Strains agg.                    | 1 : 100 | 1 : 300 | 1 : 1000 | 1 : 3000 | 1 : 5000 | 1 : 10,000 |
|---------------------------------|---------|---------|----------|----------|----------|------------|
| <i>L. icterohaemorrhagiae</i> . | 4       | ..      | 6        | ..       | ..       | 1          |
| <i>L. canicola</i>              | ..      | 1       | 5        | 1        | 5        | 2          |

Only three samples showed clinical agreement with serological examination ; in others no definite clinical history could be obtained.

In case of different animals tested, the serological results obtained were as under :—

**Table 2.**

| Animal.   | Total No. tested. | Positive (percentage) | Titre Range. | Strains agglutinated.                       |
|-----------|-------------------|-----------------------|--------------|---------------------------------------------|
| Horses .. | 100               | 10                    | 100-3000     | <i>L. ictero</i> and <i>L. pomona</i> .     |
| Sheep ..  | 150               | 20                    | 100-30,000   | <i>L. pomona</i> and <i>L. hebdomadis</i> . |
| Dogs ..   | 150               | 10                    | 100-3000     | <i>L. ictero</i> and <i>L. canicola</i> .   |
| Rats ..   | 100               | 12                    | 100-10,000   | <i>L. ictero</i> and <i>L. canicola</i> .   |

Further Studies are in progress.

## XXII. Isolation of the Phages from *S. Typhosa*.

(Soman, Kumari Savitri and Kumari Patel).

In order to develop bacteriophage technique in this Institute preliminary work was started to isolate lysogenic bacteriophages carried by *Salmonella typhosa* cultures. First, the *Salmonella typhosa* cultures were isolated in pure form from the clot cultures and they were subjected to the phage isolation technique.

In earlier years, there was much interest in the use of bacteriophages as therapeutic agents in the treatment of the diseases, but now they have been particularly useful as subjects for the study of host virus reactions and epidemiology.

The technique followed was essentially of J.S.K. Boyd where they isolated bacteriophages from the cultures of *Salmonella typhimurium*, obtained from the stools of cases of human infections or directly from the samples of faeces.

Six cultures were tested for phage isolation. Out of these six, three cultures showed the presence of phages. The plaques developed after one or two passages in each case and the activity of the phage was increased after several passages. The critical test dilution (C. T. D.) of the phage was determined. C. T. D. of one phage was found to be 1 : 2,000 and that of the second phage was 1 : 10,000 and the C. T. D. of the third phage is being determined.

In order to know whether the isolated phages are O phages or VI phages neutralisation test will be carried out. For this test, new phages in C. T. D. will be injected into the adult male rabbit intravenously to prepare the antisera. The work is in progress.

### **XXIII. Further Observations on the 'In Vitro' Plate Method for Determining the Toxigenicity of *C. Diphtheriae*.**

(Kumari Patel and Soman).

The work was continued during the year on the utility of "in vitro" plate technique of Elek for determining the toxigenicity of *C. diphtheriae*. As a result 66 diphtheria cultures were tested for their toxigenicity by all the three methods, previously described. This work has conclusively proved that the "in vitro" method can routinely be accepted for diagnosis even in a small laboratory where experimental animals like g.pigs may not be available. However, in order to make it more economical, rapid and simple, it was decided to work upon certain constituents used in the preparation of medium. It was observed that ultimate reactions depended on the type of haemoglobin-free serum or the brand of peptone or the concentrations of antitoxin used in carrying out the test.

Haemoglobin-free horse serum recommended by Elek was substituted by various animal sera, free from haemoglobin, including human serum. It was observed that human serum, of all, gave the most satisfactory results, as the reactions appeared as early as 24 hours and they were more distinct. Monkey serum was found the next best. With other animal sera, reactions appeared late.

Bactopeptone, tryptone or neopeptone replaced the proteos peptone of "difco" when they were found to be absolutely unsuitable. At the same time bactopeptone in presence of meat-extract as in the preparation of nutrient agar gave satisfactory results, provided the medium was properly filtered and sterilised at 100°C. for 1/2 hour.

As regards the diphtheria-antitoxin, it was used in varying concentrations, from 7-700 international units per ml. Although some reaction in the form of precipitate lines was noticed even with concentration of 28 units/ml it could be more easily detected and read at the concentration of 500 units/ml.

Since the pour plate method is subjected to frequent aerial contamination it was decided to incorporate potassium tellurite in the medium. Addition, of 0.1 ml of 1 per cent. potassium tellurite to the plate (medium 12 ml) did not interfere with the reactions but maintained the plate sterile.

#### **XXIV. Investigations of Salmonella Infection in the Laboratory Mice.**

(Soman & Kumari Ovalekar).

Of all the laboratory animals, white mice are bred on a large scale, for various experimental purposes, in this Institute. They are usually susceptible to septicemic diseases. All possible care is taken to prevent them against these rapidly spreading infections. In spite of this, at times, a considerable mortality occurs in them due to one of the bacterial diseases called "Mouse Typhoid", which results in the depletion of the breeding stock.

Therefore, it was thought essential to find out how and where this infection lurked and how it spreads from cage to cage, in the breeding colony. For this purpose, it was decided to keep the smallest colony under investigations. The colony was divided into different groups. A sample check of such groups was examined by taking rectal swabs of all mice whether healthy or sick for any evidence of Salmonella infection. Other items, namely, their food, water, cages and handlers were also to be investigated at the same time from this aspect. The work is still in progress and when complete it will help in taking permanent preventive measures in eradicating the infection from the colony.

#### **XXV. Serum Protein Fractions in the Blood of Normal Donors.**

(Smt. Venkataraman).

The various serum protein fractions have been extensively studied more recently by electrophoresis. Several workers have established by comparative studies that the so-called "HOWE" albumin fraction obtained by fractionation with 22-23 per cent. sodium sulphate contains an appreciable portion of the alpha<sub>2</sub> globulins. It has also been worked out that a correct picture of the albumin and the various globulins can be obtained by chemical fractionation techniques which have been adjusted to electrophoretic standards. Thus at a concentration of 15-16 per cent. sodium sulphate only the gamma globulin is precipitated; by increasing the concentration slightly to 19-20 per cent both gamma and beta globulins come down, while at a concentration of 26-27 per cent all the globulins are precipitated leaving the 'true' albumin in solution. This simple technique of fractionation which has been found to give results consistent with those obtained by electrophoresis (Kibrick and Blonstein *J. Biol. Chem.* 176, 983, 1948) will be very useful for the study of serum protein fractions for clinical work.

25-30 serum samples from the Blood Bank Department were pooled and fractionated with the various concentrations of sodium sulphate. The protein-N in these fractions were estimated by micro-Kjeldahl digestion and nesslerisation (Annual Report for 1950 ; p. 30-31 ; details under publication). The table below gives the results of analyses of seven such pooled samples :

TABLE

| Pool Number. |    | Total Proteins gm% | True Albumin gm% | Total Globulins gm% |      |       | A/GR Ratio. |
|--------------|----|--------------------|------------------|---------------------|------|-------|-------------|
|              |    |                    |                  | alpha               | beta | gamma |             |
| 1 (28)       | .. | 7.22               | 3.34             | 0.71                | 1.00 | 2.17  | 0.8608      |
| 2 (28)       | .. | 7.05               | 3.44             | 0.90                | 1.10 | 1.61  | 0.9530      |
| 3 (25)       | .. | 7.08               | 3.46             | 0.90                | 1.41 | 1.30  | 0.9559      |
| 4 (23)       | .. | 7.37               | 3.59             | 0.93                | 0.94 | 1.91  | 0.9497      |
| 5 (29)       | .. | 7.66               | 3.70             | 0.97                | 1.40 | 1.58  | 0.9870      |
| 6 (27)       | .. | 6.90               | 3.50             | 1.10                | 0.90 | 1.40  | 1.0290      |
| 7 (30)       | .. | 6.94               | 3.10             | 0.89                | 1.39 | 1.56  | 0.8074      |
| Mean         |    | 7.17               | 3.45             | 0.91                | 1.16 | 1.65  | 0.9347      |

Complete details will be published later.

#### XXVI. A simple method for the Estimation of Sodium in urine.

(Shrimati Venkataraman)

The procedure used for the estimation of serum sodium using the 'one-piece' reagent containing the Uranyl Magnesium Acetate in 80 per cent. alcohol, described by us earlier (Annual Report 1952, p. 70 ; A. Venkataraman, J. Sci. Ind. Res. 1954, Vol. 13B, p. 713-718), is found to be applicable to urine also. The phosphates in urine are removed first with calcium oxide before the sample is treated with the reagent. The precepitation of the triple salt is complete in 10-15 minutes, when the supernatant is ready for the color development with potassium ferrocyanide. A standard calibration curve can be used for reading the result of the unknown.

#### XXVII. A study of serum amylase activity in the blood of normal donors.

(Bharucha, Joshi and Smt. Venkataraman)

The determination of serum amylase activity is of interest in the diagnosis of acute pancreatitis wherein high values may be found. Low values are often reported in liver diseases and pancreatic insufficiency. Amylase activity is determined by the ability of the enzyme to degrade starch into reducing carbohydrates chiefly maltose. The results are expressed in terms of glucose.

Normal values vary over a wide range 40-160 units. Using the procedure described in STANDARD METHODS IN CLINICAL CHEMISTRY (1953) majority of the samples analysed were found to fall within a range of 40-60 in the case of blood donors. This study is being continued.

### XXVIII. Serum gamma globulin in Cirrhosis

(Smt. Venkataraman)

A few serum samples with case history of cirrhosis were picked out and analysed for total protein and gamma globulin contents by fractionating at a concentration of 15.75 per cent Sodium sulphate. The proteins were estimated by Biuret method, using the reagent recommended by Gornal *et. al* (J. Biol. Chem. 177, 751, 1949). Samples so far studied show a typical picture with a slight decrease in total protein content but a mean average of 60 per cent increase in the gamma globulin. Our previous studies have shown that in such cases there is a definite decrease in the albumin content with a corresponding increase in the total globulins, the major change being in the gamma globulin fraction.

TABLE

| Number of samples.                       | Total proteins gm per cent. |      | Gamma-globulin gm per cent. |       |
|------------------------------------------|-----------------------------|------|-----------------------------|-------|
|                                          | Range.                      | Mean | Range.                      | Mean. |
| 20 (Cirrhosis)                           | 5.95—7.45                   | 6.45 | 1.84—3.68                   | 2.69  |
| Seven pools representing normal samples. | 6.94—7.66                   | 7.17 | 1.36—2.17                   | 1.65  |

Thus, the estimation of gamma globulins in addition to total proteins and 'true' albumin will be very useful for clinical diagnosis.

### XXIX. Plague immunity in the rhesus monkey

(Dutta, Habbu, Colah and Wagle)

Using the method of repeated cross circulation, it has been possible to transfer 87.5 per cent. of "immune" blood to a normal monkey and vice versa. In this way the role played by "cellular" and "humoral" agencies in monkey plague has been studied.

When about 87.5 per cent. of the blood of monkey immune to plague, was replaced by normal blood, the monkey resisted a challenge dose of 30,000 million plague bacilli.

When the blood of a normal monkey was replaced by 70.0 per cent. or more with "immune blood", the monkey resisted a challenge dose of 30,000 million plague bacilli.

It seems cellular as well as humoral agencies play important role in plague immunity, though the former may be more important than the latter.

### XXX. A method for testing leech repellents

(Tamhane and Dutta)

Leech repellents have been tested for their efficacy by field trial in human beings or in the laboratory by allowing the leech to cross either the cloth or paper strips impregnated with the drug. The efficacy is judged by noting down the unsuccessful attempts made by the leech in crossing the strips.

It has been observed that Indian leech, *Hæmadipsa sylvestris*, from which food has been withheld for a week, readily attaches itself on the dorsal epilated surface of the rabbit's ear. The leech refuses to suck blood if any drug is smeared on the surface which is not congenial to it. This fact is taken advantage of in developing a method for evaluation of leech repellent activity of drugs. This method, appears to represent the field condition where hundreds of Indian labourers have to work daily in leech infested area, particularly bare-footed.

The method is as follows: dorsal surfaces of the ears of healthy rabbits were shaved to remove the hairs. On the following day the test was carried out. On one of the ears, definite measured area was smeared uniformly with a suitable quantity of the drug to be tested. The other ear served as a control. The leech was gently put on the painted surface. If the drug was a strong repellent, the leech instantaneously recoiled, curled up and then it dropped down. If the repellent activity was of a lower order, the leech did not recoil immediately but tried to leave the drugged surface quickly. When the repellent power was still less, the leech stayed on the surface but refused to suck. In the absence of any repellent activity of a drug, the leech did not mind sucking the blood.

Several substances like turpeneol, dimethylphthalate, diethylphthalate, D. D. T., kerosene, gammexane dust, eucalyptus oil were tested by this method. Dimethylphthalate demonstrated a strong repellent activity.

### XXXI. Investigations on the probable presence of Reserpine in *Alstonia Scholaris*.

(Sastry and Dutta)

The bark of *Alstonia Scholaris*, commonly known as Dita bark has been reputed in the Hindu medicine since ancient times. The earlier chemical investigations have resulted in the isolation of four principle alkaloids besides non-nitrogenous constituents.

In view of the recently reported isolation of Reserpine from *Alstonia constricta*, the plant indigenous to Australia, attempts were made to explore the possible occurrence of Reserpine in *Alstonia scholaris*, indigenous to India.

The alcoholic extract of the bark free from the main alkaloidal constituents was defatted with petroleum ether (40-60°C) and the dry residue soluble in chloroform was taken for investigations. The chloroform soluble part was extracted with dry benzene and the extract freed from the solvent under reduced pressure.

The dry resinous residue was subjected to electrophoresis and examination under ultra violet light. The green fluorescent zone observed in the case of pure Reserpine was absent in the case of the sample under investigation.

The process was repeated with methanolic extract.

The present investigations showed the absence of Reserpine in *Alstonia scholaris*, grown in Bombay region.

### XXXII. Determination of Methyltestosterone in Tablets.

(Madiwale and Anantnarayanan).

Several methods have been described for the assay of methyltestosterone, but none of them seemed to be very satisfactory, from the point of view of simplicity, reliability and reproducibility. One of the colorimetric methods that is based on the formation of coloured 2, 4-dinitrophenyl-hydrazone by reaction with 2, 4-dinitrophenyl-hydrazine has been investigated and suitably modified for the purpose.

Methyltestosterone was quantitatively extracted by light petroleum ether (B pt. 40°—60° C) from tablets. When a surface active agent like 'sapamine' was present, it was necessary to wash the petroleum ether extract with 2NHCl. The petroleum ether extract was then shaken with 4 successive portions of methyl alcohol (previously saturated with petroleum ether). The methanol layers together were washed once with saturated petroleum ether. The aliquots of methanol solution (expected to contain 200 to 600 mcg. of methyltestosterone) were taken and the 2,4-dinitrophenyl-hydrazone was precipitated. The precipitate was collected on a Whatman No. 40 filter-paper, washed first with 2NHCl, then with water and dried. The dry precipitate was dissolved in chloroform and suitably diluted so as to contain 20 to 60 mcg. of the drug. The intensity of the coloured chloroform solutions and blanks was determined on a Beckman model DU spectrophotometer at 390 m $\mu$ . The concentration of the active substance was found out by reference to a standard curve.

### XXXIII. Chemotherapeutic investigation of Streptonivicin (Albamy-cin) in experimental Cholera.

(Dutta and Colah).

Streptonivicin is one of the recently discovered antibiotics isolated from *Streptomyces niveus*, nov. sp. This drug was tested both *in vitro* against *Vibrio cholera* and *in vivo* on experimental cholera in infant rabbit.

The drug was found to be bacteriostatic and bacteriocidal in concentrations of 5  $\mu$ g/ml., and 10  $\mu$ g/ml. respectively against 10<sub>6</sub> V. cholera in case in hydrolysate medium.

Evaluation of its anticholera activity was determined according to the method recommended by Dutta and Habbu (1955). 10 day old infant rabbits were infected intrajestinally with 10,000 vibrios per 100 gm. of bodyweight. Streptonivicin was administered orally every six hours starting at the end of 16 hours following the infection. The dose of streptonivicin used was 40 mg/kg of bodyweight. In all, 9 doses of the drug were administered for each experiment. 2 out of the 14 rabbits died. The mortality was 14.3 per cent. It is suggested that therapeutic trial of the drug may be undertaken.

#### XXXIV. A Process for the preparation of Para-Nitro-Acetophenone.

(Deliwala, Kulkarni, Sabnis).

This work was particularly undertaken with a view to develop a cheap and practicable process for the manufacture of the very important and useful antibiotic Chloromycetin.

A very convenient route for the manufacture of Chloromycetin lies in the procedure starting from para-nitro-acetophenone and work was therefore initiated to develop a cheap and easy method for the preparation of *para*-nitro-acetophenone.

We have observed that in the preparation of *para*-nitro-acetophenone by the condensation of *beta*-ketonic esters with acid chloride, the use of sodium, potassium or magnesium-alkoxide, hitherto employed, can be easily dispensed with and instead the reaction could be advantageously carried out in good yields by the use of anhydrous sodium or potassium hydroxide in the presence of a non-polar solvent. The advantages of this method are obvious.

The use of sodium or potassium alkoxide adopted in the customary methods for the preparation of *para*-nitro-acetophenone involves the handling of metallic sodium or potassium and the interaction of these highly reactive metals with alcohol calls for considerable skill and special precautions due to extreme risk of dangerous fires and explosions; whereas the use of magnesium, although more convenient than sodium or potassium, still introduces an element of close control and demands very careful disposal of the large quantity of hydrogen gas evolved. Apart from the risks involved in the use of sodium, potassium or magnesium, the cost of alkali metal alkoxide is invariably greater than the cost involved in the use of caustic soda or caustic potash. It is thus evident that the use of caustic soda or caustic potash instead of the use of alkali metal alkoxides is a distinct advantage both economically and practically.

#### XXXV. Studies in the Preparation of Isonicotinyl Hydrazide.

(Deliwala, Shah & Mhasalkar)

The efficacy of Isonicotinyl hydrazide in the treatment of tuberculosis prompted us to investigate various experimental routes and standardise working conditions for its economic production. Starting from basic material, viz., *gamma* picoline and 4-ethyl pyrimidine, the various stages involved in the preparation of isonicotinyl hydrazide were worked out in detail and standardised to furnish average yields of more than 75 per cent. in each stage which compares favourably with yields reported in literature. Furthermore an efficient method for the preparation of hydrazine sulphate (an important raw material required in the production of isonicotinyl hydrazide) has also been systematically adapted for pilot-plant runs. The production of the drug-isonicotinyl hydrazide can therefore be taken up as an economic proposition with the data on hand.



### XXXVI. Studies in the Preparation of Diaminodiphenyl Sulphone (DDS) and its Soluble derivative

(Deliwala, Shah & Mhasalkar).

DDS and Solapsone (B. P. C.), (a soluble derivative of di-amino-diphenyl sulphone) play an important role in the treatment of Leprosy. The various stages involved in the preparation of these drugs, starting from nitrochlorobenzene were systematically investigated and standardised. The yields in each stage being between 75 to 90 per cent of theory.

### XXXVII. N1-Alkyl-Sulphathiazole.

(Deliwala, Bapat and Sardesai)

The Patent No. 29093 describes the reaction between the dicondensation product 2-Bis (acetyl sulphanilamido) thiazole and 2-aminothiazole to yield one extra mole of acetylsulphathiazole. As a further extension to this reaction, experiments were carried out to condense the discondensation product with guanidine salts to obtain acetylsulphathiazole and acetyl-sulphaguanidine.

In the course of these investigations, it was observed that the reaction proceeds smoothly in a suitable solvent e.g., acetone, but if an alcohol, e.g., methanol, ethanol, etc., is used as a medium, the course of the reaction is changed, and instead of acetylsulphaguanidine, a product identified as N1-alkyl-N4-acetyl-sulphathiazole is obtained.

A study of the mechanism of this reaction reveals that the acetyl-sulphanil radical liberated from the dicondensation product has a greater affinity for the alcohol and reacts with it rather than with the guanidine salts. An alkyl ester of acetyl sulphanilic acid is formed, and this ester by virtue of its power as an alkylating agent reacts with acetyl sulphathiazole formed in the reaction to finally yield N1-alkyl-N4-acetyl sulphathiazole, which on hydrolysis affords N1-alkylsulphathiazole.

The interesting mechanism of this reaction is being further studied for its profitable utilisation in the alkylation of other important primary and secondary amines.

### XXXVIII. Effect of Splenectomy on Antibody Production in Rabbits

(Kulkarni.)

Treatment of isoimmunised Rh negative women during pregnancy is still a problem. The process of antibody production goes on during pregnancy in the mother and these antibodies in turn have adverse effect on the foetus. So, it was thought that a means of suppressing this antibody production, if found, would be a great step forward in the treatment of such cases. With this idea in view the following experiments were being conducted in rabbits.

Healthy female adult rabbits (weighing more than 2 kg) were chosen and splenectomy was performed under general (ether) anaesthesia. After about a week these rabbits were immunised by injecting 10 ml of alum precipitated pooled human serum intramuscularly (following the method of Proom) for production of anti-human globulin serum (Coomb's reagent). A course of two injections was given at an interval of a fortnight to one month. Control rabbits were also treated similarly. It has been observed that antibody response was found to be absent in some of the splenectomised rabbits and in some the response was much less than in the control animals. The experiments are in preliminary stage. Further studies are in progress to study the time interval between splenectomy and immunisation, nature of antigen and electrophoretic studies on antibodies.

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