



सत्यमेव जयते

**GOVERNMENT OF MAHARASHTRA**

**ANNUAL REPORT**  
**OF THE**  
**HAFFKINE INSTITUTE**  
**FOR THE YEAR 1973-74**



**1975**

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## PART I

### GENERAL REVIEW

Haffkine Institute celebrated Platinum Jubilee this year having entered seventy-fifth year of its existence. Right from its very inception, the Institute has devoted itself to researches in communicable diseases. The Platinum Jubilee Year was inaugurated on the 10th of August 1973 and the jubilee was celebrated in a befitting manner by holding scientific seminars on topics of great interest to the scientists of the Institute. A separate special report on the significant events during the Platinum Jubilee Year is appended separately.

Haffkine Institute continued its expansion during this year, both in the field of production and research. Due to the petroleum crisis, there was an unprecedented rise in the prices of raw materials and consequential shortages in supplies. But the production programme was maintained as per schedule. The supplies to various hospitals and dispensaries were kept up at the existing price level although it meant a tremendous strain on the finances of the Institute. Thus, the annual turnover of various products such as vaccines, toxoids, antisera against tetanus, diphtheria, snake venoms and also gas gangrene and non-biologicals which included essential drugs, such as anti-amoebic agents and nutritional supplements such as vitamins, reached 124 lakhs. The Institute continued to maintain a steady supply of diagnostic reagents and laboratory-bred animals of the required quality to various research institutions in Maharashtra and the rest of the country.

This Institute is affiliated to the University of Bombay and is involved in training of scientists in various disciplines. There are 96 students on its roll, all working for their Master's or Doctorate Degrees in various branches, such as Microbiology, Biochemistry, Applied Biology, Pharmacology, Zoology and Organic Chemistry. The Institute has reorganised its training programme of the D.M.L.T. and M.L.T. students. The number of seats for D.M.L.T. is also proposed to be increased. Several short-term training courses, each of three months' duration, are offered to science graduates. Total of 75 took advantage of these courses.

The Department of Immunology was split up into two departments, viz. Department of Antitoxins and Sera which took over the production of antitoxic sera and the Department of Immunology devoted to development of research in the field of immunology. The entire fleet of horses from Bombay was transferred to Pimpri Farm. A veterinary graduate was appointed to cultivate land at Pimpri for the production of lucerne grass needed by the department. This project is quite a success; thanks to the efforts put in by the Farm Supervisor and his colleagues at Pimpri Farm. For this, they need to be complimented.

The Department of Bacteriology has pursued very vigorously its production activity in spite of the fact that it was required to shoulder additional responsibilities of taking over the production of diphtheria and tetanus and pertussis vaccines. Owing to the prevailing scarcity conditions, there were heavy

demands for cholera vaccine and these were fully met. Tetanus toxoid had to be supplied. In large amounts for immunisation of persons involved in scarcity works. The Institute rose to the occasion and these demands were fully met.

A Task Force under the Chairmanship of Director was established for undertaking the production of D. P. T. Vaccine. The Task Force met regularly and reviewed the progress and took corrective measures required, if any. With the advice rendered by W. H. O. Experts, Dr. Standfast and Dr. Van Hemert and the efforts put in by the leaders of the group, particularly Shri Nimbkar, Dr. Saldanha, Shri Karbhari and their younger colleague scientists, it was possible to standardise the production of D. P. T. Vaccine in a short time. The Institute is now on its way for a regular production of D. P. T. Vaccine and will reach a production target of about 2 million doses annually.

A Radiation Biology Unit was established with a view to study the effects of radiation on biological systems and it works under the direct supervision of the Director. The unit started with a meagre equipment and has, therefore, been mainly involved in rendering public service by undertaking such tests as hyoid function tests, which include  $^{131}\text{I}$  uptake studies and  $\text{PB } ^{131}\text{I}$  esti-

mation. Patients are referred to this unit by private medical practitioners and hospitals. The unit is on its way to standardise methods for estimation of hormones by radio-immuno-assay technique. The activities of this unit are expected to expand rapidly, so that the unit will attain the status of a full-edged department.

Toxicology and Human Pharmacology Section was organised under direct supervision of the Director during the year, with a view to study in detail the toxicity of drugs prepared by the Institute and referred by various industrial firms. There was a great need to develop such facilities in this part of the country. The Institute has made a beginning and the unit has carried out acute, sub-acute and chronic toxicity studies on a number of drugs and chemicals referred to it. It is expected that this unit will also develop into a full-edged department, so that it can undertake extensive toxicological studies on drugs as well as industrial toxic materials referred to it from time to time.

The programme for production of oral polio vaccine has gained momentum. Dr. K.H.Dave, Assistant Director, was deputed to U.S.A. and Japan on a W.H.O. Fellowship programme to study recent advances in the production, control and standardization of oral polio vaccine. A team of W.H.O. Consultants visited the Oral Polio Vaccine Unit and made several recommendations before a regular production programme could be undertaken. These recommendations are being implemented.

The Institute pursued vigorously the various research projects initiated in the previous years. The entire research programme has been completely reorganised so as to concentrate on the following aspects of research.

*Epidemiological research.*—Work on the epidemiology of enteroviruses under the leadership of Dr. K.H.Dave, Officer I/c of the Enterovirus Unit of the I.C.M.R. was continued.

During the period January-December 1973, 727 cases of paralytic poliomyelitis were recorded in Greater Bombay. About 94 per cent of the cases occurred in the age group of 3 months to 3 years and less than 1 per cent of the cases were recorded in the age group below 3 months. Studies on the efficacy of oral polio vaccine were pursued vigorously. A preliminary study on the anti-polioviral activity in human milk and faeces was also initiated. Three hundred and twenty stool samples were studied. Twenty-one per cent of the samples showed presence of Copro-antibodies against all the three types of polio viruses.

Studies in the epidemiology of filariasis, plague and cholera were also continued. Over 1,000 rodent serum samples were collected for plague studies. Out of these serum samples, samples from Dhokri, Murud and Jalgaon showed positive titres indicating the presence of anti-bodies to F1 antigen of *Y. pestis*. The total number of rodent serum pools positive was fifteen. Out of 240 samples of dog serum, 15 were found to be positive.

Filariasis surveillance work was carried out in close collaboration with the public health department in various areas in Maharashtra. It has been established that the prevalence rate of filaria varies from 3% to 15% in the areas in close vicinity of Bombay city. More extensive work, on control of filarial infection by mass treatment with Diethyl Carbamazine has been planned for the next year. Simultaneously, it was possible to study the epidemiology of filarial infection by skin test and haemagglutination test, using Swada's antigen. Attempts are being made to prepare a specific antigen from the infective larvae of *W. bancrofti*.

Epidemiological study of Dracunculosis was also undertaken in collaboration with the Public Health Department in various parts of Kolaba District of Maharashtra. A field trial, using Mobandazole, Jonit and Metronidazole was carried out in Dracunculosis. The results are awaited with interest.

Cholera studies started in Parbhani were successfully completed and a report was submitted. It was noted that there were several cases of active cholera carriers. Advice has been given to the health authorities with respect to eradication of such endemic foci.

Extensive study on the pattern of intestinal parasitic infections was carried out in four districts of Maharashtra. This revealed that the average infestation rate was 69.4%. In Bombay, it was 45.4%, 90.6% in Thana and 100% in Kolaba and Parbhani districts. Epidemiological studies on Burcellosis and Leptospirosis were continued. These three important collaborative projects have shown substantial results.

*Collaborative Research.*—Three important collaborative research projects initiated in previous years have shown substantial progress and provided interesting leads for further research.

The study group on "snake venoms" has made considerable progress in various aspects of venom research. Venoms of Cobra and Russell's viper were fractionated. Each fraction was studied biochemically by Shri Chatwal, a Research Fellow in Immunology Department. A near purified protein with a substantial neurotoxic activity was obtained. Two fractions were

isolated, one exhibiting predominantly neurotoxic and the other cardiotoxic activity. The neurotoxic fraction produced reversal neuromuscular paralysis. Several of these fractions were studied for their effects on pooled coagulation mechanisms. It was possible to pinpoint the site of action of the various fractions of venoms obtained from *Naja naja* and Russell's viper. A precise and very promising work in this regard was done by Shri Muthe of the Immunohaematology Department. On the basis of preliminary evidence, it appears that anticoagulant activity is due to an action on the intrinsic system, particularly at the stage of activated factor.

Fibrinolytic or fibrinogenolytic activity was also located in the venoms and a simple qualitative method to study these was devised.

*Venom Toxoid*.—Cobra venom toxoid has been prepared and is undergoing trials for estimating its immunogenicity. This has been done under direct supervision of the Director.

*Scorpion Venom*.—Deaths due to scorpion bite were reported from one of the districts in Maharashtra. A study on the toxicity and immunogenicity of scorpion venoms was, therefore, taken up by the Director's Laboratory. It has been established that the scorpion venom, though a weak immunogen, can produce specific antibodies. Attempts are being made to increase the antibody titre with a view to find out their protective nature and to prepare a specific anti-serum. It was also established that the black scorpion venom contains a large amount of histamine and a neurotoxic factor responsible for toxicity. A venom toxoid is being attempted. A number of drugs were screened for their prospective action against envenomation. A tranquilizer with anti-histamine properties appears promising.

The "Filariasis Group" also continued its activities vigorously. *L. carini* filaria model in cotton and white rats has been established. Compounds synthesized by the Chemotherapy Department were screened for their potential antifilarial activity, both *in vitro* and *in vivo*. Some of the compounds have shown very promising activity, particularly against microfilaria and this is being pursued more vigorously.

The Chemotherapy Department has geared up its activities totally to seek agents with potential anti-parasitic and anti-bacterial activities. Anti-tumour work has been suspended. Triazine and imidazole derivatives were synthesized and screened for anti-inflammatory, anti-helminthic and anti-filarial activities. Some compounds have shown very powerful anti-helminthic activity with a wider spectrum, i.e. both against nematodes and cystodes.

A project was undertaken in collaboration with the Radiation Medicine Centre of the B. A. R. C. to assess the possibility of standardizing a method for early detection of contaminations.  $C_{14}$  labelled glucose was incorporated in the medium and the amount of labelled  $CO_2$  evolved by the contaminating organisms served to detect the contamination. The method has been well-standardized and the work is being pursued more vigorously. This is of great significance in the production of vaccines and particularly serves to detect

contamination at an early stage in production, thus minimising further losses. A small electronic detector device is being designed in collaboration with B.A.R.C.

Dr. M. V. Shirodkar took over as Head of the Department of Virology. Researches in this department were reorganised. Studies on *in vitro* blockade by rabies virus of Rous Sarcoma virus were initiated and completed. Studies on viral hepatitis have been undertaken. Transmission of rabies virus to common water snakes was attempted. The results of preliminary experiments are very encouraging. The possible risk of incidence of human rabies through rat bite is being studied. A serological survey of 101 normal children belonging to age group of 10-16 years from a local school for Rubella infection showed an incidence of 75 per cent

A new antirabic vaccine developed in human diploid cell culture at the Wistar Institute, Philadelphia, was given clinical trials in 38 normal human volunteers. Further studies in larger number of volunteers are necessary before deciding on the acceptability of the vaccine in preference to the currently produced one. Further studies in monkeys and post-exposure trials have been planned.

Thus, the entire research programme of the Institute falls into four categories :—

1. Production-oriented research, especially with a view to improve on the quality of vaccines. This is being attempted in the field of rabies and cholera.
2. Collaborative research in order to study in totality the various aspects of the problem as is being done in the case of filariasis and snake venoms.
3. Basic research : Basic research in the field of microbiology, pharmacology, biochemistry and immunology is conducted by different departments.
4. Epidemiological research : This is pursued in collaboration with Public Health Department of Government of Maharashtra. Thanks to the keen interest shown by Dr. V. N. Rao, Director of Health Services, it has been possible to undertake various epidemiological projects of public health importance.

The library continued to render its service to the scientists of this Institute in particular and to others. A new section on communicable diseases like cholera was completed. A Documentation Centre on Dr. Waldemar Haffkine is being organised ; thanks to the valuable donation of books and papers received from Madam Edithe Lutzker.

During this year, it has been proposed to make far-reaching changes in the structure and functions of the Haffkine Institute. Thus, on the occasion of the inauguration of the Platinum Jubilee Celebrations of this Institute, the Minister for Public Health announced the Government's intention to convert the Haffkine Institute into two autonomous bodies : Haffkine Biopharmaceutical Corporation and the Haffkine Institute Society for Research, Training and Testing. The production activity will be taken over by the Haffkine Biopharmaceutical Corporation and the Society will continue to devote itself to researches in



public health problems. With the formation of these two bodies, it is expected that the impediments, which were hitherto responsible for the rapid growth of the Institute, will be eliminated and the scientists of the Institute will be able to pursue vigorously their scientific activities in the areas of their interest.

The Labour Welfare activities of the Institute continued with full vigour. A member of the staff was deputed to the Worker Teachers' Training Course at Bandra. A Balwadi for the benefit of the children of the employees was started. A sewing class was organised for the benefit of the workers. A television set was installed in the employees' colony.

The dispensary was further reorganised to meet the increasing needs of the patients. About 37,000 patients attended the dispensary during the year. Mass miniature radiography of over 1,000 staff members of the Institute was carried out. Family planning programme was continued with vigour during the year.

Heads of departments and senior members of the staff contributed to the different activities in the fields of research, production and training. Without their active co-operation, enthusiasm and zeal in shouldering a joint responsibility, the Institute would not have been able to succeed in all its undertakings.

The success of the Institute depends upon the drive, integrity, consciousness and the skill of all the members of the staff, the scientists, technical staff, the administrative staff and last but not the least, the workers. It is gratifying to note that all the employees of this Institute have shown a sense of responsibility towards their jobs and have tried their best to contribute to the success of this great institute. The Director takes this opportunity to thank all the fellow scientists, administrators and the workers, who are comrades in arms, for their unstinted support and loyalty and sense of involvement, which alone was responsible to enable him to discharge his onerous duties, both administrative and scientific.

## PART II

### RESEARCH

#### A. RESEARCH PROJECTS

#### B. DEPARTMENTAL RESEARCH

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### CHOLERA PROJECT

#### Cholera carrier studies

Cholera carrier studies were undertaken at Parbhani, in the Marathwada region of the State of Maharashtra during the year 1972-73, since there were reports of cholera epidemic every year in the District for the last 5 years. The area of focus in Parbhani was mainly Kadrabad plot from where every epidemic is presumed to commence.

The main object of the study was to investigate the prevalence of human reservoirs i.e. the 'Cholera carriers'. Water samples and animal excreta from the selected spots were also studied for their possible role in the maintenance and spread of cholera infection.

It was shown from these studies that cholera epidemic season in the study area corresponds with the monsoon season. Also, cholera-like diarrhoea cases occur in an explosive manner in the pre-monsoon and monsoon seasons. The highest incidence is seen in the younger age groups, especially the children.

Ninety-eight strains of *V. cholerae* biotype *El-tor* and serotype *Ogawa* were isolated from 6,014 human faecal samples. They belonged to phage types 2 and 4. *V. cholerae* were isolated from 72 index cases, five convalescent and 12 asymptomatic carriers. Non-agglutinable (NAG) vibrios—6 from animal faecal samples (pools) and 4 from water samples were isolated. The pathogenicity test results showed that 100 per cent isolates from human cases were pathogenic to infant rabbits. Moreover, 50 per cent of the NAG strains isolated from animals and water produced cholera-like syndrome in the animals, which were indistinguishable from those produced by *El-tor* vibrio strains isolated from clinical cases of cholera in the area.

#### Antibody response to two cholera vaccines in volunteers

It has been shown in the field trials that the vaccines prepared by different methods differ from each other considerably in their protective action. The present work was undertaken to study the antibody response of the two vaccines prepared from the same strains of the organisms and having comparable mouse protective ability. The two vaccines prepared in two different laboratories contained the same number of vibrios. The difference in the method of preparation was that, in one, formalin was used as a killing agent and phenyl mercuric nitrate (P. M. N.) as a preservative, whereas in the other, phenol was used both as a killing agent and as a preservative.

A two dose schedule of 0.5 ml. and 1.0 ml. respectively, at an interval of 14 days, was used to administer the vaccines in the volunteers. The production of agglutinins and bactericidal antibodies was studied up to a period of six months.

It was observed that agglutinins and vibriocidal antibodies were produced in majority of volunteers. However, failure of response to agglutinating production was also observed in some volunteers (even after the second dose) who responded in production of vibriocidal antibodies. The second dose did not show any rise either in agglutinating or vibriocidal antibodies. The agglutinins lasted for a period of about one month, while the bactericidal antibodies persisted a little longer. Pre-immunization sera samples showed vibriocidal activity, the cause of which cannot be explained.

The two vaccines showed difference in agglutination titres to a marked extent against *Inaba*, but against *Ogawa*, the difference was less (Tables I and II). Non-responders to both the vaccines included those who were in direct contact with vibrio, having been engaged in vaccine production.

### Live Cholera Vaccine

With a view to find out the efficacy of live cholera organisms as a prophylactic agent, three-hour old culture of the 569B *Inaba* strain of *Vibrio cholerae* was tested for its antigenicity by active mouse protection test along with a lyophilized standard cholera vaccine and freshly prepared cholera vaccine. Both these vaccines contained  $6 \times 10^9$  organisms per ml. which were killed by formalin and preserved with phenyl mercuric nitrate.

The results of tests presented in Table 3 show that the level of immunity conferred by 4,000 to 5,000 viable organisms is almost the same as that induced by about 300,000 to 534,000 killed organisms. This shows that the live organisms are almost 100 times more potent than the killed organisms. The factor responsible for the high efficacy of live vibrios might be that the organisms may be persisting and multiplying at the site of injection and in the other body tissues. To confirm this presumption, a number of mice were inoculated intraperitoneally and intramuscularly with a three-hour old culture of *Vibrio cholerae* 569B *Inaba* strain. Each mouse received about one million viable organisms. The mice were killed at an interval of 1, 4, 8, 16, 32 and 48 hours and an attempt was made to isolate the vibrios from the site of injection, peritoneal cavity, spleen, liver and heart. In the case of mice inoculated intramuscularly, no organisms could be detected at the site of injection or in any of the organs even within one hour. In the case of mice inoculated intraperitoneally, a few organisms could be isolated from the peritoneal cavity within a period of only four hours. The other tissues did not show the presence of any organisms at periods varying from 1 to 48 hours. It is essential to have more experimental data to come to a definite conclusion in this respect.

### Radiation-killed cholera vaccine

It has been previously reported that the cholera vaccine, prepared by irradiating the organisms either for one or two hours, was more potent than the usual formalin-killed and phenyl-mercuric nitrate preserved cholera vaccine.

It was also reported that the radiation-killed cholera vaccine did not show any deterioration in antigenicity even after a period of 18 months of storage at 4° to 10°C.

The efficacy of the radiation-killed cholera vaccines and their storage stability were determined by the active mouse protection test only. It was thought worthwhile to test the efficacy of these vaccines by other methods also, such as agglutinin response in rabbits, vibriocidal activity of immune sera and also by ileal loop challenge method in adult rabbits. With this object in view, adult rabbits, weighing about 1,600 gms. were immunized at an interval of ten days, with two intravenous doses of 0.5 ml. and 1.0 ml. of a vaccine containing  $6 \times 10^9$  organisms per ml. of the strain 569 B Inaba of *Vibrio cholerae*, inactivated by exposing to a Co<sup>60</sup> source for one hour. Fourteen days after the second dose, each of the rabbits was challenged with 0.1 ml. of a three-hour old culture containing one million organisms of the strain 569 B Inaba of *Vibrio cholerae*, by ileal loop method. Eighteen hours after the challenge, the loops were found to be normal without accumulation of fluid, indicating complete protection. Similar experiments were also carried out with formalin-killed vaccine containing an equivalent number of organisms of the same strain as in the case of irradiated vaccine. In case of normal rabbits, which were used as controls, accumulation of fluid was observed in loops. In all, three rabbits were used for each type of experiment and three were kept as controls.

All the rabbits were bled before and after immunization for vibriocidal and agglutinin antibody titrations prior to challenging them by ileal loop method. The first sample of blood was collected just before the first dose, the second ten days after the first dose and the third 14 days after the second dose. All the rabbits showed 1 : 20 vibriocidal titre before immunization and  $10^6$  titre before and after the second dose, showing no increase in titre after the second dose. The agglutinin titre of the sera of all the rabbits was 1 : 10 before immunization and 1 : 640 after the first and second dose, showing no rise after the second dose. A similar pattern of vibriocidal and agglutinin titres was seen also in the case of rabbits immunized with formalin-killed vaccine.

The three tests, viz. the agglutinin response in rabbits, vibriocidal activity of immune sera and the ileal loop challenge method used in the above experiments, showed that the irradiated vaccine was as potent as the formalin-killed vaccine. The active mouse protection tests used in the work reported previously showed that the irradiated vaccine was superior to formalin-killed vaccine. However, the relative efficacies of these two types of vaccines could not be distinguished by the methods adopted in the present study.

#### Use of peanut protein hydrolysate for the growth of cholera organisms

Peanut protein was found to be a suitable substitute to casein for the preparation of medium to cultivate all the four virulent strains of *V. cholerae* used in the production of cholera vaccine. Even on a large scale, the growth of the organisms was found to be the same as in the case of casein hydrolysate medium (Table 1). The organisms were grown in the media of casein and peanut protein hydrolysates under different physical conditions (stationary, shaken and

urface) at 37° C. Cellular composition, such as macro and micro molecules and mineral elements, was determined in the dry cells. The activities of glycolytic enzyme (LDH) and transaminase enzymes (GOT and GPT) were also determined in the cells of *V. cholerae*. It was seen from the results that peanut rotein did not produce appreciable variations in the cell composition and nzyme activities of the organisms. Therefore, the medium of peanut protein ydrolysate was used to prepare quadruple cholera vaccine and tested for tency. The vaccine was found to be as potent as that prepared from the ase in hydrolysate medium.

### **biochemical studies on proteins and lipid components of *V. cholerae***

A detailed study of the protein pattern and phospholipid composition of the whole cell and plasma membrane of two strains of *V. cholerae* (Inaba 569B and biotype *El-tor* Inaba) was carried out. Different chemical components, fatty acids of the whole cell and plasma membrane were also determined. The results indicated that the whole cell contained 10-12 per cent total lipids, whereas, 30 per cent lipid was detected in plasma membrane of both the strains. It was also seen from the pattern of fatty acids that C 16 (palmitic acids), C18:0 and C18:1 were the major components of the lipids of the whole cell and plasma membrane of the organisms.

### **Effect of certain drugs on the synthesis of macro-molecules of *V. cholerae***

Action of certain drugs, such as Berberine, Furazolidone, Chloromycetin and Ampicillin were studied, using labelled compounds-glucose, amino-acids and nucleotides, on *Vibrio* and *El-tor* cholerae. Berberine is found to damage the cell membrane, cause increase in protein synthesis and detoxify the toxin produced. Chloromycetin and Furazolidone affect the pattern of ribosomal proteins and RNA resulting in abnormal protein synthesis. Ampicillin causes lysis of *vibrios*.

### **Study on the mechanism of action of Berberine in experimental cholera**

The action of the alkaloid 'Berberine' on cholera toxin was investigated in the adult rabbit ideal loop model.

The alkaloid was administered prior to the administration of toxin into the loops and the dose response curves of toxin and toxin plus berberine were obtained. The alkaloid was given into the loops at two-dose levels, viz. 3 mgs. and 5 mgs. Three animals were used for each dose level of the drug. Toxin was given in 4 doses, viz. 2.5, 5.0, 10.0 and 12.5 mg. levels.

Dose response for cholera toxin on the basis of fluid accumulation in the loop was established. It was found that berberine produced a shift to the right of the dose response curve. Regression lines for toxin effect and the toxin and berberine did not differ significantly in slopes, indicating that the lines were parallel.

It appears that berberine antagonises the cholera toxin effect in isolated ileal loop. The nature of antagonism is non-competitive. Thus, it appears that the antagonism between Berberine and cholera toxin is of a non-competitive nature.

Drugs, such as Neostigmine, Ephedrine, Caffeine and Nicotinic Acid were tested on fluid accumulation in order to ascertain the mechanism by which Berberine reduced the loop fluid. It was found that none of these drugs caused significant and consistently reproducible reduction in fluid accumulation caused by cholera toxin.

#### **Preparation of an oral cholera vaccine**

Studies are being carried out on the feasibility of preparing an oral cholera vaccine of good immunising potency. Conventional formalin-killed vaccine has been used to immunise different animal species by intra-intestinal route. The immunity developed against live vibrio challenge as well as against challenge due to toxin is being studied.

Non-pathogenic strains of vibrios, viz., Bhaskaran's strain C<sub>14</sub>-S<sub>5</sub> and the water vibrio *El-tor* water 6, given intra-intestinally, are being observed for the length of persistence in the gastro-intestinal tract and the grade of immunity developed against pathogenic strains of cholera vibrios.

ABLE  
*Agglutinin response of volunteers to two vaccines after two doses*  
INABA

| Vaccine | Number of<br>Volunteers | Number of<br>non-<br>responders | Time of collection of<br>samples after first dose | Number of volunteers showing the<br>following titres |    |    |    |    |     |     | Geo-<br>metric<br>mean<br>titre |    |      |
|---------|-------------------------|---------------------------------|---------------------------------------------------|------------------------------------------------------|----|----|----|----|-----|-----|---------------------------------|----|------|
|         |                         |                                 |                                                   | 10                                                   | 10 | 20 | 40 | 80 | 160 | 320 |                                 |    |      |
| A       | ..                      | 18                              | 1                                                 | Pre-immunization                                     | .. | 14 | 3  | .. | ..  | ..  | ..                              | .. | 1.3  |
|         |                         |                                 |                                                   | 14 days after 1st dose                               | .. | 1  | 1  | 4  | 2   | 6   | 2                               | 1  | 42.8 |
|         |                         |                                 |                                                   | 1 month                                              | .. | 1  | 5  | 5  | 2   | 2   | 1                               | 1  | 23.2 |
|         |                         |                                 |                                                   | 2 months                                             | .. | 14 | 2  | 1  | ..  | ..  | ..                              | .. | 1.5  |
|         |                         |                                 |                                                   | 6 months                                             | .. | 16 | 1  | .. | ..  | ..  | ..                              | .. | 1.1  |
| B       | ..                      | 26                              | 7                                                 | Pre-immunization                                     | .. | 17 | 1  | 1  | ..  | ..  | ..                              | .. | 1.3  |
|         |                         |                                 |                                                   | 14 days after 1st dose                               | .. | 3  | 2  | 4  | 1   | 6   | 2                               | 1  | 26.8 |
|         |                         |                                 |                                                   | 1 month                                              | .. | 2  | 3  | 4  | 4   | 3   | 3                               | .. | 26.1 |
|         |                         |                                 |                                                   | 2 months                                             | .. | 13 | 2  | 2  | 2   | ..  | ..                              | .. | 3.3  |
|         |                         |                                 |                                                   | 6 months                                             | .. | 14 | 1  | 1  | 1   | ..  | ..                              | .. | 1.6  |

TABLE 2  
*Agglutinin response of volunteers to vaccines after one and two doses*  
OGAWA

| Vaccine | Number of<br>Volunteers | Number of<br>non-<br>responders | Time of collection if samples<br>after 1st dose | Number of volunteers showing the<br>following titres |    |    |    |    |     |     | Geo-<br>metric<br>mean<br>titre |     |      |
|---------|-------------------------|---------------------------------|-------------------------------------------------|------------------------------------------------------|----|----|----|----|-----|-----|---------------------------------|-----|------|
|         |                         |                                 |                                                 | 10                                                   | 10 | 20 | 40 | 80 | 160 | 320 |                                 |     |      |
| A       | ..                      | 18                              | 1                                               | Pre-immunization                                     | .. | 13 | 3  | 2  | ..  | ..  | ..                              | 2.0 |      |
|         |                         |                                 |                                                 | 14 days after 1st dose                               | .. | .. | 1  | 3  | 4   | 3   | 2                               | 5   | 77.0 |
|         |                         |                                 |                                                 | 1 month                                              | .. | 1  | 2  | 4  | 4   | 7   | ..                              | ..  | 31.3 |
|         |                         |                                 |                                                 | 2 months                                             | .. | 11 | 3  | 3  | 1   | ..  | ..                              | ..  | 3.0  |
|         |                         |                                 |                                                 | 6 months                                             | .. | 17 | 1  | .. | ..  | ..  | ..                              | ..  | 1.1  |
| B       | ..                      | 26                              | 3                                               | Pre-immunization                                     | .. | 20 | 2  | 1  | ..  | ..  | ..                              | 1.0 |      |
|         |                         |                                 |                                                 | 14 days after 1st dose                               | .. | 1  | 2  | 2  | 5   | 4   | 4                               | 5   | 64.1 |
|         |                         |                                 |                                                 | 1 month                                              | .. | 6  | 3  | 5  | 3   | 4   | ..                              | 2   | 12.1 |
|         |                         |                                 |                                                 | 2 months                                             | .. | 14 | 2  | 5  | ..  | 2   | ..                              | ..  | 3.4  |
|         |                         |                                 |                                                 | 6 months                                             | .. | 19 | 1  | 1  | 1   | ..  | ..                              | ..  | 1.5  |

TABLE 3

*Potency tests on suspensions of live cholera organisms*

| Test No. | Vaccine                               |    |    | ED <sub>50</sub> |                     |
|----------|---------------------------------------|----|----|------------------|---------------------|
|          |                                       |    |    | M1               | Number of organisms |
| 1        | Live 569 B Inaba suspension           | .. | .. | ....             | 3,925               |
|          | Standard cholera vaccine              | .. | .. | 0·0000725        | 435,000             |
|          | Fresh formalin-killed cholera vaccine | .. | .. | 0·000089         | 534,000             |
| 2        | Live 569 B Inaba suspension           | .. | .. | ....             | 4,475               |
|          | Standard cholera vaccine              | .. | .. | 0·000067         | 402,000             |
|          | Fresh formalin-killed cholera vaccine | .. | .. | 0·00005          | 300,000             |

TABLE 4

*Large-scale yields of cholera cultures on the agar medium of peanut protein and casein hydrolysates***Average values of two batches**

| Strains of <i>V. cholerae</i> |    | Number of Roux bottles* | Yield in the media of protein hydrolysates (ml./bottle)** |                |
|-------------------------------|----|-------------------------|-----------------------------------------------------------|----------------|
|                               |    |                         | Casein                                                    | Peanut protein |
| Inaba 569 B                   | .. | 200                     | 112                                                       | 125            |
| Ogawa S/II/58                 | .. | 200                     | 131                                                       | 140            |

\*180 ml./bottle of agar (1·85%) impregnated protein hydrolysate (200 mg.N/100 ml.

\*12,000 million organisms/ml.



## FILARIA PROJECT

The project was initiated in 1972. The project committee consisting of Director (Chairman), Deputy Director(R) in charge Zoonosis, Deputy Director in charge Chemotherapy, Assistant Director, Pharmacology, Assistant Director, Biochemistry, Dr. Gatlwar, Senior Scientific Officer (Class I) meet every month and evaluate the progress of the project.

### Epidemiology

Epidemiological studies in filariasis in the villages of Murdhe, Rai, Morva and Dongri revealed 96 microfilaria carriers and 52 filarial disease positive cases in 1,440 people examined. Similar epidemiological study is being carried out at the village Uttan in Thana District.

### Immunology

Skin tests were undertaken with Sawada's FST antigen in the known non-endemic area of Parbhani Town. These tests showed that the people unexposed to filarial infection do not react to the antigen.

### Drug evaluation

Drug trials were undertaken with Mebendazole. Trials were undertaken in forty-one microfilaria carriers from the villages of Murdhe, Rai, Morva and Dongri.

### Laboratory studies

Maintenance of *L. carinii* culture in cotton rats and albino rats was continued. One-hundred and twenty-one rodents were infected. *L. carinii* antigen was prepared for serological studies.

### Transmission studies

Infection of *Culex P. fatigans* in the laboratory with *W. bancrofti*, using microfilaria carriers, is being attempted. Development of *W. bancrofti* in the vector species is also being studied. When sufficient number of infective larvae become available, attempts will be made to prepare an antigen for diagnosing wucheriasis.

### Studies on testing of drugs against Filariasis

(a) *In vitro* screening, using, *L. carinii*.—A method has been standardised to maintain microfilariae of *L. carinii* *in vitro*.

The white rats infected with *L. carinii* were bled and microfilariae were separated by centrifuging. They were then maintained in Tyrode containing 20 per cent horse serum at 28° C for 96 hours. It was observed that most of the microfilariae remained motile for a considerable time. The results are presented in Table 1. The effect of the drug on motility and mortality of microfilariae will be studied for over 72 hours. The following drugs were tested for primary screening :—

L-tetramisole, R-1396, R-2392, R-3333 and R-3362.

(b) Maintenance of *W. bancrofti* on vero cell line.—The transmission of *W. bancrofti* to animals has not yet been successful. It was, therefore, decided

to maintain the microfilariae of *W. bancrofti* on vero cell line. Vero cell line is the tissue culture of monkey kidney cells, which is being maintained satisfactorily for the last 10 months. Microfilariae of *W. bancrofti* were alive up to 96 hours on vero cell line. The results of motility and mortality are shown in Table 2.

(c) *In vitro* screening of drugs as anti-filarial agents in white rats. Rats with high microfilarial counts and those showing progressive increase in the count were chosen for this study. The drugs were given orally for 6 days. Doses were 6 mg./kg./day for Hetrazan and 8 mg./kg./day for *L-tetramisole* and Mebendazole. The microfilarial count was checked for 6-28 days. The rats were sacrificed at the end of this period and the adult worms from pleural cavity were taken out and counted. The results are presented in Table 3 and 4 respectively. On treatment with Hetrazan, microfilarial level in the peripheral blood was reduced and after discontinuing the Hetrazan treatment the microfilarial count recovered to 60 per cent of the original. On treatment with Litmosoides also, the microfilarial level was reduced and after discontinuing the drug, the microfilarial count recovered to 30 per cent of the original. Whereas, on treatment with Mebendazole, the microfilarial level went on decreasing, the trend followed even when the drug was discontinued on the 28th day, the microfilarial count was 28 per cent of the original.

TABLE 1

*Microfilariae of L-tetramisole carinii alive in vitro*

| Hours | Number of Microfilariae<br>per tube |     |     |     | Percentage of motile Microfilariae<br>per tube |    |    |     |
|-------|-------------------------------------|-----|-----|-----|------------------------------------------------|----|----|-----|
|       | 1                                   | 2   | 3   | 4   | 1                                              | 2  | 3  | 4   |
| 0     | 227                                 | 229 | 192 | 242 | 96                                             | 99 | 99 | 100 |
| 24    | 206                                 | 183 | 220 | 232 | 81                                             | 90 | 86 | 86  |
| 48    | 216                                 | 212 | 200 | 203 | 79                                             | 81 | 85 | 94  |
| 72    | 221                                 | 198 | 199 | 186 | 80                                             | 82 | 90 | 90  |
| 96    | 197                                 | 215 | 183 | 176 | 50                                             | 16 | 40 | 50  |

TABLE 2  
*Microfilariae of Wuchereria bancrofti alive in vitro on vero cell line*

| Hours | No. of microfilariae |    |    |    |    |    |    |    | Percentage of motile microfilariae |    |     |     |     |     |     |     |
|-------|----------------------|----|----|----|----|----|----|----|------------------------------------|----|-----|-----|-----|-----|-----|-----|
|       | Tube Nos.            |    |    |    |    |    |    |    | Tube Nos.                          |    |     |     |     |     |     |     |
|       | 1                    | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 1                                  | 2  | 3   | 4   | 5   | 6   | 7   | 8   |
| 0     | 60                   | 57 | 58 | 66 | 64 | 53 | 60 | 56 | 100                                | 95 | 100 | 100 | 100 | 100 | 100 | 100 |
| 24    | 52                   | 37 | 44 | 37 | 47 | 38 | 41 | 42 | 96                                 | 97 | 60  | 90  | 80  | 84  | 80  | 95  |
| 48    | 66                   | 43 | 33 | 40 | 41 | 41 | 42 | 31 | 80                                 | 75 | 17  | 10  | 25  | 10  | 10  | 20  |
| 72    | 52                   | 55 | 43 | 40 | 45 | 59 | 41 | 53 | 20                                 | 25 | 5.0 | 7   | 7   | 9   | 2   | 10  |
| 96    | 49                   | 52 | 56 | 49 | 37 | 55 | 43 | 51 | 0                                  | 0  | 2   | 0   | 0   | 200 | 0   | 0   |

At 72 hours, the motility was reduced to + = negligible motility.

TABLE 3  
*Microfilarial count after drug treatment*

| Days | Control | Hetrazan | L-Tetramisole | Mebendazole |
|------|---------|----------|---------------|-------------|
| 0    | 100     | 100      | 100.0         | 100         |
| 3    | 110     | 8.4      | 7.5           | 63.0        |
| 7    | 83      | 1.5      | 1.5           | 42          |
| 10   | 104     | 10.5     | 9.6           | 30          |
| 14   | 112     | 42.0     | 10.5          | 31          |
| 17   | 79      | 37.0     | 15.0          | 43          |
| 21   | 105     | 57.0     | 27.0          | 33          |
| 24   | 83      | 54.0     | 21.5          | 27          |
| 28   | 101     | 60.0     | 29.5          | 28          |

Notes.—(1) The microfilarial count at the beginning is taken as 100 per cent and the result is expressed as percentage of the same.  
(2) No. of rats used = 5 rats/drug.

TABLE 4  
*Adult worms recovered from drug-treated rats*

| Treatment     |    |    | Adult worms recovered from rats |      |        |      | Calcification of adult worms in rats |
|---------------|----|----|---------------------------------|------|--------|------|--------------------------------------|
|               |    |    | Male                            |      | Female |      |                                      |
|               |    |    | Alive                           | Dead | Alive  | Dead |                                      |
| Control       | .. | .. | 26                              | 12   | 22     | 4    | 2/2                                  |
| Hetrazan      | .. | .. | 33                              | 11   | 28     | 5    | 3/5                                  |
| L-tetramisole | .. | .. | 25                              | 26   | 31     | 6    | 3/5                                  |
| Mebendazole   | .. | .. | 49                              | 11   | 40     | 10   | 5/5                                  |

## PLAGUE PROJECT

Plague continues to remain absent from the State of Maharashtra for the last twenty-five years. However, several studies have shown the likelihood of presence of foci of plague in Maharashtra and other parts of India. These foci, under favourable conditions, may lead to the flare-up of an epizootic, which may consequently culminate in an outbreak. In view of this possibility, the plague project studies were initiated in the year 1965 to detect these foci and to initiate surveillance measures in collaboration with the State Public Health Department.

### Epidemiological studies

During the period under report, epidemiological studies were continued in 'D' and 'E' ward areas of Greater Bombay. In all, 945 rats from 'D' and 602 rats from 'E' ward areas were examined. *R. rattus*, *R. norvegicus* and *B. bengalensis* together were found to constitute the prevalent rodent fauna. *Mus musculus* and *B. gigantea* were also recovered in good numbers. Prevalence of various types of rodents during different seasons is under study.

*X. cheopis* showed its predominance, as reported earlier, over the other species of fleas recovered, viz. *X. astia*. 185 fleas from ward 'D' and 63 fleas from ward 'E' were examined. Other ectoparasites harboured by these rodents are being studied.

### Serological studies

A programme to assess serological status of *R. rattus* in various parts of the Maharashtra State was undertaken. Rodents from various municipal areas were collected for these studies. Erythrocytes, sensitised with fraction I antigen of *Yersinia pestis* were received from Middle Asian Research Institute of Plague Control, Zavodskaya, (Ministry of Health, U.S.S.R.) for plague serological studies.

Over 1,000 serum samples of rodents were collected from different parts of the Maharashtra State and screened. Out of these, fifteen pools, collected from Murud, Dhoki, Barsi, Jalgaon and Malegaon, were found to be positive in 1:2, 1:40, 1:60 dilutions respectively. These findings indicate the likelihood of presence of inapparent foci of plague infection among commensal rodents in these parts.

Apart from these, 240 serum samples from dogs and 100 serum samples from pigs were collected and examined serologically. Out of these, fifteen dog sera samples and three pig sera samples were found to be positive by HA tests. Further studies are in progress.

### Laboratory trials with IN-100

Laboratory trials were undertaken with IN-100—a new rodenticide developed by Messrs. Rohm and Haas, Philadelphia. The rodenticide was assessed for its efficacy against *R. rattus*, *R. norvegicus*, *B. bengalensis* and *Mus musculus*. It was found to be effective against all the four species of rodents in the laboratory.

### Susceptibility of rats to anticoagulant rodenticides

Assessment of susceptibility status of various species of rodents to Warfarin and other anticoagulant rodenticides was continued. So far, rodents from 'B', 'C', 'D' and 'E' ward areas have been screened. The studies showed presence of tolerance to Warfarin among *B. bengalensis* population in 'D' and 'E' ward areas.

### Investigations on the susceptibility of rodent population in the Maharashtra State to *Y. pestis* and related serological studies.

The work related to the assessment of susceptibility of rats to plague collected from different parts of the State was continued. Approximately equal number of rats from 'D' ward, belonging to different species, viz. *B. bengalensis*, *R. norvegicus* and *R. rattus* were used. These were challenged with a fixed dose of approximately 5,000 organisms of the virulent strain 195/ of *Y. pestis* and their susceptibility was determined. Results presented in Table 1 show that rats belonging to the species *B. bengalensis* are highly susceptible to plague, their susceptibility being 93.3 per cent, whereas *R. norvegicus* and *R. rattus* show high resistance of 60 per cent and 50 per cent respectively.

### Fluorescent antibody staining technique

To develop a rapid method for the detection of plague infection, two methods (one direct and the other indirect) were tried. In the direct method, the rabbits were immunised with the whole cell antigen and Fraction I antigen of *Y. pestis*. Serum conjugate was then prepared by conjugating the sera of the immunised rabbits with fluorescein isothiocyanate at 4°C. The conjugate was then used to stain the smears of *Y. pestis* (195/P) and *Y. pseudotuberculosis* (PR-I) grown on blood agar. Smears of 195/P strain of *Y. pestis* exhibited good fluorescence, whereas *Y. pseudotuberculosis* (PR-I) showed weak fluorescence. In the indirect method, serum of guinea-pigs, injected with normal rabbit serum, was conjugated with fluorescein isothiocyanate at 4°C. The smears of *Y. pestis* (195/P), *Y. pseudotuberculosis* (PR-I) and (PR-II strains) and *Y. septica* were first stained with the Fraction I antiserum at 37°C for 30 minutes. The slides were then thoroughly washed with buffered saline and then stained with the serum conjugate. In this technique, 195/P strain of *Y. pestis* exhibited good fluorescence, whereas the strain PR-I of *Y. pseudotuberculosis* showed weak fluorescence. The fluorescence exhibited by PR-II strain of *Y. pseudotuberculosis* and *Y. septica* was non-specific.

### Identification of ten strains of plague

Identification of ten strains of plague, received from Indonesia through the H. O. Regional Office, for South East Asia, New Delhi, was undertaken. For this purpose, studies were carried out on their morphology biochemical and serological aspects, phage susceptibility, virulence and fraction, I content as shown in Table 2.

TABLE 1

*Susceptibility of Rats from 'D' Ward to P. pestis (195/P)*

| Place of collection | Species used          | No. of rats inoculated |        |       | No. of rats died within 15 days |        |       | No. of rats found Positive |        |       | Per cent susceptibility | Per cent resistant |
|---------------------|-----------------------|------------------------|--------|-------|---------------------------------|--------|-------|----------------------------|--------|-------|-------------------------|--------------------|
|                     |                       | Male                   | Female | Total | Male                            | Female | Total | Male                       | Female | Total |                         |                    |
| D—Ward              | <i>B. bengalensis</i> | 10                     | 20     | 30    | 9                               | 19     | 28    | 9                          | 19     | 28    | 93.3                    | 6.7                |
|                     | <i>R. norvegicus</i>  | 10                     | 20     | 30    | 5                               | 7      | 12    | 5                          | 7      | 12    | 40                      | 60                 |
|                     | <i>R. rattus</i>      | 10                     | 20     | 30    | 5                               | 10     | 15    | 5                          | 10     | 15    | 50                      | 50                 |

ABLE

*Identification of the strains received from Indonesia through  
W.H.O., New Delhi.*

| Strain         | Morphology and staining |         | Biochemical reactions |         |          |           |           |          |                   | Susceptibility to phage i.e. lysis |          | Serology                             | Virulence LD <sub>50</sub> | Fraction content by Gel diffusion |
|----------------|-------------------------|---------|-----------------------|---------|----------|-----------|-----------|----------|-------------------|------------------------------------|----------|--------------------------------------|----------------------------|-----------------------------------|
| J-2            | Gram negative           | Glucose | Sucrose               | Lactose | Mannitol | Bul-citol | Rham-nose | Glyc-rol | Nitrate reduction | 28°C                               | 37°C     | Agglutination with antiplague serum  | 43·5                       |                                   |
| J-2 a          | .. —                    | +       | —                     | —       | +        | —         | —         | —        | +                 | +                                  | +        | —                                    | 5·0                        | +                                 |
| J-3            | .. —                    | +       | —                     | —       | +        | —         | —         | —        | +                 | +                                  | +        | —                                    | 2·850                      | +                                 |
| J-3 c          | .. —                    | +       | —                     | —       | +        | —         | —         | —        | +                 | +                                  | +        | —                                    | 4·4                        | +                                 |
| J-4            | .. —                    | +       | —                     | —       | +        | —         | —         | —        | +                 | +                                  | +        | —                                    | 12·7                       | +                                 |
| J-4 d          | .. —                    | +       | —                     | —       | +        | —         | —         | —        | +                 | +                                  | +        | —                                    | 7·4                        | +                                 |
| J-5            | .. —                    | +       | —                     | —       | +        | —         | —         | —        | +                 | +                                  | +        | —                                    | 9·2                        | +                                 |
| J-5 a          | .. —                    | +       | —                     | —       | +        | —         | —         | —        | +                 | +                                  | +        | —                                    | 28·2                       | +                                 |
| J-6            | .. —                    | +       | —                     | —       | +        | —         | —         | —        | +                 | +                                  | +        | —                                    | 1·124                      | +                                 |
| J-6 b          | .. —                    | +       | —                     | —       | +        | —         | —         | —        | +                 | +                                  | +        | —                                    | 0·22                       | +                                 |
| Control 195/P. | —                       | +       | —                     | —       | +        | —         | —         | —        | +                 | +                                  | +        | —                                    | 1·805                      | +                                 |
| Control PR—L   | Gram-ve Ovoid form      | +       | —                     | —       | +        | —         | —         | —        | +                 | No lysis                           | No lysis | Do not agglutinate with plague serum |                            |                                   |

+ = Positive reaction  
— = Negative reaction

## PROJECT STUDY ON RICKETS

It is reported that the incidence of rickets in infants and children in different age groups is high and, in many cases, they do not respond to Vitamin D therapy. It was, therefore, thought important to carry out investigations in rickets with respect to their early diagnosis and prevention. The project, jointly under taken with the Department of Pediatrics, J. J. Group of Hospitals, includes the following aspects :—

- (a) to determine the incidence of rickets in children ;
- (b) to establish a reliable criteria for early diagnosis ;
- (c) to study the metabolism of Vitamin D in children with or without rickets ;
- (d) to evaluate the treatment schedule of Vitamin D in the prevention and treatment of rickets.

About fifty blood samples from infants and children of different age groups, ranging from one month to three years, were received from the Department of Pediatrics, J. J. Group of Hospitals. Using micro-analytical methods, these samples were analysed for calcium (total and ionized), phosphorus, alkaline phosphatase and proteins (see Table 1). Excess serum was stored for estimation of Vitamin D. The following scientists are the collaborators :—

Dr. B. B. Gaitonde.  
 Dr. M. M. Wagle.  
 Dr. Y. K. Ambedkar.  
 Shri H. S. Ved.

TABLE 1  
*Studies in Rickets*

| Age group           | Total Calcium<br>mg./100 ml.<br>serum | Ionized Calcium<br>mg./100 ml.<br>serum | Proteins<br>gms./100 ml.<br>serum | Phosphorus<br>mg./100 ml.<br>serum | Alkaline<br>phosphatase<br>activity<br>in Bodonsky<br>Units |
|---------------------|---------------------------------------|-----------------------------------------|-----------------------------------|------------------------------------|-------------------------------------------------------------|
| 0 to 3 months       | 8.73<br>(7.8—10.1)                    | 4.38<br>(3.52—5.23)                     | 5.86<br>(4.8—6.4)                 | 3.87<br>(3.51—4.3)                 | 5.89<br>(4.62—8.26)                                         |
| 4 to 6 months       | ....                                  | ....                                    | ....                              | ....                               | ....                                                        |
| 7 to 9 months       | 11.00<br>(8.5—13.5)                   | 6.17<br>(4.57—7.76)                     | 5.25<br>(4.6—5.9)                 | 5.10<br>(3.2—7.0)                  | 6.76<br>(5.15—8.38)                                         |
| 10 to 12 months     | 9.41<br>(7.9—12.3)                    | 4.78<br>(4.32—5.28)                     | 6.22<br>(4.9—7.9)                 | 5.15<br>(3.0—6.86)                 | 9.75<br>(3.2—18.53)                                         |
| 13 to 18 months     | 8.87<br>(7.9—11.4)                    | 4.79<br>(3.65—6.32)                     | 5.84<br>(5.1—7.2)                 | 3.84<br>(2.6—5.3)                  | 9.36<br>(4.2—17.16)                                         |
| 19 months and above | 8.60<br>(7.9—9.5)                     | 4.54<br>(4.32—4.73)                     | 5.46<br>(4.6—7.2)                 | 4.48<br>(2.5—7.3)                  | 8.27<br>(4.0—14.66)                                         |

- (1) Figures above the brackets indicate the mean value.  
 (2) Figures in the brackets indicate the value range.



## B. DEPARTMENTAL RESEARCH

### DEPARTMENT OF BACTERIOLOGY

During the year under review, the department continued its studies on the development of submerged and continuous culture methods for the growth of micro-organisms. Studies on "Preparation of live Cholera vaccine", "Susceptibility of Rodent Population in Maharashtra State to *Y. pestis*", "Radiation-killed Cholera Vaccine" and "Use of Labelled Glucose for Sterility Testing of Biologicals", were also continued. A new programme, viz., "A study of Anti-body response to Cholera Vaccines in Volunteers" was initiated. In view of the difficulties experienced in importing Casamino acids, experimental work was also taken up to seek a substitute. Attempts were made to study the toxin production by *C. diphtheria* using deep and submerged culture method.

#### **Development of submerged and continuous culture method for the growth of micro-organisms.**

Work on the preparation and standardization of various types of typhoid vaccines, prepared from the growth of *S. typhi* in fermentor and on solid fortified medium, was reported last year. During the year under review, few more batches of similar vaccines, i.e. formalised, heat-killed, alcoholised and acetone-killed and dried vaccines, were prepared from the whole culture as well as washed cells of *S. typhi* grown in fermentor and on solid fortified medium. Most of these vaccines as also those prepared last year, have been repeatedly tested for their efficacy by active mouse protection test. Cell-free supernatants (separated from the culture and preserved with 0.5 per cent. phenol) have also been subjected to potency test.

It will be seen from the results presented in Table 1 that all the three, viz., formalised, heat-killed and alcoholised vaccines, prepared from the whole fermentor culture are more potent than the standard A.K.D. Vaccine, since their relative potency is above 1.0. However, the A.K.D. and other vaccines prepared from the washed cells, yield poor protection, i.e. about 1/10th of the standard A.K.D. Vaccine (Tables 1 and 2). Poor antigenicity of these vaccines may be attributed to the loss of some protective antigenic components in the supernatant.

It will also be seen from the results (Table 1) that the supernatants are almost as potent as the whole culture vaccines, suggesting that most of the protective antigens go with the supernatant.

Apart from the potency studies, work on antigenic response of these vaccines in rabbits was continued. Using the W.H.O. method, formalised, heat-killed and alcoholised typhoid vaccines, prepared from the whole fermentor culture, have been injected along with the standard A.K.D. Vaccine.

Sera separated from the blood of the immunized rabbits were analysed periodically for H, Vi and O antibody titres. These compare well with those obtained by immunizing rabbits with standard A.K.D. Vaccine.

Work on the same lines as mentioned above for *S. typhi* was also started for *S. paratyphi* B, with a view to prepare T.A.B. Vaccine. Few batches of different types of Paratyphoid B vaccines were prepared. These will be subjected to potency test after determining LD<sub>50</sub> of *S. paratyphi* B, a challenge strain received from the Central Research Institute, Kasauli.

### Live Cholera Vaccine

The studies on the development of a live cholera vaccine were continued. with a view to find out the efficacy of live cholera organisms as prophylactic agents, 3 hour-old culture of the strain 569 B Inaba of *Vibrio cholerae* was tested for its antigenicity by active mouse protection test along with a standard cholera vaccine and a freshly prepared cholera vaccine. Both these vaccines contained  $6 \times 10^9$  organisms/ml. of the strain 569 B Inaba. They were killed by formalin and preserved by the addition of phenyl mercuric nitrate. The standard vaccine was preserved in a lyophilised form. The results of the tests are given in Table 3.

The results of the tests show that the level of immunity conferred by only 4,000 to 4,500 viable organisms of the strain 569 B Inaba of *Vibrio cholerae* is almost the same as that induced by about 3,00,000 to 5,34,000 killed organisms. This shows that the live organisms are almost 100 times more potent than the killed organisms.

One of the factors, which may be responsible for the high efficacy of the live cholera organisms acting as an immunizing agent, might be that the organisms may be persisting and multiplying at the site of injection and in the other body tissues. In order to confirm this presumption, a number of mice were inoculated intraperitoneally and intramuscularly with a three-hour old culture of the strain 569 B Inaba of *Vibrio cholerae*. Each mouse received about one million viable organisms. The mice were killed at intervals of 1, 4, 8, 16, 32 and 48 hours and an attempt was made to isolate cholera organisms from the site of injection, peritoneal cavity, spleen, liver and heart. In case of mice inoculated intramuscularly, no organisms could be detected at the site of injection or in any of the organs even within one hour. In case of mice inoculated intraperitoneally, a few organisms could be isolated from the peritoneal cavity up to a period of only four hours. The other tissues did not show the presence of any organisms at periods varying from 1 to 48 hours. It is necessary to have more experimental data to come to a definite conclusion in this respect.

### Radiation-killed Cholera vaccine

It has been previously reported that the colera vaccine prepared by irradiating the organisms either for one or two hours was more potent than the usual formalin-killed and phenyl-mercuric nitrate preserved cholera vaccine. It was also reported that the radiation killed cholera vaccine did not show any deterioration in antigenicity even after period of 18 months of storage at 4 to 10°C.

The efficacy of the radiation-killed cholera vaccines and their storage stability were determined by the active mouse protection test only. It was thought worthwhile to test the efficacy of these vaccines by other methods also,

such as agglutinin response in rabbits, vibriocidal activity of immune sera and also by ileal loop challenge method in adult rabbits. With this object in view, adult rabbits weighing about 1600 g. were immunized with two intravenous doses of 0.5 ml. and 1.0 ml., 10 days apart, of a vaccine containing  $6 \times 10^9$  organisms per ml. of the strain 569 B Inaba of *Vibrio cholerae*, inactivated by exposing to  $^{60}\text{Co}$  source for one hour. Fourteen days after the second dose, each of the rabbits was challenged with 1.0 ml. of a 3-hour old culture containing one million organisms of the strain 569 B Inaba of *Vibrio cholerae*, by ileal loop method. Eighteen hours after the challenge, the loops were found to be normal without the accumulation of fluid indicating complete protection. Similar experiments were also carried out with formalin-killed vaccine containing an equivalent number of organisms as in the irradiated vaccine. In case of normal rabbits, which were used as controls, accumulation of fluid was observed in loops. Three rabbits were used for each type of vaccine and three were kept as controls.

All the rabbits were bled before and after immunization for vibriocidal and agglutinin antibody titrations prior to challenging them by ileal loop method. The first sample of blood was collected just before the first dose, the second 10 days after the first dose and the third 14 days after the second dose. All rabbits showed vibriocidal titre less than 1:20 before immunization and  $10^{-6}$  titre before and after the second dose, showing no increase in titre after the second dose. The agglutinin titre of the sera of all the rabbits was less than 1:10 before immunization and 1:640 after the first and second dose, showing no rise after the second dose. A similar pattern of vibriocidal and agglutinin titres was seen also in the case of rabbits immunized with formalin-killed vaccine.

The three tests, viz. the agglutinin response in rabbits, vibriocidal activity of immune sera and the ileal loop challenge method used in the above experiments, showed that the irradiated vaccine was as potent as the formalin-killed vaccine. The active mouse protection tests used in the work previously reported showed that the irradiated vaccine was superior to formalin-killed vaccine. However, it was not possible to distinguish between the efficacies of these two types of vaccines by the methods used in the present work.

#### **Antibody response to two cholera vaccines in volunteers**

It has been shown in the controlled field trials that the vaccines prepared by different methods differ considerably in their protective effect, depending upon the method of preparation. The present work was undertaken to study the antibody response of two vaccines prepared from the same strains of organisms and having comparable mouse protective ability, but differing in the method of preparation. These two vaccines were prepared from the same strains in two different laboratories and contained the same number of organisms.

The difference in the method of preparation of these vaccines was that in one method, formalin was used as an inactivating agent and phenyl mercuric nitrate (P.M.N.) as a preservative, whereas the other vaccine contained phenol both as a killing agent and as a preservative. There was no significant difference in their mouse protective activity. The vaccine were coded as 'A' and 'B' before using them for administration.

## Volunteers

The volunteers included were the members of the staff varying in age from 23 years to 54 years. The volunteers for each vaccine group were allotted by reference to random table. The sex and age distribution is shown in Table 4.

## Dosage Schedule

A two-dose schedule was used. 0.5 ml. of the vaccine was injected subcutaneously and before injection a sample of blood was collected. Again, 14 days after the first dose, a blood sample of the volunteer was collected and the second dose of 1.0 ml. of the respective vaccine was administered. Three more blood samples were collected 1, 2 and 6 months after the first dose of the vaccine.

## Agglutinin response

The agglutinin response was determined by tube agglutination test using heated suspensions of the vaccine strains. The titres, therefore, represent the 0 antibody titres. Tables 5 and 6 show the agglutinin response pattern of the two vaccines against Inaba and Ogawa serotypes.

Out of the 18 volunteers inoculated with the vaccine A, 14 had pre-immunization titres less than 1/10 and 3 had a titre of 1/10 for Inaba (Table 5). One of the volunteers having a titre less than 1/10 did not show any response even to two doses of the vaccine.

In case of vaccine B, 7 out of 26 volunteers, having a titre less than 1/10, failed to show any response to *Inaba* even after two doses of the vaccine. Seventeen out of the remaining 19 had pre-immunization titre less than 1/10 and one had a titre of 1/10. Only one showed a titre of 1/20.

Fourteen days after the first dose of the Vaccine A, all the volunteers, except one, showed titres varying between 1/20 and 1/320, giving a geometric mean titre of 1/42.8.

In case of vaccine B, 3 of the 19 volunteers had a titre less than 1/10, 2 showed a titre of 1/10 14 days after the first dose. The titres of the rest of the 14 volunteers varied between 1/20 and 1/320 giving a geometric mean titre of 1/26.8. The second dose of 1.0 ml. of the vaccine, given 14 days after the first dose, did not show any significant rise in the titres. In fact, the geometric mean titre fell to 1/23.2 in the volunteers inoculated with the vaccine A and was maintained at a level of 26.1. 14 days after the second dose of vaccine B.

With Ogawa antigen, similar pattern of agglutinin response was observed (Table 6). After the first dose of vaccine A, the titres varied between 1/20 and 1/320 in all the cases except one, making a geometric mean titre of 1/77. The vaccine B showed almost a similar pattern after the first dose, giving a geometric mean titre of 1/64.

The second dose again did not show any rise in titres, but the geometric mean titres dropped significantly to 31.3 and 12.1, 14 days after the second dose of vaccines A and B respectively.

These results show that the two vaccines show some difference in response to both the antigens in the production of agglutinins. However, in the case of Inaba, the vaccine A shows better response than that seen after administration of vaccine B. In case of Ogawa this difference is not significant.

As far as absence of response to Inaba antigen is concerned, the vaccine B failed to produce response to greater number of volunteers than that in the case of vaccine A (7 against 1). Whether this is attributable to the vaccine or response of the individuals, needs further investigations. One worthwhile observation that one individual, who failed to respond with Vaccine A and 3 volunteers who failed to respond to vaccine B were individuals who are actually engaged in the production of cholera vaccine over the past five years or engaged in potency testing of the vaccine and are heavily exposed to *V. cholerae* f both the types.

### **vibriocidal response**

The vibriocidal antibody content of the samples of sera was determined by using the method described by Finkelstein.

The pattern of vibriocidal antibody differed considerably from that of the agglutinin production in all the volunteers.

Most of the volunteers showed a vibriocidal titre of less than  $10^{-2}$  to  $10^{-4}$  before immunization (Table 7) against both Inaba and Ogawa antigens. These titres increased by 1 to 4 logs in majority of the volunteers inoculated either with A or B vaccine (Table 8). Highest number of volunteers showed 2 log increase in titre with both antigens and with both the vaccines. There was only one volunteer in the vaccine A group who failed to show a rise in vibriocidal titre against both the antigens, but he had  $10^{-4}$  pre-immunization titre. In addition, there was one volunteer who did not show any rise in titre to Inaba only, but showed rise in Ogawa titre with vaccine A.

In case of vaccine B, the one volunteer, who failed to show response, had already a titre of  $10^{-3}$  to  $10^{-4}$  against both the antigens.

### **Diphtheria toxin production in casein-hydrolysate medium**

Acid hydrolysate of Casein-Casamino acids (DIFCO) is being used for the production of diphtheria toxin. Due to difficulties experienced in importing casamino acids, an attempt was made to find out a suitable substitute for the same. After initial experimentation to determine the optimal quantities of all the ingredients of the medium, it was found that acid hydrolysate of casein used for the production of cholera vaccine can be successfully used for diphtheria toxin production. Using this medium, toxin titre of 70 to 80 I.f/ml. can be routinely obtained.

### **Diphtheria toxin production in submerged culture**

To study the toxin production by *C. diphtheriae* under submerged conditions, five different media were employed. These differed primarily in their nitrogen source. The carbon sources tried were glycerine, maltose, glucose and

sodium acetate. The other ingredients of the media were cystine, di-sodium hydrogen phosphate, calcium chloride, ferric chloride, sodium chloride, magnesium sulphate, copper sulphate, zinc sulphate, magnesium chloride, nicotinic acid, betaalanine and pimelic acid. Optimal concentrations of all these ingredients were worked out. All the media were adjusted to pH 7.2 to 7.5 prior to sterilization at 115° C for 15 minutes.

Experiments were carried out both under shaken conditions and in fermentor. In shaken culture, experiments were conducted in 50-100 ml. broth culture in 250 ml. Erlenmeyer flasks placed on a rotary shaker.

In fermentor, the experiments were conducted in 5-8 litres broth culture in 15-litre vessel with aeration through sparger at the bottom or swaying over the surface of the medium at the rate of 2-8 litres of air per minute and agitation at 200-500 r.p.m.

The strain used for toxin production was *C. diphtheriae* PW 8 Albany 103, maintained on Löffler's serum medium. The volume of the inoculum was 1 to 1.5 percent of the culture medium. The incubation period was 48-72 hours at 35°-37° C. It was observed that the media based on papain-digest of meat supported maximum toxin production, while media based on hydrolysate of casein gave a very poor yield. The toxin titres obtained after 48 hours of incubation under submerged conditions are summarised in Table 9.

### Studies in Pertussis Vaccine

Five different strains of *Bord. pertussis* were tested for the preparation of a potent pertussis vaccine and out of these, only two strains seemed to be suitable for the purpose.

A hypoglycemic factor in *Bord. pertussis* has already been reported. Experiments were carried out with Kasauli strain of mice, 6 weeks' old and weighing about 18-20 g.

Normal fasting blood sugar values, after fasting mice for 20 hours, 28 hours and 48 hours respectively, were checked and found to be within the range of 40-70 mg%.

No appreciable fall in fasting blood sugar levels of mice, immunized with pertussis vaccine (20 bill. orgms.) given intravenously was noted.

Glucose tolerance test was carried out in normal and immunised mice. Normal mice showed the usual glucose tolerance curve, showing a peak, half an hour after the glucose administration. Immunized mice showed no rise in the blood sugar level even after glucose injection.

The effect of adrenaline on the blood sugar level of normal and immunized mice was also tested. The absence of hyperglycemia in immunized mice, as compared to the normal mice, was quite specific and outstanding since the normal group values rose to twice those of the immunized group. This effect of absence of hyperglycemia in immunized mice was found to set in completely, 4 hours after the immunization and remained for more than five days.

### **Preparation of allergenic extracts from the pollens of Parthenium hysterophorous L.**

A batch of fatty portion of the pollens of Congress Grass, prepared in bulk (about 5.0 litres), was supplied to the Pharmacology department of this Institute for study. About 3.0 litres of coca solution extract of the pollens, defatted with ether, has also been prepared and Seitz-filtered. A portion of this extract has been filled into 5 ml. ampoules and forwarded for pursuing studies in different aspects.

### **Investigations on the susceptibility of rodent population in the Maharashtra State to *Y. pestis* and related serological studies**

The work related to susceptibility of rats collected from different parts of the State was continued. Approximately equal number of rats from 'D' ward of Bombay belonging to different species, viz. *B. bengalensis*, *R. norvegicus* and *R. rattus* were used. These were challenged with a fixed dose of approximately 5,000 organisms of the virulent strain 195/P of *Y. pestis* and their susceptibility was determined. Results presented in Table 10 show that rats belonging to the species *B. bengalensis* are highly susceptible to plague, their susceptibility being 93.3 %, whereas *R. norvegicus* and *R. rattus* show high resistance (60 % and 50 % respectively).

### **Fluorescent antibody staining technique**

To develop a rapid method for the detection of plague infection, two methods (one direct and the other indirect), were tried. In the direct method, the rabbits were immunized with the whole-cell antigen and Fraction I antigen of *Y. pestis*. Serum conjugate was then prepared by conjugating the sera of the immunised rabbits with fluorescein isothiocyanate at 4°C. The conjugate was then used to stain the smears of *Y. pestis* (195/P) and *Y. pseudotuberculosis* (PR-I) grown on blood agar. Smears of 195/P strain of *Y. pestis* exhibited good fluorescence, whereas *Y. pseudotuberculosis* (PR-I) showed weak fluorescence. In the direct method, serum of guineapig, injected with normal rabbit serum, was conjugated with fluorescein isothiocyanate at 4°C. The smears of *Y. pestis* (195/P), *Y. pseudotuberculosis* (PR-I and PR-II strains) and *Y. septica* were first stained with the Fraction I anti-serum at 37°C for 30 minutes. The slides were first thoroughly washed with buffered saline and then stained with the serum conjugate. In this technique, 195/P strain of *Y. pestis* exhibited good fluorescence, whereas the strain PR-I of *Y. pseudotuberculosis* showed weak fluorescence. The fluorescence exhibited by PR-II strain of *Y. pseudotuberculosis* and *Y. septica* was non-specific.

### **Identification of ten strains of plague**

Identification of ten strains of plague received from Indonesia through the W.H.O. Regional Office, New Delhi, was undertaken. For identification, studies were carried out on their morphology, biochemical and serological aspects, phage susceptibility, virulence and Fraction I content as shown in table 11.

TABLE I

*Potency tests of typhoid vaccines prepared from whole cultures, washed cells and supernatants*

| Type of vaccine                       | Prepared from                     | Batch No. | ED* 50 of the vaccine | ED* 50 of the standard A.K.D. Vaccine | Relative Potency |
|---------------------------------------|-----------------------------------|-----------|-----------------------|---------------------------------------|------------------|
| Formalin-killed and P.M.N. preserved  | Whole culture                     | 1         | 0·001295              | 0·002426                              | 1·87             |
|                                       | Do.                               | 5         | 0·002135              | 0·002426                              | 1·13             |
|                                       | Washed cells                      | 13        | 0·02008               | 0·002426                              | 0·12             |
|                                       | Do.                               | 16        | 0·06579               | 0·006579                              | 0·1              |
| Heat-killed and Phenol-preserved      | Whole culture                     | 2         | 0·0009                | 0·003094                              | 3·44             |
|                                       | Do.                               | 6         | 0·001330              | 0·001528                              | 1·14             |
|                                       | Washed cells                      | 14        | 0·02360               | 0·002648                              | 0·11             |
|                                       | Do.                               | 17        | 0·4791                | 0·005773                              | 0·12             |
| Alcohol-killed and alcohol preserved. | Whole culture                     | 3         | 0·001921              | 0·003658                              | 1·85             |
|                                       | Do.                               | 7         | 0·002677              | 0·005718                              | 2·14             |
|                                       | Washed cells                      | 15        | 0·07640               | 0·006081                              | 0·08             |
|                                       | Do.                               | 18        | 0·04791               | 0·005773                              | 0·12             |
| Cell-free supernatants                | From Batch No. 4, 13, 14 and 15   |           | 0·002485              | 0·002821                              | 1·13             |
|                                       | From Batch Nos. 8, 16, 17 and 18. |           | 0·002765              | 0·007761                              | 2·80             |
| Acetone-killed and dried vaccines     | Washed cells                      | 4         | 0·06533               | 0·002745                              | 0·042            |
|                                       | Do.                               | 8         | 0·05874               | 0·007795                              | 0·14             |

\* —Mean of 3 tests.—



TABLE 2

*Potency tests of typhoid vaccines prepared from whole cultures and washed cells grown upon solid fortified medium*

| Type of vaccine                  | Prepared from | Batch No. | ED* 50 of the vaccine | ED* 50 of the standard A.K.D. Vaccine | Relative Potency |
|----------------------------------|---------------|-----------|-----------------------|---------------------------------------|------------------|
| Heat-killed and Phenol preserved | Whole culture | 10        | 0·002313              | 0·005005                              | 2·16             |
|                                  | Washed cells  | 20        | 0·009922              | 0·005955                              | 0·59             |
| Alcohol-killed and preserved     | Whole culture | 11        | 0·004297              | 0·006306                              | 1·23             |
|                                  | Washed cells  | 21        | 0·01917               | 0·003489                              | 0·15             |

\*—mean of 3 tests.

TABLE 3

*Potency tests on suspensions of live cholera organisms*

| Test No. | Vaccine                                     | ED 50     |                  |
|----------|---------------------------------------------|-----------|------------------|
|          |                                             | ml.       | No. of Organisms |
| 1        | Live 569-B Inaba suspension .. ..           | ..        | 3,925            |
|          | Standard cholera vaccine .. ..              | 0·0000725 | 4,35,000         |
|          | Fresh formalin-killed cholera vaccine .. .. | 0·000089  | 5,34,000         |
| 2        | Live 569-B Inaba suspension .. ..           | ..        | 4,475            |
|          | Standard cholera vaccine .. ..              | 0·000067  | 402,000          |
|          | Fresh formalin-killed cholera vaccine .. .. | 0·00005   | 300,000          |

TABLE 4

*Age and sex distribution of volunteers*

| Vaccine used | Total number of volunteers | Age in years | Sex  |        | Number of non-responders to |       |
|--------------|----------------------------|--------------|------|--------|-----------------------------|-------|
|              |                            |              | Male | Female | Inaba                       | Ogawa |
| A            | 18                         | 20—40        | 9    | 6      | ...                         | ...   |
|              |                            | 40           | 3    | ...    | 2                           | 1     |
| B            | 26                         | 20—40        | 10   | 12     | ...                         | ...   |
|              |                            | 40           | 3    | 1      | ...                         | ...   |

Non-responders — Volunteers showing a titre of less than 1/20, 14 days and 1 month after immunization.

TABLE 5  
*Agglutinin response of volunteers to two vaccines after one and two doses*  
*INABA*

| Vaccine | Number of volunteers | Number of non-responders | Time of collection of samples after 1st dose | Number of volunteers showing the following titres |    |    |    |    |     |     | Geometric mean titre |
|---------|----------------------|--------------------------|----------------------------------------------|---------------------------------------------------|----|----|----|----|-----|-----|----------------------|
|         |                      |                          |                                              | 10                                                | 10 | 20 | 40 | 80 | 160 | 320 |                      |
| A       | ..                   | 18                       | 1                                            | Pre-immunization                                  | .. | 14 | 3  | .. | ..  | ..  | 1.5                  |
|         |                      |                          |                                              | 14 days after 1st dose                            | .. | 1  | 1  | 4  | 2   | 6   | 42.8                 |
|         |                      |                          |                                              | 1 month                                           | .. | 1  | 5  | 5  | 2   | 1   | 23.2                 |
|         |                      |                          |                                              | 2 months                                          | .. | 14 | 2  | 1  | ..  | ..  | 1.5                  |
|         |                      |                          |                                              | 6 months                                          | .. | 16 | 1  | .. | ..  | ..  | 1.1                  |
| B       | ..                   | 26                       | 7                                            | Pre-immunization                                  | .. | 17 | 1  | 1  | ..  | ..  | 1.3                  |
|         |                      |                          |                                              | 14 days after 1st dose                            | .. | 3  | 2  | 4  | 1   | 6   | 26.8                 |
|         |                      |                          |                                              | 1 month                                           | .. | 2  | 3  | 4  | 4   | 3   | 26.1                 |
|         |                      |                          |                                              | 2 months                                          | .. | 13 | 2  | 2  | 2   | ..  | 3.3                  |
|         |                      |                          |                                              | 6 months                                          | .. | 14 | 1  | 1  | 1   | ..  | ..                   |

TABLE 6  
*Agglutinin response of volunteers of two vaccines after one and two doses*  
*OGAWA*

| Vaccine | Number of volunteers | Number of non-responders | Time of collection of samples after 1st dose | Number of volunteers showing the following titres |    |    |    |    |     |     | Geometric mean titre |
|---------|----------------------|--------------------------|----------------------------------------------|---------------------------------------------------|----|----|----|----|-----|-----|----------------------|
|         |                      |                          |                                              | 10                                                | 10 | 20 | 40 | 80 | 160 | 320 |                      |
| A       | ..                   | 18                       | 1                                            | Pre-immunization                                  | .. | 13 | 3  | 2  | ..  | ..  | 2.0                  |
|         |                      |                          |                                              | 14 days after 1st dose                            | .. | .. | 1  | 3  | 4   | 3   | 77.0                 |
|         |                      |                          |                                              | 1 month                                           | .. | 1  | 2  | 4  | 4   | 7   | 31.3                 |
|         |                      |                          |                                              | 2 months                                          | .. | 11 | 3  | 3  | 1   | ..  | 3.0                  |
|         |                      |                          |                                              | 6 months                                          | .. | 17 | 1  | .. | ..  | ..  | 1.1                  |
| B       | ..                   | 26                       | 3                                            | Pre-immunization                                  | .. | 20 | 2  | 1  | ..  | ..  | 1.0                  |
|         |                      |                          |                                              | 14 days after 1st dose                            | .. | 1  | 2  | 2  | 5   | 4   | 64.1                 |
|         |                      |                          |                                              | 1 month                                           | .. | 6  | 3  | 5  | 3   | 4   | 12.1                 |
|         |                      |                          |                                              | 2 months                                          | .. | 14 | 2  | 5  | ..  | 2   | 3.4                  |
|         |                      |                          |                                              | 6 months                                          | .. | 19 | 1  | 1  | 1   | ..  | 1.5                  |

TABLE 7

*Distribution of vibriocidal antibody before immunization*

| Vaccine | Inaba                        |      |      |      |   | Ogawa                |      |      |      |
|---------|------------------------------|------|------|------|---|----------------------|------|------|------|
|         | Number of volunteers showing |      |      |      |   | the following titres |      |      |      |
|         | 10-2                         | 10-2 | 10-3 | 10-4 |   | 10-2                 | 10-2 | 10-3 | 10-4 |
| A       | ..                           | 7    | 4    | 6    | 1 | 4                    | 5    | 7    | 2    |
| B       | ..                           | 5    | 2    | 11   | 2 | 4                    | 3    | 9    | 4    |

TABLE 8

*Vibriocidal antibody response of the two vaccines after one and two doses of vaccine**INABA*

| Vaccine used | Number of volunteers | Number of non-responders | Time of collection of sample after 1st dose |    | Number of volunteers showing the following difference in log titres |   |   |   |    |
|--------------|----------------------|--------------------------|---------------------------------------------|----|---------------------------------------------------------------------|---|---|---|----|
|              |                      |                          |                                             |    | 0                                                                   | 1 | 2 | 3 | 4  |
| A            | 18                   | 2                        | 14 days                                     | .. | 2                                                                   | 3 | 5 | 3 | 5  |
|              | 16                   | ....                     | 1 month                                     | .. | 4                                                                   | 3 | 2 | 5 | 2  |
|              | 18                   | ....                     | 2 months                                    | .. | 5                                                                   | 4 | 5 | 4 | 0  |
|              | 17                   | ....                     | 6 months                                    | .. | 5                                                                   | 3 | 7 | 2 | .. |

*OGAWA*

|   |    |      |          |    |   |   |   |    |    |
|---|----|------|----------|----|---|---|---|----|----|
| A | 18 | 2    | 14 days  | .. | 2 | 3 | 7 | +2 | 4  |
|   | 16 | 2    | 1 month  | .. | 2 | 5 | 5 | 3  | 1  |
|   | 18 | 2    | 2 months | .. | 3 | 6 | 6 | 3  | 0  |
|   | 17 | .... | 6 months | .. | 8 | 5 | 4 | .. | .. |

*INABA*

|   |    |   |          |    |    |   |    |    |    |
|---|----|---|----------|----|----|---|----|----|----|
| B | 20 | 1 | 14 days  | .. | 1  | 5 | 11 | .. | 3  |
|   | 16 | 1 | 1 month  | .. | 2  | 7 | 7  | .. | .. |
|   | 19 | 1 | 2 months | .. | 5  | 9 | 3  | 2  | .. |
|   | 18 | 1 | 6 months | .. | 10 | 5 | 3  | .. | .. |

*OGAWA*

|   |    |      |          |    |    |   |   |   |   |
|---|----|------|----------|----|----|---|---|---|---|
| B | 20 | 1    | 14 days  | .. | 1  | 7 | 8 | 1 | 3 |
|   | 16 | 1    | 1 month  | .. | 5  | 6 | 3 | 2 | 0 |
|   | 19 | 1    | 2 months | .. | 7  | 7 | 4 | 1 | 0 |
|   | 18 | .... | 6 months | .. | 14 | 1 | 3 | 0 | 0 |

TABLE 9  
Yields of diphtheria toxin in different media

| Nitrogen source                                 |  |  |  | Carbon source  |    | After 48 hours, |       |
|-------------------------------------------------|--|--|--|----------------|----|-----------------|-------|
|                                                 |  |  |  |                |    | pH              | Lf/m. |
| <i>Acid hydrolysate of Casein</i> .. .. .       |  |  |  | gm %           |    |                 |       |
| (a) Casamino acids, DIFCO .. .. .               |  |  |  | Maltose 2.0    | .. | 7.8             | 10    |
| (b) Media section of Haffkine Institute .. .. . |  |  |  | Do.            | .. | 7.8             | 10    |
| <i>Enzyme-hydrolysate of casein—</i>            |  |  |  |                |    |                 |       |
| (a) Tryptone T (Oxoid) .. .. .                  |  |  |  | Do.            | .. | 8.0             | 10    |
| (b) Tryptone TS (Bengal Immunity) .. .. .       |  |  |  | Do.            | .. | 8.0             | 10    |
| (c) NZ case (Sheffield, England) .. .. .        |  |  |  | Do.            | .. | 7.5             | 40—50 |
| Papain-digest of beef .. .. .                   |  |  |  | Do.            | .. | 7.5             | 60—65 |
|                                                 |  |  |  | Maltose 2.0 .. | .. | 7.8             | 75—80 |
|                                                 |  |  |  | + glucose 0.1  |    |                 |       |

TABLE 10  
Susceptibility of rats from 'D' ward to *Y. pestis* (195/P)

| Place of collection | Species used          | Number of rats inoculated |        |       | Number of rats found dead within 15 days |        |       | Number of rats found positive for plague |        |       | Per cent susceptibility | Per cent resistance |     |
|---------------------|-----------------------|---------------------------|--------|-------|------------------------------------------|--------|-------|------------------------------------------|--------|-------|-------------------------|---------------------|-----|
|                     |                       | Male                      | Female | Total | Male                                     | Female | Total | Male                                     | Female | Total |                         |                     |     |
| 'D' Ward            | <i>B. bengalensis</i> | ..                        | 10     | 20    | 30                                       | 9      | 19    | 28                                       | 9      | 19    | 28                      | 93·3                | 6·7 |
|                     | <i>R. norvegicus</i>  | ..                        | 10     | 20    | 30                                       | 5      | 7     | 12                                       | 5      | 7     | 12                      | 40                  | 60  |
|                     | <i>R. rattus</i>      | ..                        | 10     | 20    | 30                                       | 5      | 10    | 15                                       | 5      | 10    | 15                      | 50                  | 50  |

TABLE 11

*Identification of strains received from Indonesia through the W. H. O. Regional Office, New Delhi*

| Strain        | Morphology and staining               | Biochemical reactions |         |         |          |         |
|---------------|---------------------------------------|-----------------------|---------|---------|----------|---------|
|               |                                       | Glucose               | Sucrose | Lactose | Mannitol | Dulciti |
| 1             | 2                                     | 3                     | 4       | 5       | 6        | 7       |
| J-2           | .. Gram—negative cocobacillary forms. | +                     | —       | —       | +        | —       |
| J-2 a         | .. Do.                                | +                     | —       | —       | +        | —       |
| J-3           | .. Do.                                | +                     | —       | —       | +        | —       |
| J-3 c         | .. Do.                                | +                     | —       | —       | +        | —       |
| J-4           | .. Do.                                | +                     | —       | —       | +        | —       |
| J-4 d         | .. Do.                                | +                     | —       | —       | +        | —       |
| J-5           | .. Do.                                | +                     | —       | —       | +        | —       |
| J-5 a         | .. Do.                                | +                     | —       | —       | +        | —       |
| J-6           | .. Do.                                | +                     | —       | —       | +        | —       |
| J-6 b         | .. Do.                                | +                     | —       | —       | +        | —       |
| Control 195/P | .. Do.                                | +                     | —       | —       | +        | —       |
| Control PR-I  | .. Gram—ve ovoid forms                | +                     | —       | —       | +        | —       |

| Rhamnose | Glycerol | Nitrate reduction | Susceptibility to plague phage |          | Serology                                           | Virulence LD50 (No. of organisms) | Fraction I content (by gel diffusion) |
|----------|----------|-------------------|--------------------------------|----------|----------------------------------------------------|-----------------------------------|---------------------------------------|
|          |          |                   | at 28°C                        | at 37°C  |                                                    |                                   |                                       |
| 8        | 9        | 10                | 11                             | 12       | 13                                                 | 14                                | 15                                    |
| —        | —        | +                 | Lysis                          | Lysis    | Shows agglutination with anti-plague serum. i      | 43.5                              | +                                     |
| —        | —        | +                 | Do.                            | Do.      | Do.                                                | 5.0                               | +                                     |
| —        | —        | +                 | Do.                            | Do.      | Do.                                                | 82.05                             | +                                     |
| —        | —        | +                 | Do.                            | Do.      | Do.                                                | 4.4                               | +                                     |
| —        | —        | +                 | Do.                            | Do.      | Do.                                                | 12.7                              | +                                     |
| —        | —        | +                 | Do.                            | Do.      | Do.                                                | 7.4                               | +                                     |
| —        | —        | +                 | Do.                            | Do.      | Do.                                                | 9.2                               | +                                     |
| —        | —        | +                 | Do.                            | Do.      | Do.                                                | 28.2                              | +                                     |
| —        | —        | +                 | Do.                            | Do.      | Do.                                                | 1.124                             | +                                     |
| —        | —        | +                 | Do.                            | Do.      | Do.                                                | 0.22                              | +                                     |
| —        | —        | +                 | No Lysis                       | No Lysis | Does not show agglutination with anti-plague serum | 1.805                             | +                                     |

+ Positive reaction  
 — Negative reaction

### Sterility testing by use of labelled glucose

With a view to develop quicker method for ascertaining the sterility of biologicals, the last year's work was continued. During the year, 101 samples of biological products were tested for their sterility by employing labelled glucose as well as by routine method. These included 40 samples of rabies vaccine, 36 samples of cholera vaccine, 10 samples of pertussis vaccine, 10 samples of tetanus toxoid and 5 samples of anti-tetanus serum. Results of the sterility testing with labelled glucose were obtained within 18 hours of incubation at 37° C. Discrepancy arose only in one sample of rabies vaccine. The discarded sample gave no counts in a flask with labelled glucose, whereas the routine method showed contamination. In all other cases, results of both the methods tallied. These are given in Table 1.

TABLE 1

*Sterility testing by using labelled glucose and I. P. method*

| Sample               | Total No. | By using labelled glucose |             | By I.P. method |             | Percentage of agreement between the two methods |
|----------------------|-----------|---------------------------|-------------|----------------|-------------|-------------------------------------------------|
|                      |           | Sterile                   | Non-sterile | Sterile        | Non-sterile |                                                 |
| Rabies vaccine ..    | 40        | 29                        | 11          | 28             | 12          | 9.75                                            |
| Cholera vaccine ..   | 36        | 32                        | 4           | 32             | 4           | 100                                             |
| Pertussis vaccine .. | 10        | 8                         | 2           | 8              | 2           | 100                                             |
| Tetanus vaccine .    | 10        | 10                        | ..          | 10             | ..          | 100                                             |
| Anti Tetanus serum.. | 5         | 5                         | ..          | 5              | ..          | 100                                             |

## DEPARTMENT OF BIOCHEMISTRY

**Biochemical Studies on *Vibrio cholerae***

So far, detailed studies on lipids (Phospholipids and fatty acids) of *V. cholerae* have not been reported in the literature. However the occurrence of unusual fatty acids and phospholipids in the lipids of pathogenic bacteria has made us intensify our efforts to find out the relationship of these unique molecules to the disease process and virulence of the strains. Recently, several pharmacological and biological properties of bacterial lipids have been reported in the literature. However, very little is yet known about the biochemical aspects of lipids of the *V. cholerae* organism, in spite of all the emphasis laid on the epidemiology and toxins of this organism. In view of these facts, a detailed study of lipids of whole cell, cell wall and membranes of two antigenically different strains of *V. cholerae* 569B Inaba and *El tor* Inaba (Haemolytic) were carried out to differentiate them biochemically. Phospholipids of the two strains were identified and characterized. Five different components were determined quantitatively. The major phospholipids of *V. cholerae* thus identified were phosphatidyl ethanolamine (PE), phosphatidyl glycerol (PG) and lysophosphatidyl ethanolamine (lyso-PE) comprising more than 90% of the total phospholipids. Phosphatidyl ethanolamine (68-70%) is the most abundant phospholipid of *V. cholerae*. Quantitative differences were noted as regards this phospholipid fraction in *V. cholerae*. In *V. cholerae* 569B strain, it is 66%, whereas in *El tor* Inaba, it is 74%. The presence of larger amounts of phosphatidyl ethanolamine in the strains showed increased virulence of that particular strain. Lyso-phosphatidyl ethanolamine (6.8-7.0 per cent) is the characteristic of *V. cholerae*. In other bacteria, it is less than 1 per cent. The chemical composition of cell wall and membrane of both the strains was of a pattern similar to that found in Gram-negative bacteria. Membrane proteins were resolved by 7.5 per cent SDS-polyacrylamide gel electrophoresis. Eighteen to twenty distinct protein bands were identified according to their molecular weights ranging between 17,000 and 90,000. Proteins split into a few major and several minor bands.

**Use of peanut protein hydrolysate for cholera vaccine preparation**

Biochemical studies of *V. cholerae* in relation to cellular composition, oxygen uptake and protein synthesis and nucleotide composition of RNA were reported last year. The results revealed no appreciable differences in the biochemical aspects of cholera organisms grown on peanut protein hydrolysate medium as compared with those grown on casein hydrolysate medium. The work was further extended to study some enzymatic systems—lactic dehydrogenase (LDH), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvate transaminase (GPT) of cholera organisms grown on the media of casein and peanut protein hydrolysates, and chemically defined synthetic media. The specific activities of LDH and GOT in cholera vibrios were found to be more in the synthetic media whereas, in the case of peanut protein hydrolysate medium, the enzyme activities remained unchanged as in the case of casein hydrolysate medium (Table 1).

: After considering the growth responses and biochemical aspects, peanut protein hydrolysate was found to be suitable for the cultivation of cholera cultures. In the laboratory, a Quadruple cholera vaccine was prepared in the peanut protein hydrolysate medium by the standard procedures adopted in the Bacteriology Department of this Institute. The vaccine thus prepared was referred to the same department for mouse protection tests. The results indicated that the vaccine was as potent as the one prepared in the casein hydrolysate medium.

With a view to try the production of cholera vaccine on a larger scale, about 600 litres of peanut protein hydrolysate (200 mg. N per 100 ml.) were prepared for the cultivation of cholera vibrios by the improved procedure of digestion with hydrochloric acid alone. A noteworthy feature of this procedure is that a highly expensive and imported chemical, viz. Barium carbonate, can be totally eliminated from the existing procedure of digestion with hydrochloric and sulphuric acids, thus reducing the cost of the medium considerably. The growth response of the organisms in Roux bottles (100 bottles per batch) containing peanut protein hydrolysate (200 mg. N per 100 ml.) was found to be the same as in case of casein hydrolysate.

#### **Effect of dietary Carbohydrates (Starch/Sucrose) on the metabolism of glucose— $-U^{14}C$ , acetate $-1^{14}C$ and the turnover of cholesterol and phospholipids**

The lipid metabolism in the starch/sucrose-fed animals was studied further by using labelled precursors at the end of the feeding period. Glucose incorporation was more in the total liver lipids of sucrose group. Acetate incorporation was high in total lipids and cholesterol fraction of the liver lipids of sucrose-fed animals. Using labelled cholesterol, it was observed that the retention of cholesterol was not altered in the liver and intestine of both the groups. It is inferred that cholesterol synthesis is enhanced on sucrose diet, while cholesterol catabolism is unaffected. Studies with  $32p$  indicated that the phospholipid synthesis is lowered in the sucrose group.

#### **Effect of long-term treatment of some antidiabetic drugs on lipid metabolism**

The long-term effect of sulfonylurea drugs (tolbutamide in particular) and insulin was studied on lipid metabolism in human diabetic and non-diabetic subjects. The same was also studied in normal rabbits by carrying out biochemical and histo-pathological investigations. The difference in the lipid pattern, when compared with the controls, was found to be variable according to the type of treatment, inspite of having well-controlled blood sugar levels. The study provides evidence to the effect that these anti-diabetic drugs are likely to be atherogenic inspite of their property of controlling the blood sugar levels. This being an interesting observation, the mechanism and metabolic pathway will be studied in detail.

#### **Studies on the immunological aspects of nematode infections**

This work was undertaken, as very little is known about the immunological responses of the host in parasitic diseases. Studies on the immunological aspects of the parasitic infections will yield more information about the immune status of the host and also give us better means of diagnosis and immunization in parasitic diseases.



Humoral and cell-mediated immune responses are being studied, using *Nematospiroides dubius* infected mice as the laboratory model. Standardization of techniques in cell-mediated immunity is in progress.

### Prevalence of night blindness in Sirur taluka of Poona District

It was reported that in Sirur taluka of Poona district, night blindness was prevalent as a consequence of continued scarcity conditions on a wide scale. A research programme, including diet and nutrition surveys and examination of blood samples for vitamin A, R.B.C., haemoglobin, serum proteins etc., was undertaken in collaboration with the Directorate of Health Services. Fifty-five serum samples received from four different villages were analysed for the above-mentioned tests. The results clearly indicated that people in Sirur taluka were suffering from Vitamin 'A' deficiency during scarcity conditions prevalent in the area (Table 2).

### Analysis of "Sukhadi" and other food samples

The Government of Maharashtra had initiated a programme to supply "Sukhadi" to the scarcity labour above the age of eighteen years. To assess its nutritive value, the Government had created a temporary nutrition cell at his Institute for a period of two months. Fifty-eight 'Sukhadi' and other food samples received from Collectors of various districts of Maharashtra State were analysed for proximate principles, minerals and Vitamins. Results are given in Table 3.

### Standardization of a method for estimating cyclic AMP for studying the effect of drugs

It is now well known that cyclic AMP plays an important role in certain diseases. To understand the mechanism of action of certain drugs, it is necessary to measure cyclic AMP levels before and after treatment with a particular drug. A micro method for estimation of cyclic AMP by competitive binding with specific protein, using  $^3\text{H}$  labelled cyclic AMP *in vitro*, has been standardized.

TABLE 1

*Effect of media on the specific enzyme activity of V. cholerae, Ogawa S/II/58*  
(1.85 per cent agar medium)

| Enzyme                                                              | Casein hydrolysate (200 mg. N/100 ml.) | Peanut protein hydrolysate (200 mg. N/100 ml.) | Chemically defined medium |
|---------------------------------------------------------------------|----------------------------------------|------------------------------------------------|---------------------------|
| 1. Lactate dehydrogenase (LDH) (Units/min./mg. protein).            | 86                                     | 110                                            | 136                       |
| 2. Glutamic Oxaloacetic transaminase (GOT) (Units/hr./mg. protein). | 89                                     | 87                                             | 117                       |
| 3. Glutamic pyruvic transaminase (GPT) (Units/hr./mg. protein).     | 22                                     | 26                                             | 22                        |

Average values of two individual experiments in duplicate.

TABLE 2

*Vitamin A analysis*

| Age Group            |    |    | Sirur Taluka,<br>Vit. A. (I.U./100 ml.<br>Plasma) | Parner Taluka,<br>Vit. (A.I.U./100 ml.<br>Plasma) |
|----------------------|----|----|---------------------------------------------------|---------------------------------------------------|
| Below 20 years       | .. | .. | 30<br>(—)                                         | 40<br>(30—50)                                     |
| 20 years to 50 years | .. | .. | 43.8<br>(20—90)                                   | 47.5<br>(25—80)                                   |
| 51 years to 69 years | .. | .. | 52.7<br>(30—100)                                  | 50<br>(—)                                         |
| 70 years and above   | .. | .. | 38.7<br>(30—60)                                   | ....<br>....                                      |
| Total                |    |    | 45.8<br>(20—100)                                  | 47.7<br>(25—80)                                   |

(1) Normal value reported in the literature for adult is 66 to 110 I.U. per 100 ml. plasma  
 (2) Figures at the top of brackets indicate the mean value, while figures in brackets indicate the value range.

TABLE 3

*Food samples Values per 100 gm. of samples*  
 ('Sukhadi' and other food samples)

| Range                       |                |    |    |    | Mean  |
|-----------------------------|----------------|----|----|----|-------|
| <i>Proximate Principles</i> |                |    |    |    |       |
| Moisture g.                 | 7.5 to 13.1    | .. | .. | .. | 9.0   |
| Protein g.                  | 10.0 to 17.2   | .. | .. | .. | 13.3  |
| Fat g.                      | 8.6 to 17.6    | .. | .. | .. | 12.2  |
| Ash g.                      | 1.4 to 2.3     | .. | .. | .. | 1.9   |
| Carbohydrates g.            | 59.3 to 69.0   | .. | .. | .. | 63.3  |
| Calories                    | 382.0 to 446.0 | .. | .. | .. | 418.2 |
| <i>Minerals</i>             |                |    |    |    |       |
| Calcium mg.                 | 92 to 152      | .. | .. | .. | 107.6 |
| Phosphorus mg.              | 200 to 315     | .. | .. | .. | 240.1 |
| Iron mg.                    | 4 to 14        | .. | .. | .. | 6.4   |
| <i>Vitamins</i>             |                |    |    |    |       |
| Vitamin A.I.U.              | 1124 to 1919   | .. | .. | .. | 1058  |
| Vitamin B1 ug.              | 304 to 467     | .. | .. | .. | 351   |

## DEPARTMENT OF CHEMOTHERAPY

**Synthesis and study of compounds for their possible anti-parasitic activity**

As mentioned in the last annual report, a large number of compounds were synthesised and submitted for the study of their antihelmintic activity, both *in vitro* and *in vivo*. These studies were completed during the year. Many of these compounds showed a good order of anthelmintic activity comparable to tetramisol.

A compound from 5-nitrobenzthiazolyl piperazine series, viz. 5-nitro-2-[(2-thiazolyl)-1'-piperazinyl]-benzthiazole, was found to be active *in vitro* against *A. lumbricoides* at a dose level of 0.1 µg./ml. It also showed activity against *N. dubius* in mice at 0.2 mg. dose level (92 per cent mortality). Similarly, 5-nitro-2-[(4'-methyl)-1'-piperazinyl] benzthiazole exhibited 94 per cent activity in *in vivo* screening at a dose level of 0.2 mg. Two more compounds from the pyridothiazolyl piperazine series and 2-arylamino-pyrido (3, 2-d) thiazole series have also shown activity at a dose level of 0.5 mg. (90-92 per cent mortality of the adult worms).

**2-Heterocyclic aminopyrido(3, 2-d)thiazoles**

Since thiazolyl pyridothiazole derivative exhibited high order of anthelmintic activity, other heterocyclic rings such as quinoline, pyrimidine, morpholine, thiazoline, etc. and adamantyl in place of thiazole, have been incorporated into the pyrido(3, 2-d)thiazole ring. Ten compounds were synthesized, of which six were studied and evaluated for their anti-parasitic activity. Out of these, 2-adamantylaminopyrido(3, 2-d)thiazole has shown activity against *N. dubius* in mice at a dose level of 0.2 mg. with 92 per cent mortality of adult worms.

**2-Aryl-imidazo (2,1-b)thiazolo (3,2-d)pyridines**

While synthesizing new structures for testing against *Ascaris lumbricoides*, a series of tricyclic compounds, obtained by reacting 2-aminopyrido(3, 2-d)thiazoles with different phenacyl bromides followed by cyclization, were prepared. Out of the seven compounds synthesized, five have shown antihelmintic activity against *A. lumbricoides* from human host *in vitro* testing. 2-Phenylimidazo(2, 1-b)thiazolo(3, 2-d) pyridine hydrobromide was the most active at a dose level of 1 µg./ml. in the above series of compounds. The activity of this compound appears to be comparable to that of tetramisole and is also irreversible.

**2-aryl-pyrido(3, 2-d) thiazolo(3, 2-a)s-triazine-1,3-diones**

In view of the interesting biological properties, especially the trypanocidal and anti-filarial activities of *s*-triazine compounds, a novel fused ring systems, involving *s*-triazine and pyrido (3, 2-d) thiazole moieties was synthesized and evaluated. These compounds were synthesized by reacting 2-amino-pyrido (3, 2-d) thiazole with aryl isocyanates. Three representative compound were screened against *N. dubius* in mice. All the three have exhibited activity.

The most active of these is *o*-methoxyphenyl-pyrido (3, 2-d) thiazolo (3, 2-a)-*s*-triazine-1, 3-dione, which showed activity at 0.1 mg. dose with 94 to 95 per cent mortality of the adult worms.

### Piperazinylpyridines

In view of the well-known activity of 2—(2-methoxyethyl)-pyridine against gastro-intestinal tract nematodes, a series of related compounds containing piperazine moiety has now been prepared. 'Bis [ $N^1$  carbamoyl- $N^4$  aryl (or alkyl) piperazinyl] pyridines and L, L<sup>1</sup>-bis [ $N^1$ -alkyl- $N^4$ -aryl (or alkyl) piperazinyl]-pyridines have been prepared and are being studied for their anti-parasitic activity.

### Synthesis and study of compounds for anti-tubercular activity

In view of the importance of nitrofuran moiety for anti-bacterial activity, 5-nitrofuroyl chloride was condensed with different mono-substituted piperazines to obtain  $N^1$ -(5-nitro-2-furoyl)- $N^4$ -substituted piperazines. By *in vitro* studies,  $N^1$  (5-nitro-2-furoyl)- $N^4$ -(pyrimidyl)-piperazine was found to be the most active (0.5  $\mu$ g. ml.) from among the compounds tested from both these series. Two compounds of this series are undergoing tests for antiamoebic activity.

Another series of Mannich bases from 5-nitro-2-furamide and furfural acetone with different substituted piperazines were also prepared. While the compounds from the first series showed *in vitro* anti-tubercular activity of high order, the Mannich bases were found to be completely inactive.

### Synthesis of anti-inflammatory compounds

*New Schiff bases for anti-inflammatory activity.*—Indole and the thiazole ring systems seem to be important from the point of view of anti-inflammatory activity as inferred from Indomethacin and Fenclozic acid. In view of this, a series of new Schiff bases were prepared from Indole and other aldehydes and thiazole amines. However, only two compounds from this series have shown significant anti-inflammatory activity.

### Synthesis and study of compounds for anti-tumor activity

*Schiff bases from benzaldehyde bi-methane sulphonates and 4-(*p*-aminophenyl) thiazoles and aromatic amines.*—In view of the clinical utility of Busulfan (or Myleran), as an anti-tumor drug, various schiff bases containing bi-methane sulphonate moiety were synthesized and submitted for screening from this laboratory.

Out of the above 65 compounds of bi-methanesulphonates submitted for anti-tumor screening, reports of 53 compounds have been received from Cancer Chemotherapy National Service Centre (CCNSC), Bethesda, U.S.A. Thirty compounds showed significant activity in L-1210 Lymphoid leukemia in animal screening and KB cell culture. Further, these compounds are being tested in P-388 Lymphocytic leukemia.

C.C.N.S.C. has further confirmed the activity of three compounds tested in different biological laboratories. The following three compounds were reported as cures in L-1210 Lymphoid leukemia the tumor system which has the greatest predictability regarding the clinical effectiveness.

- (i) NSC 166252      ..      1/6 cures at 125 mgs./kg. dose level ;
- (ii) NSC 16623      ..      2/6 cures at a dose of 12·5 mgs./kg.
- (iii) NSC 166234      ..      1/6 cures at 12 mgs./kg. dose level.

One of the compounds, NSC 166224, ethanol, 2, 2'-[4-[3-(chlorophenyl limino, methyl] phenyllimino) bis, bi-methanesulphonate mono-hydrochloride, showed broad safety margin in animal test, having the highest non-toxic dose at 40 mgs./kg. and a minimum effective dose at 2·5 mgs./kg.

In general, it is found that these compounds are less toxic and more effective than the analogous Schiff bases from benzaldehyde nitrogen mustard as reported earlier.

## DEPARTMENT OF CLINICAL PATHOLOGY

## Typhoid Scheme

Under the Typhoid Scheme unit, 897 calls were attended, blood samples were collected for blood cultures and Widal tests. An analysis of the data showed the following results :

| Total No. of samples |       |       | Widal+ve Culture +ve | Widal+ve Culture -ve | Widal -ve Culture +ve | S. typhi- | Para A isolated | Stool Culture +ve |
|----------------------|-------|-------|----------------------|----------------------|-----------------------|-----------|-----------------|-------------------|
| Blood                | Urine | Stool |                      |                      |                       |           |                 |                   |
| 978                  | 24    | 204   | 72                   | 279                  | 61                    | 100       | 33              | 1                 |

This year, with a great effort, 204 samples of stool were collected from the patients. Very few samples could be obtained from the contacts of the patients in spite of repeated requests. No *Salmonella* strains were isolated from the stool samples of contacts. Urine cultures were negative.

Fourteen strains of *S. typhi* were sent to the *Salmonella* Phage Typing-Centre at Lady Hardinge Medical College, New Delhi.

The following phage types were reported :—

|      |    |    |            |
|------|----|----|------------|
| A    | .. | .. | 11 samples |
| E1   | .. | .. | 1 sample   |
| 42   | .. | .. | 1 sample   |
| D1 N | .. | .. | 1 sample   |

Four hundred and seven strains of *S. typhi* were screened for antibiotic sensitivity and transferable drug resistance. Thirty eight strains were found to possess multiple drug resistance, while five showed resistance against Chloramphenicol. The Chloramphenicol resistance was lost on subculture.

Since we could not succeed in getting repeated stool samples, the VI haemagglutination test was standardized to find out whether this test could be made use of in the detection of carrier state.

The endotoxin of *S. typhi* was extracted and labelled by chromium <sup>51</sup> in collaboration with the scheme sponsored by the International Atomic Energy Agency at this Institute. It is proposed to use the labelled antigen for the radio-immunoassay of sera from suspected cases of enteric fever.

## Gonorrhoea

A good serological test is not available for the diagnosis of chronic gonococcal infections, particularly in female patients. Haemagglutination test, using alkali extract of gonococci as an antigen, was standardised. Comparison of the results of this test with the fluorescent antibody test is in progress.

The work on the antibiotic sensitivity of *N. gonorrhoeae* to Penicillin and Septtran has been undertaken and is in progress.

### **Antibiotic sensitivity testing and studies on R-factor**

Antibiotic sensitivity testing is one of the most frequent bacteriological investigation in this department. Antibiotic sensitivity tests were carried out on 297 urine samples. Ninety-one per cent of the strains isolated were found to possess multiple drug resistance. One-hundred and two of these M. D. R. strains were screened for the presence of R-factors and 71.7 per cent of these were proved to possess R-factor.

One hundred stool samples from healthy individuals, who had no recent history of treatment with antimicrobial drugs, were screened for R-factor. Twenty-five per cent of these stools were found to carry organisms with transferable drug resistance. Stool samples from 37 cases undergoing antibiotic therapy were screened for the presence of R-factor. Eighty per cent of them harboured bacilli with transferable drug resistance. Thus, the effect of selective pressure of antibiotics was noted.

### **Tuberculous Meningitis**

Thirty-one samples of C. S. F. collected from suspected cases of Tuberculous Meningitis were investigated by culture and guinea-pig inoculation test. Of these, 2 were found positive by guinea pig inoculation method and none by culture methods. Thus, guinea pig inoculation method seems to be the most sensitive and superior to the culture method for the diagnosis of tuberculous meningitis.

### **brucellosis**

A sero-epidemiological investigation was undertaken to assess the prevalence of Brucellosis in man and animals. Nine-hundred and sixty human sera were analysed from professionally pre-disposed persons, clinically suspected cases of U. O. and blood donors. Four-hundred and fifty-four sera from cattle, goat, pigs, sheep, dog and horses were investigated. This study indicated a high incidence of Brucellosis in the occupationally pre-disposed population, viz. : from dairy and piggery workers, 12 per cent and 9.5 per cent respectively. The incidence in animals varied from 6 to 70 per cent.

### **adenomyosis**

Six-hundred and fifty-six uterine specimens were studied. Adenomyosis was detected in 150 uteri, giving an incidence of 22 per cent. Associated lesions in the uterine adnexa were also studied.

### **anaerobic culture**

Attempts at isolating and identifying *anaerobic* organisms were made, using Hrome-Acid Jar method and by modifying other techniques.

### **identification of M. tuberculosis in tissue sections by fluorescent microscopy**

Identification of mycobacteria in tissue section is a crucial means of confirming the diagnosis of tuberculous granuloma. The conventional carbol-uchsin staining procedures have been largely unsatisfactory. There is a conclusive evidence that fluorescent microscopy is far superior to the conventional Ziehl-Neelsen staining procedure in the identification of mycobacteria. Hence,

fluorescent staining method was standardised using Auramine Rhodamine mixture (Kuper and May, 1960). The results were compared with modified Fite-Faraco's method (carbol fuchsin method). It was observed that the fluorescent staining method was more sensitive and superior to the Fite-Faraco's method for the detection of *M. tuberculosis* in tuberculous granulomatous lesions.

### **Rheumatoid Arthritis**

The work on the detection of R. A. factor in the sera of Rheumatoid Arthritis cases, by using locally prepared reagents, was continued and it is being compared with the Rose-Waler Test.

The results obtained by the locally assembled reagents showed 100 per cent agreement with the Hyland R. A. test kit. (imported).

### **Comparison between Rose-Waaler and Latex Test**

Fifty normal sera and one hundred and eleven sera from cases of Rheumatoid arthritis and other diseases were tested by Rose Waaler test and Latex fixation test. Although, latex fixation test has proved to be a more sensitive test in the diagnosis of Rheumatoid Arthritis, it is less specific. Latex fixation test has shown positive results in lepromatous leprosy, in non-rheumatoid collagen diseases and 4 per cent of normal sera.



## DIRECTOR'S LABORATORY

The pharmacological aspects of tetanus toxin were investigated. Crude toxin obtained in a lyophilised form from the Immunology Department was first standardised for its potency. The effect of toxin was then studied in various species of animals, particularly with a view to elucidate the toxin action on the autonomic nervous system. Various autonomic effector organs, such as rat fundal strip, isolated guinea-pig's or rat's vas-deference, blood pressure and respiration in rats and guineapigs, lingomandibular effects etc. were also studied, after administration of toxin in various doses over different periods of time. Prof. Kryznovsky of U.S.S.R., who has been working in the field of mechanism of tetanus toxin, visited this laboratory. His visit provided us opportunities for discussions in the field of tetanus toxin mechanism.

Pharmacological aspects of pertussis toxin, consisting of two fractions, viz. the heat-labile and the heat-stable, were studied. The toxin is shown to produce a number of biological actions. Administration of one of the fractions produces hypoglycemia. Heat-stable fraction, when injected into animals, renders rats more susceptible to the action of his-tamine. Since the method of bioassay to test the potency of vaccine is not very satisfactory, it was decided to investigate the possibility of using one of these biological activities to seek a more suitable method for bioassay of pertussis vaccine. The results obtained so far are encouraging.

Choleraenic toxin of *V. cholerae* has been studied by using ileal loops of rabbits. It was decided to find out whether the toxin as well as the vaccine potency can be evaluated by using ileal loop preparation of rat as an experimental model. This method has the advantage of simplicity, besides being economical. We found that the cholera toxin gives a good dose response curve in case of ileal loop of rats. Further, the mechanism of action of cholera toxin in producing fluid accumulation was studied, using a number of pharmacological agents, such as Nicotinic Acid, Theophylline, Prostaglandin, Berberine etc. The mechanism of action of choleraenic toxin is being further evaluated with a view to study the action of cyclic AMP in fluid accumulation.

A new plant R-10—an indigenous plant obtained from areas nearabout Bombay—was collected and identified. Different extracts, such as chloroform, ether, alcohol and cold water, were subjected to preliminary testing for antifertility activity in rats, mice and rabbits. The results indicate that the crude extract and the alcohol extract administered orally for a period of 3 days during the stage of diestrous in rats and mice and immediately after mating in rabbits, showed a significant antifertility activity. Further work on its chemical analysis and antifertility activity is in progress.

Oral contraceptive pills have been reported to produce toxic psychosis in a number of persons. Further, this toxic psychosis has been thought to be due to an action of estrogen or probably progesteron on pyridoxin metabolism in the body. It was, therefore, decided to investigate in rats the effects of oral contraceptives on neuro-humoral mechanism of the brain. Biochemical

methods for assaying neuro-humoral transmitters, such as gamma-amino butyric acid, noradrenaline, Dopamine and 5-hydroxy tryptamine, by fluorometric method have been standardised.

A series of 3-4 di-substituted 5-mercapto-1, 2, 4-(H)-triazoles were studied for their activity on the central nervous system. Most of the compounds in this series showed a mild depressant action. However, 3, 4-diethyl-5-mercapto-1, 2, 4-(H)-triazole was found to possess a marked C.N.S. stimulant activity. This compound was found to antagonize the central nervous system depression induced by various narcotic agents like pentobarbitone, diazepam, ethyl alcohol, chloralose and epentol—a new steroidal anaesthetic. The drug was, however, not found to antagonize the effects of morphine. The margin of safety appears to be good. The drug is being investigated further as a narcotic antagonist with a view to assess a possibility of its therapeutic use. Acute and chronic toxicity studies of the compounds are also underway.

## DEPARTMENT OF IMMUNO-HAEMATOLOGY

### **Studies on Australia antigen**

The screening of the sera of patients suffering from various clinical conditions for Australia antigen (HBAg) was continued, using the standard agar gel double diffusion technique (AGD) and counter-immuno-electrophoresis (CEP) technique. The results are given in Table 1.

### **Radio-immune assay for Australia antigen**

The department was fortunate in obtaining, through the courtesy of Messrs. Abbott Laboratories, U.S.A., kits for carrying out screening of Australia antigen (HBAg) by radio-immune assay technique on 500 samples. This test was standardized in collaboration with the Radiobiology Unit. This opportunity was utilised to compare the different methods of assay of HBAg used in this laboratory with the above method. Sera from four groups of persons were screened using simultaneously the A.G.D., the CEP and R.I.A. techniques. The results are given in Table 2.

The results show that the radio-immune assay technique is the most sensitive method for detection of HBAg. The high incidence (27 per cent) of positive samples among acute infective hepatitis cases, collected from Kasturba Infectious Diseases Hospital, Bombay, reveals the high prevalence of Type II virus in the population and gives credence to the suggestion about spread of his virus by faeco-oral route.

### **Radio-immune assay of plasma fractions for HBAg.**

Opportunity was also taken to screen different fractions of plasma for the presence HBAg by R.I.A. technique, as the parent plasma is drawn from random population without screening for HBAg. The results are given in Table 3.

It is interesting to note : (a) that Australia antigen, though not detected in parent plasma pool, could be isolated in fractions II+III and III, indicating thereby that it is present in very diluted amounts in random plasma pool, (b) that HBAg is separated with fraction I (fibrinogen), cryoprecipitates and fractions II+III and III (alpha beta globulins) and is not present in fraction III (gamma globulin) and fraction V (albumin) which are clinically useful fractions.

### **Assessment of liver functions in HB antigenemia in various clinical conditions**

Liver is the main target organ which undergoes pathological changes in the clinical conditions which exhibit HB antigenemia. Therefore, it was felt that the study of liver function tests in such conditions would reveal some interesting findings. The results of the work carried out are given in Table 4.

It is interesting to note that the values in cases of hepatitis are quite high, more so in HBAg +ve cases, whereas in cases of leprosy and leukemia, these values are slightly elevated, indicating thereby that the persistence of the antigen is perhaps due to the defective immune mechanism in the latter cases.

## Status of immunoglobulins in HB antigenemia

The immune response to any infection is revealed by variation in immunoglobulin patterns. These patterns were studied in sera of patients suffering from HBAG positive and HBAG negative infective hepatitis. The results are given in Table 5.

Some workers had reported higher IgA level in HBAG positive cases. However, the results arrived at with our tests do not show any significant variation between HBAG positive and HBAG negative groups.

## Studies on alpha-fetoprotein (AFP)

The appearance of an embryo-specific globulin (now known as AFP) in an adult mammalian was first shown by Abelev (1964), particularly in adult mice bearing primary hepatoma. Tatarinov (1966) showed the presence of AFP in persons suffering from primary hepatoma. Over the past few years, the testing of serum for AFP in suspected malignancy of liver has become a routine in many laboratories.

The testing procedure is similar to that used for the screening for HBAG. Dr. C. A. Linsell, Chief of Nairobi Regional Centre, investigating the malignancy of liver in Uganda (Africa), has suggested that there is a possible relationship between HBAG and AFP in malignancy of liver and therefore, suggested that such studies may be carried out here also. The antibody was kindly supplied by Dr. Abelev (U.S.S.R.).

During the year, 89 suspected cases of hepatoma and other hepatic conditions were investigated at this Institute for the AFP. Results are given in Table 6.

## Red-cell enzymes

Role of red-cell enzyme deficiencies in the causation of haemolytic anaemia has now been well-established. One of the key enzymes is Glucose-6-Phosphate Dehydrogenase (G-6-PD), the deficiency of which is responsible for the development of severe drug-induced haemolytic anaemias, often resulting in acute renal shut-down. Departmental studies carried out in 1964 here also showed that G-6-PD deficiency in new-born infants could result in severe neo-natal jaundice. The department has been investigating such patients for G-6-PD deficiency since almost a decade.

## Screening of G-6-PD deficiency

During the year, seventy-five cases of haemolytic anaemias were referred to us for the screening of G-6-PD deficiency. The results are given in Table 7.

While our earlier studies were restricted only to the quantitative screening test, since the last 3 years, quantitative assay techniques for G-6 PD have also been established. Also, other enzymes like GSH and PK have been incorporated in our enzyme studies. Our first efforts were to set up the normal values in adult males and females as well as in new-born males and females. The first part of the study was undertaken to ascertain the levels of these , enzymes in G-6-PD deficient males during haemolytic crisis and those not having the crisis as well as in G-6-PD heterozygote carriers with nearly 50 per

cent G-6-PD values in comparisons with those in normal females. Also, G-6-PD deficient males had very low GSH levels, particularly when associated with haemolytic crisis. No gross differences in PK levels were noticed.

The red-cell enzyme profile in various anaemias was completed in 1973 and summary of the data is presented in Table 8.

It is apparent from Table 8 that GSH levels are significantly increased in iron deficiency anaemia, leukemia and thalassemia. G-6-PD levels showed a slightly higher trend in all other categories of anemias studied. This may probably reflect the higher enzyme levels of younger (immature) cells in peripheral circulation. PK levels are also slightly high in all conditions, except in aplastic anaemia.

In conclusion, it may be said that Beutler's glutathione stability test is likely to prove valuable in determining the degree of haemolytic anaemia, while the enzymes G-6-PD and PK remain ineffective, though their slightly higher levels may be due to circulating immature young cells and thus not contributing to the physiological status.

### **Anticoagulants in enzyme studies**

In previous year's report, the data for assessing suitability of 4 different anti-coagulants in studying enzymes profiles was reported. Findings suggested that EDTA was the anti-coagulant of choice. This year, the study was further extended to investigate two EDTA anti-coagulants (Na and K). It was observed that K2-EDTA had marked effect on enzymes during two-week preservation, thus pointing to the fact that Na2 EDTA is a better choice.

### **Red-cell preservation**

Studies on preservation of the blood for transfusion for a sufficient length of time at 4°C have been of great value for the blood banks. Preliminary studies on this aspect were undertaken during the year for the preservation of red cells in various preservatives at 4°C and to determine the base-line changes. These preliminary observations did indicate that CPD appears to have a slight edge over ACD as judged from the results on plasma haemoglobin, osmotic fragility and blood sugar levels. Detailed programme, involving addition of purine derivatives on red cell preservation, will be undertaken in the ensuing years. These studies will also involve the estimation of red-cell enzyme intermediates like GSH, ATP and 2-3 DPG in the glycolytic pathway.

### **aptoglobins**

Haptoglobins are haemoglobin-binding X2 globulins. Earlier studies have suggested that low levels of Hp play an important role in the diagnosis of active haemolytic process. This test was, therefore, used in the samples referred to this department for various haemolytic anaemias of obscure origin. aptoglobins were identified by starch-gel electrophoresis and the bands are visualised by staining with benzidine hydrogen peroxide stain to demonstrate Hb and Hb-Hp bands. Eighteen out of 31 cases were of Hp O-O type indicating active haemolytic process. Ahaptoglobulinaemia samples usually contained very small amount of haptoglobins, i.e. 10 mgs. percent.

## Immunoglobulins

Serum samples from patients suffering from various disorders of immunoglobulin were investigated. Starchgel electrophoresis, using discontinuous buffer system of pH 8.6 was used for separation of the proteins. Majority of the cases referred, were suspected to have clinical diagnosis of multiple myeloma. However, these cases also included children suspected of hypogammaglobulinemia because of history of recurrent infections. A total of 152 cases were referred for immunoglobulin status. The results are given in Table 9.

Among the hypergammaglobulinemia cases in adults, 3 cases of monoclonal IgG myeloma were detected, while 5 of the 12 cases of raised IgM showed dysgammaglobulinemia picture. One of the male children showed clinical and laboratory evidence of agammaglobulinemia with IgG level of less than 3 units and having normal IgM and IgA. Family study of the prepositus, presented evidence of hypogammaglobulinemia in mother, while father and one sister had normal levels of immunoglobulines, suggesting possibly X-linked agammaglobulinemia.

## Specific immunoglobulins

*Tetanus immunoglobulins*—Very few prisoners take full course of immunisation and the response in those taking full course is not very satisfactory. From the discussions with foreign experts, it was gathered that only 10-12% of hyperimmunised persons develop satisfactory titre of antibody. So, another larger batch of prisoners has been taken up for hyperimmunisation.

*Anti-D immunoglobulin*.—The batch of IgD anti-D, purified by DEAE sephadex batch chromatography, has shown high activity (3 times the W. H. O. standard) as reported by the Blood Group References Centre here. Bigger batches will be processed following this procedure.

## Biochemical and immunological studies of human fibrinogen and cryoprecipitate

Anti-haemophilic factor and fibrinogen were prepared using different concentrations of Sodium Chloride.

The precipitate and supernatant at every stage have been tested both for the presence of fibrinogen and for anti-haemophilic factor. Complete removal of either anti-haemophilic factor or fibrinogen is not possible in the above methods. Fibrinogen was also prepared from contaminated plasma by Kabi laboratory method and polyethylene glycol method. It was purified by column chromatography using Sephadex G 200. Purified fibrinogen was also prepared by Blomback method from old fibrinogen ampoules. Purified fibrinogen was used for the standardization of radial immuno-diffusion, technique using 0.8 ml. of anti-fibrinogen/10 ml. of 1% agarose.

Anti-haemophilic factor was prepared by 25% NaCl method and separated from fibrinogen by polyethylene glycol method. Anti-haemophilic factor was also prepared from plasma of different blood groups and also from pooled plasma by different methods. Anti-A and Anti-B titre of plasma and anti-haemophilic factor was anti-A (1:8), anti-B (1:2) in the anti-haemophilic factor prepared from A and B group plasma. There was no titre in the anti-haemophilic factor prepared from pooled plasma.

### Studies on coagulant and anti-coagulant properties of snake venoms

Snake venom contains a complex mixture of proteins which has several enzymatic activities. Snake venom shows coagulant as well as anti-coagulant properties. Some snake venoms at a lower concentration show coagulant activity, whereas at a higher concentration cause incoagulability.

Preliminary tests were carried out with venoms of *Naja naja*, *Vipera russelli* and Krait in order to evaluate the coagulant and anti-coagulant principles present therein.

#### Results of tests performed

*Coagulant activity.*—Coagulant activity of the venom was measured by adding equal amount of venom solution to citrated plasma and fibrinogen. Only Russell's viper venom at a higher concentration (0.1 mg./ml. and above) showed coagulant activity.

*Recalcification time of plasma.*—Cobra and Krait venom showed anti-coagulant activity. Viper venom showed both coagulant as well as anti-coagulant activity.

*One-stage prothrombin time.*—Cobra and Krait venoms inhibit the extrinsic pathway of prothrombin activation. Viper venom activates the prothrombin activation.

*Thromboplastin generation test.*—Cobra venom was found to inhibit the thromboplastin generation and at the same time destroyed the formed blood thromboplastin. It is likely that venom might be destroying factors V and VIII respectively.

Viper venom accelerates the generation of thromboplastin and specifically activates factors X to Xa respectively.

Krait venom at low concentration accelerates the generation of thromboplastin but, at a higher concentration, it has got inhibitory action. It is likely that Krait venom activates factor X and also inhibits factor VIII activity.

*Euglobulin lysis test.*—Fibrinogenolytic and fibrinolytic activity of all the three venoms was tested by Euglobulin lysis time method.

A new agarose fibrin plate method was developed and standardised. In his method, the fibrinogen is converted into fibrin by thrombin in the presence of agarose which serves as the base for the fibrin. Material to be tested is added to this, punched well in a small quantity (10 ml.) and the slide is incubated at 37° C for 16 hours. Lysed zones produced after incubation were measured and reported in sq. mm. Different parameters, which affect the fibrin lysis ones, were thoroughly worked out.

Streptokinase (SK) was used in different concentrations, ranging from 100 U of SK/ml. to 1 U of SK/ml. of solution. Lysed zones produced by SK gave a dose response curve. This method was found to be very sensitive as compared to fibrin plate method.

Further in vivo studies on snake venoms are in progress.

### Immuno-biochemical aspects of rheumatoid syndrome

Rheumatoid disorders are collagen disorders supposed to be of auto-immune origin, leading to various clinical features like polyarthritis, fibrositis etc. The exact nature of the rheumatoid factor is not definitely known, but is presumed to be immunoglobulin 'M' with sedimentation of 19-22S. Many diagnostic tests have been evolved to diagnose this condition. Latex fixation test is one of the sensitive and dependable tests. The latex particles are coated with active gamma globulin molecules and tested against the suspected sera. The gamma globulin produced by this department has been found to be suitable for this test.

Sixty samples of suspected cases of rheumatoid arthritis were investigated for RA factor test, haptoglobins and immunoglobins during the last year.

The investigation was continued and one hundred seventy-two samples were tested so far. The results are given in Table 10. The work is still in progress.

TABLE I  
*Incidence of Australia (HBAG) antigen in liver disorders*

| Types of cases                            | Total No. tested | HBAG(antigen)   |            | HBAb(antibody)  |            |
|-------------------------------------------|------------------|-----------------|------------|-----------------|------------|
|                                           |                  | Number positive | Percentage | Number positive | Percentage |
| Infective hepatitis—                      |                  |                 |            |                 |            |
| Adults .. ..                              | 298              | 35              | 11.6       | ..              | ..         |
| Children .. ..                            | 27               | 2               | 7.1        | ..              | ..         |
| Liver cirrhosis—                          |                  |                 |            |                 |            |
| Adults .. ..                              | 30               | 2               | 6.7        | ..              | ..         |
| Children .. ..                            | 18               | 1               | 5.5        | ..              | ..         |
| Cooley's anaemia cases (Multi-transfused) | 36               | 4               | 11.1       | 4               | 14.3       |
| AKD (Artificial Kidney Dialysis Unit)     | 30               | ..              | ..         | ..              | ..         |
| Total ..                                  | 439              | 44              | 10.0       | 4               | 14.3       |

TABLE 2  
*Radio-immune assay for HBAG compared with A.G.D., C.E.P., techniques*

| Samples                                 | No. tested | HBAG +ve |            |        |            |        |            |
|-----------------------------------------|------------|----------|------------|--------|------------|--------|------------|
|                                         |            | A.G. D.  |            | C.E.P. |            | R.I.A. |            |
|                                         |            | No.      | Percentage | No.    | Percentage | No.    | Percentage |
| Voluntary donors                        | 75         | 1        | 1.3        | 4      | 5.33       | 5      | 6.66       |
| Professional donors                     | 100        | 1        | 1.0        | 4      | 4.00       | 12     | 12.00      |
| Infectious hepatitis patients           | 100        | 4        | 4.0        | 11     | 11.00      | 27     | 27.00      |
| Blood Bank and Kidney dialysis workers. | 114        | 2        | 1.71       | 4      | 3.42       | 6      | 5.26       |
| Total ..                                | 389        | 8        | 2.05       | 23     | 5.90       | 50     | 12.82      |



TABLE 3

*Results of testing plasma fractions for presence of HBAg by R.I.A. technique*

| Sample                  | No. tested | HBAg +ve | Remarks                                       |
|-------------------------|------------|----------|-----------------------------------------------|
| Plasma pool .. ..       | 3          | 1        |                                               |
| Fraction I (fibrinogen) | 5          | 1        | Derived from HBAG+ve plasma                   |
| Cryoprecipitate         | 5          | 1        |                                               |
| Fraction II+III         | 3          | 3        |                                               |
| Fraction III            | 2          | 2        | Derived from plasma pool not tested for HBAG. |
| Gamma globulin          | 6          | ..       |                                               |
| Fraction V albumin      | 5          | 1        |                                               |

TABLE 4

*Results of Liver Function Tests in various diseases in relations to HBAG*

| Liver Function Tests           | Names of the diseases |                  |                |                |                 |                 |
|--------------------------------|-----------------------|------------------|----------------|----------------|-----------------|-----------------|
|                                | Viral hepatitis       |                  | Leukemia       |                | Leprosy         |                 |
|                                | Au+ve<br>(22)×        | Au—ve<br>(39)×   | Au+ve<br>(8)×  | Au—ve<br>(44)× | Au+ve<br>(8)×   | Au—ve<br>(20)×  |
| SGOT (Karmen Units)            | 115·6÷<br>±8·477      | 99·4<br>±9·24    | 33·2<br>±4·79  | 28·5<br>±1·66  | 67·5<br>±12·063 | 34·4<br>±3·33   |
| SGPT (Karmen Units)            | 159·86<br>±13·36      | 127·0<br>±10·515 | 31·4<br>±5·03  | 23·7<br>±1·67  | 62·2<br>±10·08  | 19·16<br>±1·535 |
| Alk. Phos.<br>(Bodansky Units) | 4·18<br>±0·404        | 4·97<br>±0·561   | 2·8<br>±0·55   | 2·92<br>±0·09  | 2·77<br>±0·47   | 2·3<br>±0·088   |
| Bilirubin (mg. %)              | 6·54<br>±0·32         | 8·6<br>±1·065    | 0·37<br>±0·044 | 0·32<br>±0·015 | 0·5<br>±0·19    | 0·5<br>±0·097   |

*Normal values of twenty controls*

|                             |    |    |    |    |            |
|-----------------------------|----|----|----|----|------------|
| SGOT (Karmen Units)         | .. | .. | .. | .. | 21·2±1·44  |
| SGPT (Karmen Units)         | .. | .. | .. | .. | 25·7±1·84  |
| Alk. phos. (Bodansky Units) | .. | .. | .. | .. | 3·2±0·286  |
| Bilirubin (mg. %)           | .. | .. | .. | .. | 0·28±0·018 |

× Figures in parenthesis denote the number of estimations

÷ Mean ± Standard error.

TABLE 5

*Immunoglobulin and SGPT levels in viral hepatitis*

| Groups        | No. | Immunoglobulins (mg.%) |          |          | SGPT<br>(Karmen<br>Units) |
|---------------|-----|------------------------|----------|----------|---------------------------|
|               |     | IgG                    | IgA      | IgM      |                           |
| Normal .. ..  | 25  | 1350±9·7               | 172±4·9  | 159±5·5  | 25·0±10·0                 |
| HBAG Positive | 13  | 1498±87·0              | 256±24·1 | 181±18·0 | 183·0±4·8                 |
| HBAG Negative | 11  | 1722±33·0              | 265±16·9 | 192±20·2 | 193·0±9·2                 |

TABLE 6  
*Incidence of Alpha-fetoprotein (AFP) in various liver disorders*

|                    |    |    |    |    | Total<br>number<br>tested | AFP Positive |            |
|--------------------|----|----|----|----|---------------------------|--------------|------------|
|                    |    |    |    |    |                           | No.          | Percentage |
| Hepatic Coma       | .. | .. | .. | .. | 9                         | ...          | ...        |
| Hepatitis          | .. | .. | .. | .. | 14                        | 1            | 7.1        |
| Suspected Hepatoma | .. | .. | .. | .. | 27                        | 3            | 11.1       |
| Cirrhosis          | .. | .. | .. | .. | 35                        | 1            | 2.8        |
| Jaundice           | .. | .. | .. | .. | 4                         | 1            | 25.0       |
| Total              |    |    |    | .. | 89                        | 6            | 6.6        |

TABLE 7  
*G-6-PD deficiency in cases of haemolytic anaemia referred from city hospitals*

| Clinical condition |    |    |    | Number of cases | G-6-PD deficient |            |      |
|--------------------|----|----|----|-----------------|------------------|------------|------|
|                    |    |    |    |                 | No.              | Percentage |      |
| Haemolytic anaemia | .. | .. | .. | ....            | ....             | ....       |      |
| Adults (Males)     | .. | .. | .. | 65              | 8                | 12.3       |      |
| Children (Males)   | .. | .. | .. | 52              | 5                | 10.0       |      |
| Total              |    |    |    | ..              | 117              | 13         | 10.9 |

TABLE 8  
*Levels of G-6-PD, GSH and PK in different categories of anaemias*

|                         |    |    |    | GSH             |        |        | G-6-PD          |       |        | PK              |       |        |
|-------------------------|----|----|----|-----------------|--------|--------|-----------------|-------|--------|-----------------|-------|--------|
| Group                   |    |    |    | No. of<br>cases | Mean   | ±S.D.  | No. of<br>cases | Mean  | ±S.D.  | No. of<br>cases | Mean  | ±S.D.  |
| Normal Males            | .. | .. | .. | 94              | 63.31  | ±10.21 | 60              | 3.882 | ±0.893 | 56              | 38.46 | ±7.35  |
| Normal Females          | .. | .. | .. | 60              | 66.04  | ±9.99  | 50              | 3.720 | ±1.100 | 44              | 36.80 | ±7.76  |
| Cord bloods Males       | .. | .. | .. | 25              | 70.41  | ±12.07 | 20              | 3.950 | ±0.790 | 25              | 41.89 | ±7.32  |
| Iron deficiency Anaemia | .. | .. | .. | 69              | 102.60 | ±20.54 | 69              | 4.180 | ±0.92  | 69              | 48.10 | ±5.46  |
| Dimorphic Anaemia       | .. | .. | .. | 43              | 97.32  | ±23.15 | 43              | 4.140 | ±1.28  | 43              | 47.16 | ±8.00  |
| Megaloplastic Anaemia   | .. | .. | .. | 11              | 80.87  | ±15.75 | 11              | 5.160 | ±2.54  | 11              | 60.92 | ±5.31  |
| Aplastic Anaemia        | .. | .. | .. | 6               | 75.97  | ±9.49  | 6               | 4.070 | ±0.44  | 6               | 40.70 | ±4.13  |
| Haemolytic Anaemia      | .. | .. | .. | 8               | 96.90  | ±30.00 | 9               | 5.160 | ±1.82  | 9               | 49.30 | ±8.44  |
| Leukemia                | .. | .. | .. | 13              | 118.70 | ±46.28 | 13              | 4.560 | ±1.88  | 13              | 46.80 | ±10.88 |
| Thalassemia             | .. | .. | .. | 26              | 104.40 | ±27.15 | 26              | 4.370 | ±1.84  | 26              | 48.80 | ±9.77  |

TABLE 9

### *Immunoglobulines in referred cases*

| Group    |    | No. of cases | IgG | IgA  | IgM  | Ig Normal |
|----------|----|--------------|-----|------|------|-----------|
| adult    | .. | 102          | 10↓ | .... | .... | ....      |
|          |    |              | 18↑ | 10↑  | 13↑  | 51        |
| children | .. | 50           | 16↓ | 11↓  | .... | 8         |
|          |    |              | 5↑  | 2↑   | 8↑   | ....      |
| Total    |    | 152          | 49  | 23   | 21   | 59        |

TABLE 10

#### *Immunological findings in suspected rheumatoid cases*

| tex fixation test |          | Haptoglobin type |     |     | Immunoglobuline        |      |      |       |       |       |       |
|-------------------|----------|------------------|-----|-----|------------------------|------|------|-------|-------|-------|-------|
| Positive          | Negative | 2-1              | 2-2 | 0-0 | IG (Total)<br>(Normal) | IG ↑ | IG ↓ | IgG ↓ | IgG ↑ | IgM ↑ | IgM ↓ |
| 80                | 92       | 37               | 127 | 6   | 80                     | 33   | 10   | 18    | 11    | 16    | 4     |

Two 1-1 type also found

## DEPARTMENT OF IMMUNOLOGY

**Biochemical and immunological studies on the Indian snake and scorpion venoms**

The fractionation of elapid snake venoms was undertaken with a view to obtain purified fractions containing potent toxins and other enzymes. It was thought that the purified or partially purified fractions containing toxins could be used for hyperimmunization of horses to produce a high titre avid antiserum and the other fractions might be valuable as laboratory reagents and/or therapeutic agents.

The preliminary fractionation was carried out by pevikon block electrophoresis (PVC-E). In Krait venom, three toxic fractions were obtained while in Cobra venom, only one toxic fraction was obtained. It was found that in Cobra venom, non-toxic fraction contained the maximum proteolytic activity, while the toxic fraction had very little proteolytic activity. In Krait venom, out of the three toxic fractions obtained, the one with the maximum toxic activity did not contain any proteolytic activity. However, the remaining two toxic fractions were proteolytic. The results of enzyme analysis indicated that there are at least two types of proteases in the venom.

The fractionation of Krait venom was then carried out on Sephadex G-100. It was observed that some fractions obtained also contained phospholipase A (PhA) activity. This PhA was found to be heat-stable. Attempts to separate PhA from the toxin by repeated subjection of toxic fractions, obtained by PVC-E and Sephadex gel filtration, to a column of Sephadex G-50 were not successful. The molecular weights of PhA and toxin in the toxin fraction were then determined by polyacrylamide gel electrophoresis (PLA-E) taking pure standard proteins in the range of 6,000 (insulin) and 68,000 (albumin). Phospholipase A was found to have a molecular weight of 30,000 and the toxin was found to have a molecular weight between 6,000 and 7,000. PhA was then successfully separated from toxin by perchloric acid treatment. The toxin obtained by this method was found to be homogeneous by PLAE and immunoelectrophoresis (IEP) techniques against polyvalent horse antivenin serum of Haffkine Institute. The amino acid composition of this purified toxin is being determined.

**Fractionation of Russell's Viper and *Echis carinata* venoms**

The preliminary studies on the toxic and enzyme activities of the crude *Echis carinata* venom are in progress.

**Toxoidation of Cobra Venom**

Experiments were undertaken to obtain a toxoid of Cobra venom for use in the active immunization of man as also for the production of antivenin in horses.

Preliminary results showed that the toxic activity decreases considerably when venom is irradiated with Cobalt 60. The testing of the antigenicity of this irradiated venom in laboratory animals is in progress.

**Preparation of antivenin against Scorpion venom**

The possibilities of preparing an antiserum against the black Scorpion venom are being explored. In the preliminary experiments, precipitating type of

antibodies were detected in the serum of rabbits immunized with black scorpion venom by gel diffusion test. The neutralizing activity of this antiserum is being tested.

#### Studies on the improvement of antitoxin titres in hyperimmune horses

The antibody titres obtained on hyperimmunization of horses with bacterial toxins or venoms for the commercial production of antisera in the Department of Antitoxins and Sera were reported to be unsatisfactory. Hence, studies were undertaken (i) to make a systematic analysis of the horse titres obtained in that department, (ii) to assess the correlation between the antibody titres and the immunization schedule, (iii) to study the antigen used and (iv) to watch the health of the animals etc., with a view to devise suitable means for enhancing the titres in the case of the low titre horses and maintaining the titres in the case of the high-titre horses. Studies were, therefore planned accordingly. The results of preliminary analysis of the data on antitoxin titres in the hyperimmune horse antisera are given below :—

#### *Classification of horses and other details*

| Total No. of horses analysed | Type of horses    | Titre range         | Antigen used                       | Approx. Percentage response |
|------------------------------|-------------------|---------------------|------------------------------------|-----------------------------|
| 142                          | Low Responders    | 300—500 IU/ml       | Diphtheria Tetanus Toxoid or venom | 75%                         |
|                              | Medium responders | 501—800 IU/ml       |                                    | 16%                         |
|                              | High responders   | 801 IU/ml and above |                                    | 10%                         |

Graphs of antibody titres *versus* the courses of immunization were plotted in order to compare the pattern of antibody response in the three classified groups. However, no significant difference was noted in all the three groups, except some variation in the pattern of response among the high responders, i.e. the optimum antibody titre was reached in a period of 5-7 months, followed by a steep decline, while in others the optimum antibody titres were reached after 17 months, but after a small decline, they were maintained fairly well for another 17 months. Further study to substantiate the data and correlate it to the schedule of immunization is in progress.

Studies are also in progress to determine the influence of sex and age on the antibody titre. It was also proposed (i) to determine the protein content (ii) to analyse the electrophoretic patterns, (iii) to study the distribution of antibodies against the different components in the toxin or toxoid samples used for immunization of horses (intramolecular competition) and (iv) to study the distribution of antibodies against the antigenic determinants of toxin molecule using heated or enzyme-digested toxin (intramolecular competition) in the high and low-titre horse antisera. The work of collection of materials, preparation of reagents and standardization of techniques for these studies is in progress.

## DEPARTMENT OF PHARMACOLOGY

The main function of the department is to study indigenous drugs with a view to assess their usefulness in modern medical practice. Working with the assistance of the Central Council for Research in India Medicine and Homoeopathy and in collaboration with Dr. N.V. Subbarao of Osmania University, the department has demonstrated the usefulness of *Tylophora indica* and an oxidized product of the vasicine isolated from *Vasaka* in the treatment of bronchial asthma in animal experiments.

The department also undertakes screening of compounds synthesized by the Chemotherapy Department.

During the year under review, methods were standardised for screening drugs against leprosy, filariasis and intestinal parasites. Methods were also standardised to find out the possible anticonvulsant and anti-inflammatory activities of certain drugs.

### Study of new Anthelmintics

Few of the series of new organic compounds, synthesized in the Chemotherapy Department of this Institute, have been shown to possess anthelmintic activity. *In vitro* experiments carried out on round worms (*Ascaris lumbricoides*) obtained from the intestines of dead bodies at post-mortem, provided the evidence of this activity. These compounds were shown to inhibit contractile responses induced by acetylcholine of the isolated muscle strip preparations obtained from female worms. Out of the 20 compounds studied, 14 showed this activity to a variable extent. Compounds bearing code numbers R-3366, R-3365 and R-3333 have been shown to possess promising anthelmintic activity at as low a dose level as 0.1 to 0.5 µg/ml.

### Nematosporides dubius infection in mice

Mice weighing between 18 and 20 gms. were infected with 100 infective larvae. After 14 days, the drug was administered orally in 0.1 mg. and 0.5 mg. doses respectively, per mouse. The animals were sacrificed five days after the treatment and the number of worms not expelled were counted. The percentage of worms expelled was compared with the control (see Table 1).

### Hymenolepis nana infection in mice

Parasitic infection of *H. nana* was produced in mice by giving 1,000 ova/0.2 ml. orally. Seventeen days after the infection, the mice were tested for infectivity. The infected mice were then treated with standard drugs, such as Piperazine phosphate, Tetramisol and Thiabendazole. The worm counts in the control mice as well as in the treated mice were taken as the parameters to find out the effectiveness of each of the drugs used (see Table 2.)

### Studies in anti-leprosy drugs

The Indian Council of Medical Research continued to support this scheme. After standardisation of Shepard's mouse foot-pad method, the department undertook screening of new compounds and presented a paper at the VII Convention of Allergy and Applied Immunology held at Bombay. It was shown that Thalidomide prevented the release of histamine due to the interaction of human leprosy serum and the antigen from ICRC bacilli. The department

also undertook immunological studies to find out the interaction between the human leprosy serum and the antigen from ICRC bacilli from the diagnostic point of view.

### Indigenous Medicinal Plants

The beneficial effects of two indigenous medicinal plants, namely *Adhatoda vasaka* and *Tylophora indica* in the treatment of Asthma and other respiratory diseases were substantiated by means of experimental data in laboratory models. The beneficial effects of the plant *Adhatoda vasaka* are attributable to vasicinone (an oxidised form of Vasicinone)—an alkaloid obtained from the leaves of this plant. Vasicinone is shown to possess marked bronchodilating activity, both *in vitro* (isolated tracheal chain preparation method) and *in vivo* (Konzett and Rossler's method) in experimental models. It also potentiated the bronchodilating activity of Isoprenaline. In these models Vasicinone was found to be as potent as Theophylline. At effective bronchodilating doses, Vasicinone exhibited weak cardiac stimulant activity. Vasicinone was also shown to inhibit activity towards the histamine release by peritoneal mast cells of rats subsequent to interaction between IgE type antibodies and antigen.

The chloroform extract of plant *Tylophora indica* and the alkaloidal fraction isolated from this plant were shown to possess bronchodilating activity. The alkaloid fraction was found to possess more potent bronchodilating activity. Both the alkaloidal fraction and chloroform extracts showed weak cardiac stimulant activity. The chloroform extract was also shown to possess inhibitory activity towards the anaphylactic release of histamine from peritoneal mast cells of rats. The results of these studies are under publication.

TABLE 1  
Screening of anti-helminthic drugs against *N. dubius*

| Serial No. | No. of mice used | Drug No.               | Drug Dose                     | Percentage efficacy |
|------------|------------------|------------------------|-------------------------------|---------------------|
| 1          | 5                | Standard (Tetramisole) | 0.1 mg./mouse<br>(5 mg./kg.)  | 10 per cent         |
|            |                  |                        | 0.5 mg./mouse<br>(25 mg./kg.) | 100 per cent        |
| 2          | 5                | 3452                   | 0.1 mg./mouse                 | 14 per cent         |
|            |                  |                        | .5 mg./mouse                  | 26 per cent         |
| 3          | 5                | 3453                   | 1 mg./mouse                   | 16 per cent         |
|            |                  |                        | 5 mg./mouse                   | 82 per cent         |
| 4          | 5                | 3454                   | 0.1 mg./mouse                 | 22 per cent         |
|            |                  |                        | 0.5 mg./mouse                 | 94 per cent         |
| 5          | 5                | 3455                   | 0.1 mg./mouse                 | 2 per cent          |
|            |                  |                        | 0.5 mg./mouse                 | 4 per cent          |

TABLE 2  
Screening of anti-helminthic drugs against *H. nana*

| Serial No. | No. of mice used | Drug                 | Dosage mg./mouse | Percentage efficacy |
|------------|------------------|----------------------|------------------|---------------------|
| 1          | 5                | Piperazine Phosphate | 150 mg.          | 95 per cent.        |
|            |                  |                      | 200 mg.          | 96 per cent.        |
| 2          | 5                | Tetramisole          | 1 mg.            | 66.6 per cent.      |
| 3          | 5                | Thiabendazole        | 50 mg.           | 97.5 per cent.      |

## QUALITY CONTROL DEPARTMENT

### Stability of Vitamins in light

Work continued on this problem and the results indicate that riboflavin, folic acid and to a certain extent, pyridoxine hydrochloride, were destroyed by light, while thiamine hydrochloride, ascorbic acid, niacin, niacinamide and calcium pantothenate were stable. Ultraviolet (UV), violet (V), blue (B) and green (G) regions of light could destroy riboflavin, but yellow (Y) orange (O) and red (R) could not do so. Folic acid can be destroyed by UV-V-B region while, under alkaline condition, pyridoxine hydrochloride could be destroyed by UV light only.

Riboflavin can be stabilized by benzyl alcohol, phenol and, to some extent by BHA (Butylated hydroxy anisole) and BHT (Butylated hydroxy toluene), whereas folic acid becomes stable in the presence of BHA, BHT and ascorbic acid. BHA and BHT being anti-oxidants could reduce the rate of oxidation of folic acid but not of riboflavin.

On increasing the concentration of phenol, photo destruction of riboflavin got reduced, while changes in the benzyl alcohol concentration were ineffective. Alcohol promotes the destruction of riboflavin. Combination of riboflavin and folic acid could be stabilized to a maximum extent by ascorbic acid and benzyl alcohol together.

### Study on *Vibrio parahaemolyticus* in Bombay

Investigations were prosecuted on this problem during the period under review.

Four-hundred and fifty-nine rectal swabs of gastro-enteritis cases from Kasturba Hospital, Bombay and one hundred and seventy more stool samples of gastro-enteritis cases from Civil Hospital, Parbhani, were culturally studied, but *Vibrio parahaemolyticus* could not be isolated.

Two-hundred and fourteen pooled samples of animal excreta from Parbhani were processed and 11 pooled samples of faeces of dogs out of eighty-six were found to be positive for *V. parahaemolyticus* and all the rest were negative.

Twenty-five pooled samples of crabs and twenty-five pooled samples of shrimps from coastal region of Colaba, Cuffe Parade, Worli, Mahim and Thana creek were bacteriologically investigated and 9 strains of *V. parahaemolyticus* were detected in crabs and eight from samples of shrimps.

The strains isolated were studied for pathogenicity in mice, guineapigs, ligated loop of adult rabbits and in infant rabbits and were found to be pathogenic.

The strains were subjected to the test of Kanagawa phenomenon and were found to be positive.

The data amply proved that *V. parahaemolyticus* is very rampant in the coast of Bombay. The investigation is still in progress and further data could throw some light on outbreaks of gastro-enteritis of unknown etiology in Bombay.



### Studies on standardisation of cholera vaccine and allied investigations

Hyper-immune sera were produced in adult rabbits by giving series of intravenous injections of monovalent, bivalent and quadrivalent cholera vaccine, individually. They were separately tested for antibody and vibriocidal titre. The sera were studied further by passive protection test in infant rabbits. The animals were given hyper-immune serum at regular intervals prior to and after the intrainestinal challenge with virulent cultures in separate experiments with *V. cholerae* Inaba, Ogawa; (El tor Inaba and Ogawa, NAG vibrio and toxin to follow) to observe the pattern of protection. The work is incomplete to-date.

Adult rabbits immunised with the cholera vaccine were subjected to challenge by *V. cholerae* making use of ligated loop technique. The study is afoot and different aspects of the problem are being investigated to evolve a method for standardisation of cholera vaccine.

### Studies on biological standardisation of antivenine

This investigation is being carried out with a view to evolve a method for biological standardisation of multivalent antivenine. Individual determinations of mouse LD<sub>50</sub> dose of the venoms (Cobra, Russel's viper, Krait and Echis) is being carried out. Capacity of 1 ml. reconstituted multivalent antivenine (Haffkine Institute) to neutralise a fixed number of mouse LD<sub>50</sub> of the venoms individually, is being estimated.

LD<sub>50</sub> was determined by using 5 mice (18-20 gms) per dilution, injected intravenously. The animals were observed for 48 hours. Reed and Muench formula was used for calculation of LD<sub>50</sub>.

Individually pooled venom samples of Cobra, Russel's viper, Krait and Echis were collected, representing collections during different seasons of the year for several years, different parts of the country, different ages of the snakes, etc.

Similarly, multivalent antivenine (250 ml. from each sub-batch of antivenine) is being collected from several batches to get a substantive stock representative of different batches. Each sub-batch is being tested individually by the C.L.D. technique and also against fixed LD<sub>50</sub> of each pooled venom. Elaborate study will be carried out and then all the antivenine collected and tested will be pooled and re-tested, which is proposed to be ultimately used as a National Standard of antivenine. The work, which is in initial stages, is in progress.

### Dynamics of disinfection

The study covers the behaviour of two disinfectants—Formaldehyde and Phenyl Mercuric Nitrate at different pH levels (5.5, 7.5 and 9.0) and temperature levels (4°C; room temperature and 37°C) on *Staphylococcus aureus*, *Shigella dysenteriae* Flexner and *V. cholerae* Inaba and Ogawa.

At present, experiments are being conducted to find out the optimum quantity of the antagonist—Sodium Metabisulphite for formalin and Sodium Thio-glycolate for P.M.N.—required for the neutralisation, so as to get the maximum revival of the organisms. Standardisation of the techniques is being carried out. The work is in progress.

## **Studies of Immunoglobulins with special reference to antibody patterns in pooled r-globulin and purified monospecific immunoglobulins**

These studies will unravel the efficacy of the purified monospecific immunoglobulins against bacterial and viral diseases. Standardisation of Tetanus and Diphtheria Toxin-antitoxin system by counter and immunoelectrophoresis has been done. Two fractions in tetanus and 3 fractions in diphtheria have been detected. Immunoglobulins like IgG, IgA and IgM have been separated from pooled Globulin by Sephadex G-200 and DEAE cellulose column chromatography. These immunoglobulins are being tested for bacterial and viral antibody titres.

Standardisation of antibody titres in small-pox (for vaccinia and variola type virus) has been carried out by HAGI (Haemagglutination Inhibition Test). It is found out that HAI titres with and without heat treatment procedure are the same. But results are to be confirmed by further testing of separated immunoglobulins. In one batch of separated immunoglobulins ('G' type), the HAI titre was found to be less than 1:10 for vaccinia and variola.

## **A comparative biochemical and immunochemical study of antibodies against various antigens**

(a) Antibody response to :—

(1) Type of antigen, (2) Route of injection, (3) Animal species, (4) Weight of the animal.

During this period, rabbits of average weight (2.5 kgs.) were injected subcutaneously with the following dilutions of diphtheria toxoid, viz. 1:4, 1:8, 1:16 (doses given were 1 ml./kg. of body weight). Antitoxin contents were determined by bioassay. The maximum titre of 11.0 I.U./ml. was found with 1:4 dilution of the toxoid. It is known that alum-precipitated toxoid gives higher titre by intravenous route so, 1:4 and 1:8 dilutions of the toxoid were injected intravenously into rabbits. Antitoxin content was found to be less than 2.0 I.U./ml. in the same way, guinea-pigs were immunised with diphtheria toxoid subcutaneously. The maximum titre of 8.0 I.U./ml. was found with 1:16 dilution of the toxoid. Work is in progress with rats.

(b) *Isolation and characterisation of IgG.*—For isolation of IgG from immune serum, Reif's method is being used. Isolation of IgG from antidiphtheria rabbit serum and anti-diphtheria guinea-pig serum was carried out. Protein concentration of serum as well as IgG were determined. Carbohydrate content of IgG was also determined.

On the same lines, work is in progress with other animal species and antigens.

## **Synthesis of anthelmintic agents**

Literature reveals that benzimidazole derivatives, particularly thiobendazole and farbendazole, have anthelmintic activity. Piperazine citrate is an established anthelmintic agent. Realizing the importance of benzimidazole moiety and piperazine moiety, work was undertaken to synthesize the compounds which involve both the benzimidazole and piperazine moieties. Thus, 25 different compounds were prepared so far and further work is in progress. The anthelmintic activity of the synthesized derivatives is being tested.

### **Studies on Indian snakes, their external and internal parasites**

Poisonous and non-poisonous snakes are being screened for their internal and external parasites. So far, Cobra (*Naja naja*), Viper (*Vipera russelli*), Krait (*Bungarus caeruleus*) poisonous snakes and water snakes (*Natrix piscator*) a green whip snake (*Dryophis pasutus*) and non-poisonous snakes, have been dissected for detection and collection of internal parasites. Parasites have been recovered from the gut region and the tracheal tubes of the snakes. They have been preserved for identification and for further work. Ticks were collected from Cobra and transplanted on the water snake (*non-poisonous*) which have no external parasites like ticks, as they live in water. This water snake has been under observation.

Further work will cover study on parasites, parasitism in snakes and anti-parasitic treatment in snakes.

## RADIATION BIOLOGY UNIT

The recently installed Cobalt-60 Gamma Chamber has been calibrated for its dose rate and irradiation of Cobra venom is in progress in relation to the production of venom toxoid. Irradiation of *V. cholerae* cultures is also in progress in order to study the possible effects of toxicity and immunogenicity in relation to the production of the vaccine.

Four-hundred and sixty samples of sera received from various groups were tested for the presence of Australia antigen by radio-immuno-assay. This was done in collaboration with Immunohaematology Department. The results showed prevalence of hepatitis associated antigen in 27 out of 100 cases of infective hepatitis, 12 out of 100 cases of professional blood donors and 6 out of 114 cases of Blood Bank and kidney dialysis workers. These results conclude that radio-immuno-assay is the most sensitive method for detection of Australia antigen. The high incidence of positive samples of acute infective hepatitis cases, collected from Kasturba Hospital for Infectious Diseases, reveals the continued prevalence of type B virus in the population and gives credence to the suggestion about spread of this virus by the faeco-oral route.

Clinical trial of cholera vaccine prepared at the Haffkine Institute and at the Central Research Institute, Kasauli, was initiated in volunteers at the Haffkine Institute and the Primary Health Centre, Virar (Thana District). The dose schedule in each group was different. The study was carried out in association with the Department of Bacteriology and the results were presented at the International Seminar on Cholera held at this Institute as a part of the Platinum Jubilee celebrations programme.

A study of immune response in mal-nourished individuals was initiated in a group of population of Virar (Thana District). Injectable cholera vaccine and tetanus toxoid were used as the antigens. Serial periodic samples were collected from 90 mal-nourished individuals before and after injection and 50 normal persons served as controls. The parameters described in the W.H.O. protocols were used, which consisted of anthropological measurements besides other clinical, biochemical and immunological tests. The results are being critically evaluated.

Blood samples from Adiwasī Tribes from Jawhar (Thana District) were collected for despatch to Prof. Kirk at Canberra, Australia, for collaborative research work on enzyme-deficient genotypes.

A study on the effect of irradiation on Cobra venom in various doses was initiated with special reference to the production of Antivenin. This is still in progress.

A study on the effect of protein mal-nutrition stress on adrenal function has been undertaken as a research problem. This study was carried out in male rats. The animals were experimentally made protein deficient in a period of 4 weeks. Biochemical parameters, such as serum proteins, urinary creatinine, urinary 17-ketosteroid excretion and radio-immuno-assay of plasma corticosterone by competitive protein-binding technique, were studied. All these methods were well-standardized. Histo-pathological study of tissues, such as liver and adrenals, was also carried out. The results of the work done so far,

are presented in Tables Nos. 1 and 2. It is evident from these results that the reduction in the body weight among protein deficient rats after 4 weeks was significant as compared to the initial body weight of these rats, also with that of the normal rats, which showed a marked increase in body weight after 4 weeks. The absolute weight values of the adrenal and liver indicated significant difference between the two groups and the same was true of the total serum proteins as well as the serum albumin, which indicated a marked reduction in the mal-nourished animals. The twenty-four hour urinary 17-ketosteroid excretion and the creatinine excretion revealed no significant change between the two groups. However, the plasma corticosterone level showed an increase in the protein-deficient group, which was statistically significant. ( $P < 0.02$ ).

Further studies on the stress of mal-nutrition and other types of stress ; phenomena in relation to psychomotor behaviour of the animals and the adrenal function are being continued.

TABLE 1

*Showing average values for body weight, organ weights and serum proteins, in normal and protein-deficient rats after four weeks*

| Group             | No. of obs. | Body weight  |                    | Organ weights |             | Serum proteins |                |
|-------------------|-------------|--------------|--------------------|---------------|-------------|----------------|----------------|
|                   |             | Initial gms. | After 4 weeks gms. | Adrenal mgs.  | Liver gms.  | Total gms. %   | Albumin gms. % |
| Normal            | 12          | 88.20        |                    |               |             |                |                |
|                   |             | $\pm 1.99$   |                    |               |             |                |                |
|                   | 10          |              | 139.60             | 19.80         | 4.330       | 8.21           | 2.86           |
|                   |             |              | $\pm 4.03$         | $\pm 0.75$    | $\pm 0.174$ | $\pm 0.18$     | $\pm 0.17$     |
| Protein-deficient | 13          | 90.40        |                    |               |             |                |                |
|                   |             | $\pm 1.68$   |                    |               |             |                |                |
|                   | 12          |              | 77.25              | 15.50         | 3.262       | 6.10           | 1.60           |
|                   |             |              | $\pm 1.47$         | $\pm 0.245$   | $\pm 0.150$ | $\pm 0.28$     | $\pm 0.198$    |

TABLE 2

*Showing average values for 24 hour urinary creatinine excretion, 24 hour urinary 17 ketosteroid excretion and plasma corticosterone in normal and protein-deficient rats after four weeks*

| Group             | No. of obs. | Urinary creatinine excretion gms./1 ltr. | Urinary 17-ketosteroid excretion mgs./24 hrs. | Plasma corticosterone ugs./100 ml. |
|-------------------|-------------|------------------------------------------|-----------------------------------------------|------------------------------------|
| Normal            | 6           | 0.213                                    | 0.0108                                        |                                    |
|                   |             | $\pm 0.0176$                             | $\pm 0.0027$                                  |                                    |
|                   | 10          |                                          |                                               | 3.402                              |
|                   |             |                                          |                                               | $\pm 0.413$                        |
| Protein-deficient | 6           | 0.258                                    | 0.0188                                        |                                    |
|                   |             | $\pm 0.0216$                             | $\pm 0.0032$                                  |                                    |
|                   | 12          |                                          |                                               | 5.030                              |
|                   |             |                                          |                                               | $\pm 0.463$                        |

## DEPARTMENT OF VIRAL VACCINES

**Poliomyelitis surveillance**

During January-December 1973, seven hundred and twenty-seven cases of paralytic poliomyelitis were recorded in Greater Bombay. The corresponding figure for the year 1972 was 1,338. It is a consistent observation that the number of paralytic poliomyelitis cases occurring during the post-epidemic year are usually 50 per cent of those during the epidemic year. This may be explained on the basis that the heavy dissemination of wild paralytogenic virus during an epidemic year leaves fewer susceptibles and consequently, during the following year, the occurrence of paralytic poliomyelitis is low. The lower incidence during the post-epidemic year results in lower dissemination of the virus and consequently results in the increase in the number of susceptibles. The periodicity of epidemics of paralytic poliomyelitis is explained on this basis.

The progressive rise in the incidence of paralytic poliomyelitis can be clearly seen from the following figures for the last five epidemic years :

| Year |    |    |    |     | No. of cases |
|------|----|----|----|-----|--------------|
| 1960 | .. | .. | .. | ..  | 514          |
| 1964 | .. | .. | .. | ... | 723          |
| 1966 | .. | .. | .. | ..  | 820          |
| 1968 | .. | .. | .. | ..  | 1,110        |
| 1972 | .. | .. | .. | ..  | 1,338        |

In spite of the progressive increase in the incidence of paralytic poliomyelitis in Greater Bombay, the age pattern and seasonal distribution have remained constant. Approximately 94 per cent of the cases are observed in the age group of 3 months to 3 years. Less than one per cent of the cases are recorded in the age group below 3 months. This is explained on the basis of the high level of maternal immunity in the first three months of life. Age distribution of cases of paralytic poliomyelitis during 1973 is shown in Table 1.

The seasonal distribution of cases in 1973 is shown in Table 2. The heavy incidence of paralytic polio-myelitis during the late summer and the monsoon months is a characteristic pattern. As many as 50 per cent of the total cases are recorded during the four months period of June-September.

**Status of residual paralysis**

In paralytic poliomyelitis, an accurate parameter for judging the extent of morbidity is the residual paralysis. For this purpose, cases are followed up for a period of 60 days from the date of onset of paralysis. Out of a total of 727 cases recorded during 1973, 478 cases could be followed up. The details of this study are as follows :—

|                                           |       |                           |
|-------------------------------------------|-------|---------------------------|
| Complete recovery (No residual paralysis) | ..    | 105 cases (22 per cent)   |
| Partial recovery                          | .. .. | 309 cases (64·5 per cent) |
| No recovery                               | .. .. | 64 cases (13·4 per cent)  |

### Paralytic illness in vaccinated individuals

Of the 727 cases recorded during 1973, there were 106 cases i.e. 14.6 per cent approximately, in which there was a history of administration of Oral Polio Vaccine in the past. Thirty-two cases had received only one dose of vaccine and in 23 cases there was history of two doses of vaccine. These cases are considered as inadequately immunized. All the three doses were administered in 51 cases. Paralytic illness in these 51 cases is considered as failure of immunisation. The age distribution and seasonal incidence of these cases did not show any difference when compared with case in which oral polio vaccine was not administered.

### Virological studies in paralytic poliomyelitis

(a) *Non-vaccinated group.*—To keep a watch on the pattern of polio viruses, approximately 10 per cent of the total cases (not vaccinated) were studied virologically. Stool samples and/or rectal swabs were collected for isolation and identification of the virus. During 1973, there were 621 cases of paralytic poliomyelitis with no history of polio immunization. Of these, 90 cases were studied virologically. The selection of these cases is based on weekly incidence throughout the year. For virus isolation, tissue cultures as well as infant mice were used. Of the 90 cases, 63 were found to be excreting a virus. Details of these virological studies are presented in Table 3. It would be seen from this table that all the three types of polio viruses are equally distributed.

The distribution of the three types of polio viruses shows varying patterns during epidemic and non-epidemic years. While, in an epidemic year, there is a gross predominance of type 1 poliovirus, during a non-epidemic year, all the three types of polio viruses are found to be responsible for the disease. This will be clearly seen from the data for two epidemic years (1960 and 1972) and three non-epidemic years (1961, 1963 and 1973) presented below :—

| Year  | Poliovirus isolated |        |        |
|-------|---------------------|--------|--------|
|       | Type 1              | Type 2 | Type 3 |
| 1960* | 173                 | 10     | 5      |
| 1972* | 39                  | 8      | 4      |
| 1961  | 20                  | 35     | 10     |
| 1963  | 87                  | 55     | 23     |
| 1973  | 15                  | 13     | 11     |

\*Epidemic year.

(b) *Vaccinated group.*—In contrast to the 10 per cent cases studied in the non-vaccinated group, efforts were made to study every case of paralytic illness occurring in an individual who had been administered oral polio vaccine in the past. During 1973, a total of 106 cases of paralytic illness amongst vaccinated individuals were recorded. Of these, 104 cases were studied virologically. The details of these studies are presented in Table 4.

From both tables 3 and 4, it will be seen that in a significant number of cases of paralytic illness, which are clinically labelled as paralytic poliomyelitis, one fails to demonstrate excretion of poliovirus and instead a non-polio virus is detected. The etiological relationship of these non-polio viruses to paralytic illness needs to be elucidated.

### Studies on stability of Oral Polio Vaccine

Oral Polio Vaccine contains the three types of attenuated polio viruses in live form. These vaccine viruses are thermolabile. Vaccines under field conditions are exposed to unfavourably high temperatures and can result in drop in the potency. This is particularly true in the summer months in India. It is now established that certain divalent and trivalent cations impart thermostability of a high order to the vaccine viruses in the oral polio vaccine. Concentration of one molar magnesium chloride under acidic pH (6.4 to 6.8) seems to give the highest thermostability.

Attenuated poliovirus type 1 (Strain LSc, 2ab) was grown in monkey kidney tissue culture bottles. The harvested virus was centrifuged to remove all cell debris and the clear virus suspension was mixed with equal quantity of 2 molar magnesium chloride solution. Virus suspension without magnesium chloride served as control. Aliquots in vials were prepared and stored at three different temperatures, viz. incubator temperature—37°C, room temperature 28°—31°C, and refrigerator temperature 8°—10°C. At an interval of 7 days, aliquots were tested for potency. The details of this study are presented in Table 5.

It will be seen from Table 5 that unstabilised virus shows significant drop in potency within 7 days if stored at 37°C. When stored at room temperature (28°—31°C), it takes about two weeks for the corresponding drop in potency. The stabilised virus requires approximately four weeks to show the corresponding drop in potency at 37°C and 28°C. Unstabilised virus, stored at refrigerator temperature, maintains the potency for about 11 weeks, whereas magnesium chloride stabilised virus maintains the potency for as long as 162 days (5, 1/2 months) when stored at refrigerator temperature. Vials of stabilised and unstabilised vaccine virus were also subjected to high temperature (50°C) for one hour and the effect on potency studied. Stabilised virus did not show any drop in the potency when subjected to 50°C. for one hour. Unstabilised virus, under similar conditions, showed a drop of almost 5 logs (Loss in potency of 99·999 per cent).

### Transport of Oral Polio Vaccine

Oral polio vaccine needs refrigeration even during transport. Experiments were designed to find out the adequacy of the containers used during transport, the type and quantity of refrigerant to be used and the duration for which proper temperature is maintained.

Results of these experiments show that when the thermocole containers, containing 1·5 to 1·8 kgs of wet ice, are stored at incubator temperature (37°C) for a period of 24 hours, the temperature of the vaccine at the end of this storage period is found to be between 3·5°C. to 5·5°C. Similar containers containing 1·5 to 2 kgs. of dry ice (solid carbon dioxide) maintain the vaccine in frozen state for a period of 24 hours.

### Efficacy of Oral Polio Vaccine

Efficacy of oral polio vaccine, as judged by sero conversion rates, is of high order in countries with temperate climate. This is not always the case in countries with warm climate. Various reasons have been advocated



poor seroconversion rates, viz. use of a vaccine with poor potency, interference by other enteroviruses, adverse effects due to breast feeding, high levels of pre-existing anti-bodies, inadequate time interval between two doses, etc.

Taking into consideration all the above factors, the following study was undertaken to assess the efficacy of oral polio vaccine:—

Pre-tested trivalent oral polio vaccine was used. Vials used for administration of the vaccine were brought back to the laboratories and unused vaccine was re-tested for potency.

Mothers were instructed not to give breast feed two hours before the administration of vaccine and during two hours following feeding of the vaccine. (Since sub-clinical infection with polioviruses and consequently the appearance of antibodies starts at a very early age in India, infants between the age group of 3 to 6 months were selected for this study. This age group was also selected on the presumption that incidence of other enterovirus infections would be low.

Interval between two doses of the vaccine was kept at six weeks.

One-hundred and twenty-two infants were recruited for this study. To determine the pre-vaccination immunological status and intestinal virus carriage, blood and stool samples and/or rectal swabs were collected before the administration of the first dose of the vaccine. Seven days after administration of each dose of the vaccine, stool and/or rectal swabs were collected from each vaccinee. Two weeks after the third dose of the vaccine, a second blood sample was collected.

For virological studies, samples were inoculated in monkey kidney tissue cultures as well as in infant mice. For serological studies, conventional neutralization test in tissue culture was used.

This study is still in progress, but on the basis of results available so far, the following comments can be made.

Of the 122 infants recruited in this study, faecal samples were obtained from 120 infants before the administration of the first dose. Of these, as many as 69 (57.5 per cent) vaccinees were found to be excreting a virus. Twelve vaccinees (10 per cent) were found to excrete poliovirus.

An attempt was made to correlate between presence of an intestinal virus before administration of the oral polio vaccine and excretion of polioviruses after the feeding of the vaccine. The results are briefly summarised below :—

| Pre-vaccination |    | Polio virus | Post-vaccination excretion |           |          |
|-----------------|----|-------------|----------------------------|-----------|----------|
|                 |    |             | Polio<br>+Non-polio        | Non-Polio | No virus |
| Virus + 69      | .. | 18          | 23                         | 22        | 4        |
| Virus — 51      | .. | 16          | 22                         | 8         | 3        |

The details of virus excretion after administration of the first, second and third dose of the vaccine are presented in Table 6.

#### Serological studies on vaccinees

So far, 70 pairs of blood samples have been studied. In the first analysis, the results have been grouped as those who did not have detectable antibodies

against all the three types of poliovirus before administration of the vaccine and in the second analysis, the results have been shown according to the type-wise level of pre-vaccination antibodies.

Out of 70 vaccinees studied so far, there were 26 infants who did not have any detectable antibodies against all the three types of poliovirus. After administration of three doses of oral polio vaccine, their response was as follows :—

Total triple negative—26.

Triple negative to triple positive—11 (42 per cent) (antibody response to all the three types).

Triple negative to double positive—4 (15.5 per cent) (antibody response to two types).

Triple negative to single positive—7 (27 per cent) (antibody response to a single type).

Triple negative to triple negative—4 (15.5 per cent).

The type-wise response amongst these 26 triple negative infants was as follows :—

Type 1—17/26 (65.4 per cent).

Type 2—18/26 (69.4 per cent).

Type 3—13/26 (50 per cent).

The type-wise and pre-vaccination titre-wise analysis of all the 70 vaccinees studied so far is presented in Table 7.

### Study of anti-polioviral activity in human milk and faeces

Failure to get adequate seroconversion, following administration of live oral polio vaccine, is ascribed to various factors. One of these is considered to be breast feeding. In India, almost all adults have fairly high level of antibodies against all the three types of poliovirus. It is known that the circulating antibodies may find their way in breast milk and potentially interfere with the effective multiplication of vaccine viruses in breast-fed infants. To assess the magnitude of this problem, samples of breast milk from lactating mothers were collected and tested for anti-polio activity. Blood samples were also collected simultaneously for comparing the anti-polio activity in breast milk with that in the serum.

For the above study, 150 pairs of blood and breast milk samples from lactating mothers have been collected so far. The lactation period ranged from 1 day to 18 months. Results of preliminary studies on few pairs are presented in Table 8. Further studies are in progress.

Breast milk antibodies, which are of the IgA type, survive passage through the gut and could be excreted in the faeces. Encretion of antibodies is also known to occur after clinical and sub-clinical infection with poliovirus. Presence of these antibodies could neutralise the virus resulting in failure to isolate the virus. To determine the extent of excretion of such copro-antibodies, faecal samples have been collected from (a) clinical cases of paralytic poliomyelitis (b) children vaccinated with oral polio vaccine and (c) apparently healthy non-vaccinated children. Three hundred and twenty faecal samples have been tested. Twenty-one per cent of the samples showed presence of copro-antibodies. Details of these are presented in Table 9.

TABLE 1

*Incidence of Paralytic Poliomyelitis in Greater Bombay (1973)*

## Age distribution

| Age group   |    |    |    | Cases | Percentage | Cummulative percentage |
|-------------|----|----|----|-------|------------|------------------------|
| 0-3 months  | .. | .. | .. | 6     | 0·83       | 0·83                   |
| 4-6 months  | .. | .. | .. | 35    | 4·8        | 5·6                    |
| 7-12 months | .. | .. | .. | 241   | 33·1       | 38·7                   |
| 1-2 years   | .. | .. | .. | 311   | 42·7       | 81·4                   |
| 2-3 years   | .. | .. | .. | 92    | 12·6       | 94·0                   |
| 3-4 years   | .. | .. | .. | 18    | 2·4        | 96·4                   |
| 4-5 years   | .. | .. | .. | 11    | 1·5        | 97·9                   |
| 5-6 years   | .. | .. | .. | 8     | 1·0        | 98·9                   |
| 6-14 years  | .. | .. | .. | 5     | 0·6        | 99·5                   |
| Total       |    |    |    | 727   | 99·5       |                        |

TABLE 2

*Seasonal distribution of cases of paralytic poliomyelitis in Greater Bombay (1973)*

| Month     |    |    |    | Cases | Percentage | Cummulative percentage |
|-----------|----|----|----|-------|------------|------------------------|
| January   | .. | .. | .. | 26    | 3·5        | 3·5                    |
| February  | .. | .. | .. | 30    | 4·1        | 7·6                    |
| March     | .. | .. | .. | 35    | 4·8        | 12·4                   |
| April     | .. | .. | .. | 29    | 3·9        | 16·3                   |
| May       | .. | .. | .. | 40    | 5·4        | 21·7                   |
| June      | .. | .. | .. | 70    | 9·5        | 31·2                   |
| July      | .. | .. | .. | 104   | 14·3       | 45·5                   |
| August    | .. | .. | .. | 101   | 13·9       | 59·4                   |
| September | .. | .. | .. | 83    | 11·4       | 70·8                   |
| October   | .. | .. | .. | 84    | 11·5       | 82·3                   |
| November  | .. | .. | .. | 64    | 8·8        | 91·1                   |
| December  | .. | .. | .. | 61    | 8·4        | 99·5                   |
| Total     |    |    |    | 727   | 99·5       |                        |

TABLE 3

*Virological studies of 90 cases of paralytic Poliomyelitis (1973) in non-vaccinated group*

| Month     |    | Total cases recorded | Cases studied | Virus + | Poliovirus |        |        | Echovirus |        | Coxsackievirus |    |    |    |
|-----------|----|----------------------|---------------|---------|------------|--------|--------|-----------|--------|----------------|----|----|----|
|           |    |                      |               |         | Type 1     | Type 2 | Type 3 | Type 7    | Type 8 | A4             | A9 | B5 | B6 |
| January   | .. | 21                   | 4             | 4       | 0          | 2      | 1      | 0         | 0      | 0              | 0  | 1  | 0  |
| February  | .. | 26                   | 4             | 4       | 2          | ..     | 2      | ..        | ..     | ..             | .. | .. | .. |
| March     | .. | 30                   | 3             | 1       | ..         | ..     | ..     | 1         | ..     | ..             | .. | .. | .. |
| April     | .. | 26                   | 4             | 3       | 1          | ..     | ..     | 2         | ..     | 1              | .. | .. | .. |
| May       | .. | 33                   | 8             | 4       | ..         | ..     | 1      | 1         | 1      | ..             | .. | .. | .. |
| June      | .. | 58                   | 8             | 7       | 1          | 4      | ..     | 1         | 1      | ..             | .. | .. | .. |
| July      | .. | 89                   | 13            | 10      | 1          | 2      | 1      | 7         | ..     | ..             | .. | .. | 1  |
| August    | .. | 84                   | 17            | 12      | 3          | 3      | 2      | 2         | 1      | ..             | .. | .. | .. |
| September | .. | 71                   | 12            | 6       | 3          | 1      | 2      | ..        | ..     | ..             | 1  | .. | .. |
| October   | .. | 71                   | 6             | 5       | 1          | 1      | 2      | 1         | ..     | ..             | .. | .. | .. |
| November  | .. | 56                   | 7             | 4       | 2          | ..     | ..     | 1         | ..     | ..             | .. | .. | .. |
| December  | .. | 56                   | 4             | 3       | 1          | ..     | ..     | 2         | ..     | ..             | .. | .. | .. |
| Total     | .. | 621                  | 90            | 73      | 15         | 13     | 11     | 18        | 3      | 1              | 1  | 1  | 1  |

TABLE 4

*Virological studies of 104 cases of paralytic poliomyelitis (1973) in vaccinated group*

| Month     |    | Cases recorded | Cases studied | Virus + | Poliovirus |    |    | Echo virus |    | Coxsackie virus |    |    |     |    |    |    |    |
|-----------|----|----------------|---------------|---------|------------|----|----|------------|----|-----------------|----|----|-----|----|----|----|----|
|           |    |                |               |         | 1          | 2  | 3  | 7          | 8  | A4              | A5 | A9 | A10 | B1 | B2 | B3 | B5 |
| January   | .. | 5              | 5             | 3       | 2          | .. | .. | ..         | .. | ..              | 1  | .. | ..  | .. | .. | .. | .. |
| February  | .. | 4              | 4             | 2       | 1          | .. | 2  | ..         | .. | ..              | .. | .. | ..  | .. | .. | .. | .. |
| March     | .. | 5              | 5             | 4       | 2          | .. | .. | 1          | .. | ..              | 1  | .. | 1   | .. | .. | .. | .. |
| April     | .. | 3              | 3             | 2       | ..         | 1  | 1  | ..         | .. | ..              | .. | .. | ..  | .. | .. | .. | .. |
| May       | .. | 7              | 7             | 6       | 1          | 2  | .. | 1          | 1  | 1               | .. | .. | 1   | .. | .. | .. | .. |
| June      | .. | 12             | 11            | 11      | ..         | 4  | 1  | 5          | 2  | ..              | .. | .. | ..  | .. | 3  | 1  | .. |
| July      | .. | 15             | 14            | 11      | 3          | 4  | .. | 1          | 3  | ..              | .. | .. | ..  | .. | .. | .. | .. |
| August    | .. | 17             | 17            | 14      | 7          | 2  | .. | 3          | 1  | ..              | .. | .. | 3   | 1  | .. | .. | .. |
| September | .. | 12             | 12            | 8       | 5          | 2  | 1  | ..         | .. | ..              | .. | 1  | ..  | .. | .. | .. | .. |
| October   | .. | 13             | 13            | 12      | 2          | 2  | 3  | 3          | .. | ..              | .. | .. | ..  | .. | .. | .. | 1  |
| November  | .. | 8              | 8             | 6       | 3          | 1  | 1  | ..         | .. | ..              | .. | .. | ..  | .. | .. | .. | 1  |
| December  | .. | 5              | 5             | 5       | 2          | .. | .. | 2          | .. | ..              | .. | .. | ..  | .. | .. | .. | .. |
| Total     | .. | 106            | 104           | 85      | 28         | 18 | 9  | 16         | 7  | 1               | 2  | 1  | 5   | 1  | 3  | 1  | 2  |

TABLE 5

*Potency of oral polio vaccine**Effect of molar magnesium chloride solution on attenuate type 1 poliovirus*

| Storage Temp.  | 37 C         |              | Room Temp.<br>(28-31 C) |            | Refrigerator<br>Temp. (8-10 C) |            |
|----------------|--------------|--------------|-------------------------|------------|--------------------------------|------------|
| Storage period | Unstabilised | Stabilised   | Unstabilised            | Stabilised | Unstabilised                   | Stabilised |
| 7 days         |              | 5.1*         | 5.8                     | 6          | 6.3                            | 6.4        |
| 14 days        | ..           | 2.9          | 5.2                     | 5.7        | 6.0                            | 6.1        |
| 21 days        | ..           | 2.3          | 4.3                     | 5.4        | 6.3                            | 6.3        |
| 28 days        | ..           | Discontinued | 4.7                     | 5.2        | 6.2                            | 6.4        |
| 35 days        |              |              | 4.0                     | 4.7        | 6.1                            | 6.2        |
| 42 days        |              |              | Discontinued            |            | 6.0                            | 6.0        |
| 49 days        | ..           |              |                         |            | 6.1                            | 6.1        |
| 77 days        | ..           |              |                         |            | 6.3                            | 6.3        |
| 86 days        | ..           |              |                         |            | 5.9                            | 6.3        |
| 93 days        | ..           |              |                         |            | 5.7                            | 6.1        |
| 98 days        | ..           |              |                         |            | 5.8                            | 6.1        |
| 117 days       | ..           |              |                         |            | 5.7                            | 6.1        |
| 129 days       | ..           |              |                         |            | 5.6                            | 6.2        |
| 141 days       | ..           |              |                         |            | 5.4                            | 6.1        |
| 148 days       | ..           |              |                         |            | 5.8                            | 6.3        |
| 155 days       | ..           |              |                         |            | 5.6                            | 6.0        |
| 162 days       | ..           |              |                         |            | 5.3                            | 6.0        |
| 169 days       | ..           |              |                         |            | 4.0                            | 5.2        |
| Discontinued.  |              |              |                         |            |                                |            |

\*Titres expressed as by TCID 50/ml.

TABLE 6

*Efficacy of Oral Polio Vaccine**Virus excretion studies*

|                  |    | Vaccinees<br>studied | Virus<br>+ | Virus<br>-ve | Poliovirus |        |        | To be<br>identified |
|------------------|----|----------------------|------------|--------------|------------|--------|--------|---------------------|
|                  |    |                      |            |              | Type 1     | Type 2 | Type 3 |                     |
| So               |    |                      |            |              |            |        |        |                     |
| (Pre-vaccination | .. | 120                  | 69         | 51           | 6          | 1      | 5      | 57                  |
| S1               |    |                      |            |              |            |        |        |                     |
| (After 1st dose) | .. | 114                  | 89         | 25           | 7          | 23     | 32     | 42                  |
| S2               |    |                      |            |              |            |        |        |                     |
| (After 2nd dose) | .. | 104                  | 77         | 27           | 8          | 10     | 12     | 49                  |
| S3               |    |                      |            |              |            |        |        |                     |
| (After 3rd dose) | .. | 98                   | 83         | 15           | 7          | 2      | 10     | 66                  |

TABLE 7  
*Efficacy of oral polio vaccine*  
(Serological studies)

|        |    |    | Pre<br>vaccine<br>titre | Number of<br>vaccinees | Post-vaccination<br>(after 3 doses of oral polio vaccine) |             |
|--------|----|----|-------------------------|------------------------|-----------------------------------------------------------|-------------|
|        |    |    |                         |                        | 4 × or more<br>response                                   | No response |
| Type 1 | .. | .. | 0                       | 34                     | 21                                                        | 13          |
|        |    |    | 8                       | 14                     | 9                                                         | 5           |
|        |    |    | 16                      | 8                      | 3                                                         | 5           |
|        |    |    | 32                      | 8                      | 4                                                         | 4           |
|        |    |    |                         | 64                     | 37(58%)                                                   | 27(42%)     |
| Type 2 | .. | .. | 0                       | 39                     | 26                                                        | 13          |
|        |    |    | 8                       | 15                     | 13                                                        | 2           |
|        |    |    | 16                      | 9                      | 6                                                         | 3           |
|        |    |    | 32                      | 5                      | 3                                                         | 2           |
|        |    |    |                         | 68                     | 48(70%)                                                   | 20(30%)     |
| Type 3 | .. | .. | 0                       | 60                     | 37                                                        | 23          |
|        |    |    | 8                       | 7                      | 4                                                         | 3           |
|        |    |    | 16                      | 2                      | 1                                                         | 1           |
|        |    |    | 32                      | 1                      | 0                                                         | 1           |
|        |    |    |                         | 70                     | 42(60%)                                                   | 28(40%)     |

TABLE 8  
*Poliomyelitis*  
*Antibody titres in human milk and blood*  
(Preliminary studies)

| Case<br>No. | Breast milk |        |        | Serum  |        |        |
|-------------|-------------|--------|--------|--------|--------|--------|
|             | Type 1      | Type 2 | Type 3 | Type 1 | Type 2 | Type 3 |
| 1           | 8*          | 8      | 8      | 256    | 64     | 64     |
| 3           | 8           | 16     | 8      | 128    | 64     | 16     |
| 4           | 16          | 32     | 16     | 128    | 128    | 128    |
| 6           | 64          | 8      | 8      | 128    | 16     | 128    |
| 33          | 4           | 8      | 16     | 64     | 64     | 32     |
| 49          | 8           | 4      | 4      | 64     | 8      | 64     |
| 61          | 64          | 8      | 64     | 128    | 256    | 128    |
| 40          | 16          | 8      | 8      | ....   | ....   | ....   |
| 47          | 32          | 16     | 16     | ....   | ....   | ....   |
| 53          | 16          | 8      | 16     | ....   | ....   | ....   |

\*reciprocal of sample dilution neutralising 100 TCD<sub>50</sub> of poliovirus.

TABLE 9

*Poliomyelitis**Incidence of copro-antibodies*

(Copro-antibody pattern in faecal samples against poliòvirus)

| Source of faecal samples                     |    |    |    | No. of<br>samples<br>tested | + ve for<br>copro-anti-<br>bodies | percent<br>+ ve |
|----------------------------------------------|----|----|----|-----------------------------|-----------------------------------|-----------------|
| Vaccinated virus + ve                        | .. | .. |    | 89                          | 29                                | 33              |
| Vaccinated virus — ve                        | .. | .. |    | 37                          | 12                                | 32              |
| Un-vaccinated virus + ve                     | .. | .. |    | 13                          | 1                                 | 7               |
| Un-vaccinated virus — ve                     | .. | .. |    | 23                          | 2                                 | 8               |
| Clinical cases (vaccinated)<br>virus + ve    | .. | .. | .. | 55                          | 11                                | 20              |
| Clinical cases (vaccinated)<br>virus — ve    | .. | .. | .. | 52                          | 7                                 | 13              |
| Clinical cases (un-vaccinated)<br>virus + ve | .. | .. | .. | 31                          | 2                                 | 6               |
| Clinical cases (un-vaccinated)<br>virus — ve | .. | .. | .. | 22                          | 5                                 | 22              |
| Total                                        |    |    |    | 322                         | 69                                | 21              |

## DEPARTMENT OF VIROLOGY

### 1. HOST-VIRUS INTERACTION—BASIC STUDIES

#### *A. Rabies*

1. *In vivo blockade by rabies virus of Rous sarcoma Virus.*—The clear demonstration that a non-oncogenic virus could totally block an oncogenic one in as high a percentage of White Leghorn Chickens as 80 was given in 1960-61: West Nile virus (WNV), an arbovirus belonging to the B Group of Casals showed total blockade of the Rous virus-produced tumour. Preliminary experiments performed at that time failed to detect interferon or interferon-like substances in the West Nile-Rous blockade system.

As an extension of this work, it was reported in 1965, from the Virus Research Centre, Poona, that two other B Group arboviruses (Kyasanur Forest Disease and Japanese encephalitis viruses) could also block Rous Sarcoma Virus. In 1967, Russian workers reported that antirabic vaccine and fixed rabies virus inhabited Rous Sarcoma Virus (RSV). In 1970, it was demonstrated at the Haffkine Institute that the Paris strain of infective rabies virus (RV) could not only produce total blockade, specifically of the Bryan Master Standard strain of RSV in a considerable percentage of chicks, but that this blockade was mediated by a serum factor not showing the properties characteristic of interferon. A full-length publication on this RV-RSV blockade system appeared in 1973.

As the mechanism underlying the original WNV-RSV system has not yet been elucidated, it was initially decided to investigate this system first. Accordingly, late in December 1973, work was initiated towards the preparation of stocks of WNV and of RSV of the Bryan Master Standard strain. However, due to the scarcity of litters of three-day mice (for research purposes), it became necessary, for the time being, to lay greater emphasis on the RV-RSV system and experiments were performed in White Leghorn chicks with the RV (CVS) and RV (Street 2/73) strains to determine whether these too, could produce blockade of the RSV and, if so, to what degree, as compared to that by the RV (Paris) strain.

The methodology employed throughout the course of the investigation was essentially the same as that described for the WNV-RSV and RV (Paris)-RSV systems. This has been described in detail in previous publications (1963, 1965, 1970 and 1973) and in the Annual Reports of the Institute. In brief, the procedure for producing the blockade was to inoculate the RV strain bilaterally into the subcutaneous wing web tissue of the chick. Forty-eight hours later, the RSV was also injected bilaterally at the same site. Starting post-RSV inoculation on the fifth day, the chicks were observed daily for tumour appearance and progress. The experiments were terminated on the 21st day. Control birds received the same material as the experimental ones, except that the RV was inactivated just prior to inoculation by UV irradiation. The morbidity (presence of tumour) ratios were noted for the experimental and control groups and from these observations the percentage of tumour-free chicks on the 21st day was computed.



Table 1 presents the results of three preliminary experiments carried out to test the comparative blocking effects of RV (Street 2/73), RV (CVS) and RV (Paris) strains. The RV (Street 2/73) strain showed the highest degree of blockade (54 per cent) in this experimental series. RV (CVS) was a close second with 50 per cent, and the least degree was exhibited by RV (Paris) viz. 20 per cent.

At the time of writing this report, experiments are already in progress for confirmation of this differential blockade as shown in this preliminary work with the different strains of RV. It is of particular interest that the maximal degree of blockade was produced by RV (Street 2/73)—a strain which is derived from a human brain sent to the Department of Virology in January 1973 for investigation. It was positive for Negri Bodies. This strain is currently undergoing active investigation by the Antirabic Section of the Department of Viral Vaccines, particularly with reference to its titration in mice.

It is hoped that future experimentation along the above mentioned avenues will lead to a better understanding of the mechanism(s) underlying these blockade systems.

2. *Biochemical changes in vivo in rabies.*—Experiments were performed to note any biochemical change which might be detectable in the host animals (the Swiss-Kasauli albino mouse and the albino rabbit) subsequent to infection by RV.

The RV (Paris) strain was used throughout the study. The virus was passaged in rabbit brain, the passage levels being 76, 78, 80, 83 and 86. Their titres ranged from 6·0–6·8, 10–10, adult mouse LD<sub>50</sub> per 0·03 ml. The standard dose used in all experiments was  $9\cdot6 \times 10^2$ – $1\cdot1 \times 10^3$ , adult mouse LD<sub>50</sub> 0·03 ml. intracerebrally. It was of particular interest that RV (Street 2/73), which has been mentioned above, was also utilised in the experiments with rabbits; it was injected intramuscularly.

Blood glucose levels from mice were estimated during the pathogenesis of the disease. As shown in Table 2, the animals were hyperglycaemic when completely paralysed, the mean blood glucose levels of the untreated control animals (Group 3) being 53 mg per cent, and 97 mg. per cent, pre- and post-RV inoculation, respectively.

Table 2 also shows that reserpine treatment of the animals, pre- or post-RV inoculation (1·33–91 Kg. mg mouse for three consecutive days) had no effect on the hyperglycaemia. Such treatment was tried to determine whether the hyperglycaemia is mediated through adrenaline. Since there were no detectable differences in the length of the incubation period, the pathogenesis of the disease and the hyperglycaemic condition, it was concluded that hyperglycaemia is not mediated through adrenaline.

Attempts at reversal of RV-induced paralysis in mice and rats through neostigmine treatment were unsuccessful, the animals dying with symptoms typical of rabies.

In studies with rabbits infected with either RV (Paris) or RV (Street 2/73), the animals exhibited a marked hypokalaemia during the stage of paralysis, while their sodium levels were not altered to any appreciable extent. There was

no excessive excretion of potassium in the urine. For the correction of hypokalaemia, potassium chloride, durabolin and spironolactone administration was attempted, but without success.

Serum creatinine levels of paralysed rabbits showed no alteration in comparison with controls.

Studies of glucose tolerance curves from RV-infected rabbits are currently in progress.

### *B. Viral Hepatitis*

1. *Attempts at cultivation of agents of human hepatitis in heterologous hosts.*—Experiments were initiated with the object of finding out a suitable *in vivo* laboratory system for growth of some of the agents producing viral hepatitis in man, particularly virus B, or the agent of serum hepatitis.

Preliminary data from early passages of human virus material in two different species of animals have offered some indication of infection. Active work is currently in progress to determine whether the results can be repeatedly confirmed.

### *C. Rubella*

1. *Teratogenesis in heterologous hosts.*—Preliminary experiments with rubella virus have yielded encouraging results in the production of one type of teratogenic lesion in two different species of laboratory animals. These systems are under intensive investigation for confirmation of the results.

## II. HOST-VIRUS INTERACTION—APPLIED STUDIES : EPIDEMIOLOGY

### *A. Rabies*

1. *Transmission to snakes.*—In collaboration with the Venomous Animal Unit of the Department of Zoonosis, exploratory experiments were carried out to determine whether the CVS strain of rabies virus could be adapted to the common water snake, *Natrix piscator*. The preliminary data from two successive snake brain passages are encouraging. Further work is in progress.

2. *Human patients.*—Samples of saliva (4), urine (2), cerebrospinal fluid (2) and serum (5) were collected from cases of human rabies admitted to different hospitals in the City of Bombay. The mouse inoculation and serum neutralisation tests were done. The results are set forth in Table 3.

The balance of three samples were under test for serum neutralisation titre at the time of writing of this Report.

3. *Carrier state in dogs.*—The normal dog acquired in August 1972 and kept under surveillance (as recorded in the Annual Report 1972-73) was further observed. It had reportedly caused death of a human patient from rabies. During the total observation period of over a year, saliva samples were being collected daily and all were found negative for virus isolation. Finally, the animal was sacrificed and the brain, salivary glands and blood were harvested. No virus was isolable from either the brain or the salivary glands. Furthermore, none of the three serum samples, collected in August 1972, October 1972 and at the time of sacrifice, showed any detectable neutralising antibody against rabies virus.

4. *Transmission in rats.*—The incidence of rat bite is considerable in the highly congested City of Bombay. The possible risk of human rabies subsequent to such bites is difficult to assess in the absence of adequate epidemiological information in this particular geographical region. Hence, preliminary studies were initiated with the object of establishing the role, if any, of rats in the transmission of human rabies. White rats were chosen due to their close relationship to their wild counterparts, satisfactory susceptibility to rabies and ready availability and ease of handling.

In order to simulate, as far as possible, conditions of natural field infection, it was decided to use street virus. Four isolates of RV (Street), obtained from three different mammalian species, viz. man, dog and cow, were tried in order to allow for different degrees of adaptation due to differences in species of origin, if any. The virus isolates were adapted to white rats, the infectivity titrations being concurrently conducted in white mice. Initially, the subcutaneous route of inoculation was used, subsequent work employing the intramuscular and intracerebral routes. Finally, the right thigh muscles were chosen as the routine site of virus introduction, again for the reason that this would mimic, as closely as possible, the situation obtaining in the field.

The results of these experiments indicated that the rate of infectivity varied considerably even with potent virus suspensions (as shown by concurrent infectivity titration in white mice) and even after utilising the chosen routine intramuscular route. Hence, a considerable period of time had to be expended upon the determination of the standard working dosages of virus on the basis of these intramuscular infectivity titrations in rats.

Work currently in progress is aimed at obtaining data concerning the experimental transmission of RV (Street) from infected white rats to their uninfected cage-mates.

5. *Transmission in bandicoots.*—A study of these animals concerning their possible role as vectors and/or carriers of human rabies, with the ultimate aim of determining whether antirabic treatment should be routinely prescribed following bite of these and related rodents, was undertaken.

In the first part of the study, a total of 500 apparently healthy bandicoots, obtained from the Municipality of Bombay from their trapping operations in the City, were subjected to a ten-day quarantine period. Following this, they were bled and then sacrificed. The brains and salivary glands were harvested. Brain smears were examined for the presence of Negri Bodies, using Seller's staining technique. From the blood, sera were separated and these were examined for the presence of neutralising antibody. White mice were inoculated intracerebrally with 10 per cent. suspensions of brain and salivary gland (in 2% HSS) to detect the presence of any rabies virus and they were observed routinely for 21 days. None of these tests revealed any evidence of rabies infection. Even in the case of the few mice, which became sick, the illness was shown not to be due to rabies virus by the brain smear examination and the neutralisation test.

The second part of the study involved experiments to test susceptibility of bandicoots to experimental rabies and to determine the mode of transmission,

if any, of rabies virus in these animals. Three street virus strains, one each from dog brain and salivary gland and the third from human brain were utilised. These were confirmed as strains of RV by staining for Negri Bodies and mouse inoculation and neutralisation test using hyperimmune horse antirabic serum.

Groups of 10 bandicoots were inoculated with each of the above three strains intracerebrally. Concurrent inoculation as well was carried out in white mice as a check. All the bandicoots developed rabies within the expected period and this was further confirmed as rabies by the routine laboratory diagnostic tests already mentioned. It was found that the intracerebrally inoculated animals had serum neutralising antibody titres ranging from 1 : 5—1 : 200.

In the transmission experiments, the three strains of rabies virus were inoculated intramuscularly into groups of ten bandicoots. Along with each infected animal, a healthy cage-mate was included to test the possibility of rabies transmission to it, through bite, scratch, secretions or excreta. The uninoculated animals' sera were tested for the presence of neutralising antibody as evidence of acquired rabies infection. At the time of submission of the present report, none of the uninfected cage-mates developed any of the signs of rabies and none developed any anti-rabies neutralising antibody.

During the course of the work mentioned above, a certain strain of street virus (2/73) of human brain origin was found to exhibit unusually high titres in mice on intracerebral inoculation. The susceptibility of different species of laboratory animals to this strain was then tested. On intracerebral and intramuscular inoculation, the incubation periods were found to be similar for mice, rats and guinea pigs. Only in the rabbit the incubation period was different. This strain has seen eight serial passages in albino mice. The virus is ether-sensitive. When a mouse brain suspension was inoculated into monkey kidney tissue cultures in order to rule out the possibility of any other viral contaminant, no CPE was seen. Currently, attempts are being made to adapt this strain to HKTC and BHK 21 cells.

As a matter of interest, a small batch of vaccine (Sample Type) was prepared in mouse brain using RV (Street 2/73). The vaccine passed the sterility, toxicity and inactivation tests. A preliminary potency test was carried out in comparison with the Sample Type sheep brain vaccine prepared at the Institute. Further work along these lines is in progress.

## B. Rubella

1. *Congenital rubella syndrome*.—During the year, 11 cases of infants and children, believed to be suffering from the congenital rubella syndrome, were referred to the department from neighbouring hospitals for viral studies.

None of the throat swabs collected from 10 of these patients yielded virus in primary human amnion cultures. Serum samples from the 11 cases and from their mothers were tested for the presence of HI antibody. Of these, three mother-child pairs showed absence of demonstrable antibodies. Three other pairs showed minimal detectable titres, whilst in the remaining 5, the titres of the mother's sera were found to be higher than those of their offspring.

In another study, the general occurrence of antirubella HI antibody in a normal parturient female population and in the cord blood was tested. Of the 93 paired blood samples from the mothers and cords, 72 pairs were found positive and 17 negative. In the remaining cases, four of the mothers' samples were positive, whilst the cord specimens were negative.

A serological survey, covering normal children from a local school (age group 10-16 years), was carried out. Of the 101 samples tested, 75 showed the presence of HI antibodies yielding an incidence of 75 per cent.

Prospective studies on rubella in normal pregnant mothers during each trimester, which had been initiated in 1972-73 had to be abandoned due to logistical problems.

### C. Rubella

1. *Measles and Subacute Sclerosing Panencephalitis (SSPE)*.—A total of 34 sera and 30 CSF samples were received from neighbouring hospitals from cases suspected to be suffering from SSPE, for the estimation of antimeasles HI antibodies.

Of these, 24 sera and 21 CSF samples were tested for HI antibodies. Twelve of the sera analysed showed titres ranging from 1:80—1:320 and 6 of the CSF specimens exhibited titres within the range 1:5—1:40 (a 1:5 titre in the CSF is considered diagnostic of the infection and cases showing such titres in the CSF invariably exhibited a serum titre range of 1:160—1:320). However, such high antibody levels in the sera were not necessarily indicative of SSPE, as such maximal titres are commonly seen in the normal post-infectious states and in sera from vaccinees. Onset of symptoms in the cases studied did not date back to more than one year. In SSPE, antibody levels increase progressively with the development of the disease. Taking the CSF titre as diagnostic of CNS infection, a follow-up of these cases would be of interest in the categorical labelling of the cases as SSPE.

### D. Influenza

1. *Influenza surveillance*.—(i) *Isolation*.—No isolation of either A or B strains was accomplished during the year.

(ii) *Serology*.—As the population covered by our surveys is very small, failure to isolate new strains does not entirely rule out circulation of a variant in that population. However, given the existence of a new variant in some parts of the world (not necessarily of pandemic proportions), it is important to be able to predict the likelihood of an epidemic due to such a variant. Thus, studies of antibodies to new virus strains, as compared to titres against older strains, can be useful when considered in the light of the antigenic interrelationships of such strains.

A small-scale serological survey was undertaken in October 1973, comprising 219 sera collected during different periods of time. These were tested for HI antibodies to three prototype strains, viz., A2/Hong Kong/8/68, A2/Coonoor/99/71 and A2/England/42/72. The latter two variants are antigenically identical.

An attempt was made to forecast the likelihood of appearance of epidemics of the existing new variant, viz., A2/England/42/72 in Bombay, in the winter of 1973-1974. This variant has some degree of cross-antigenic relationship with the previous strain, A2/Hong Kong/868. Hence, cross-protection is afforded to the new variant by A2/Hong Kong/868.

Table 4 shows the incidence of HI antibodies to the two variants A2/Hong Kong/868 and A2/England/42/72 in sera collected during different months in 1973. It is evident from an examination of this table that the latter variant has had some circulation in the population studied since May 1972. However, the circulation has been rather slow and about 50 per cent of the population is still susceptible, assuming that cross reacting antibody titres greater than or equal to 1:40 are also protective. The high percentage of antibodies, viz. 80 in the sera of school children (a highly susceptible group) from the Indian Education Society, Dadar, Bombay, as can be noted from the figures for June 1973 in the table, makes it unlikely that such an epidemic will occur.

### III. HSF-VIRUS INTERACTION—APPLIED STUDIES: PROPHYLAXIS

#### A. Rabies

1. *Antirabic vaccine trial.*—The Department has undertaken trial of a new antirabic vaccine in unbiten, normal human volunteers. It is presently under scrutiny for possible candidature as a replacement for the currently-used Sample type vaccine prepared in sheep brain at the Institute.

The vaccine is a killed one and was developed in human diploid cell cultures at the Wistar Institute, Philadelphia, U.S.A. Batches sent to the department for trials were prepared at the Institute Merieux, Lyon, France.

In the trial, of the 38 volunteers, on whose sera the results of tests on neutralising antibody are available, 7 did not show significant antibody levels (1:40) on the 10th day following the first dose of the vaccine. On the 14th day, all except one of the vaccinees showed significant titres, which fluctuated between 1:100 and 1:5000. The peak titres appear to have been reached between the 14th and 30th day, following which they declined, as revealed by tests done on the 90th day.

A decision as to the acceptability of this vaccine, in preference to that currently produced by the Haffkine Institute, will depend upon the data obtained from further studies on larger numbers of volunteers. The trials continue and serum samples for antigenicity tests are being referred to the Wistar Institute.

#### B. Vaccinia

1. *Post-vaccinial complications.*—Three cases of generalised vaccinia and one of vaccinia gangrenosa were referred to the Department from different City hospitals.

Haemagglutination-inhibition antibodies in the two cases of generalised vaccinia and in the case of vaccinia gangrenosa were low, viz. equal to or less than 10.

Vaccinia-like lesions (pocks) were isolated on the chorioallantoic membrane of the chick embryo from one of the cases of generalised vaccinia (a 17-month old baby) from the pustules and from the blood. The HI antibody titre in the serum of this patient was 1 : 40.

#### IV. HOST-VIRUS INTERACTION—APPLIED STUDIES : MANAGEMENT

##### A. Rabies

1. *Trial of an indigenous drug.*—A herbal product, claimed to be effective against rabies and referred to us by a private person, was tested in albino mice.

Two groups of animals, one inoculated intracerebrally with 50-100 LD<sub>50</sub> of the CVS strain of rabies virus and the other intramuscularly with the same dosage of the same strain, were orally administered in aqueous gum acacia suspension of the drug, 24 hours post-CVS inoculation.

The drug was found to be ineffective, as the treated animals succumbed as readily as those in the virus control groups.

TABLE 1

*Comparison of degree of blockade by three different strains of RV against Bryan Master Standard Strain of RSV-RV 48 hours prior to RSV*

| Experiment | Inoculum        |     | Number of chicks |         | Per cent tumour free chicks on 21st day |         |
|------------|-----------------|-----|------------------|---------|-----------------------------------------|---------|
|            | 1st             | 2nd | Experimental     | Control | Experimental                            | Control |
|            |                 |     |                  |         |                                         |         |
| 1          | RV(Street 2/73) | RSV | 13               | 15      | 54                                      | 0       |
| 2          | RV(CVS)         | RSV | 10               | ..      | 50                                      | 0       |
|            | RV(CVS)         | RSV | 14               | 15b     | 50                                      | 0       |
| 3          | RV(Paris)       | RSV | 15               | 15      | 20                                      | 0       |

(a) All controls inoculated with UV-inactivated RV.

(b) These served as common controls for both experimental groups of experiment 2, since they and the group of 14 were inoculated with RSV of one log<sub>10</sub> higher concentration than those of the group of 10.

TABLE 2

*Hyperglycaemia in Rabies-infected, paralysed mice (18-20 g.) unaltered by reserpine treatment*

| Group | Number of mice | Pre-inoculation blood glucose (mg. %) |      | Post-paralytic blood glucose (mg. %) |      |
|-------|----------------|---------------------------------------|------|--------------------------------------|------|
|       |                | Range                                 | Mean | Range                                | Mean |
| 1a    | 30             | 44·64                                 | 52·5 | 86·112                               | 92   |
| 2b    | 30             | 44·64                                 | 51·8 | 84·114                               | 94   |
| 3c    | 30             | 44·64                                 | 53·0 | 82·120                               | 97   |

(a) Reserpine administered prior to RV inoculation.

(b) Reserpine administered subsequent to RV inoculation.

(c) No reserpine administered.

TABLE 3

*Human rabies ; Mouse inoculation and serum neutralization tests*

| Sample | Number obtained | Test                 | Result               |
|--------|-----------------|----------------------|----------------------|
| Saliva | 4               | Mouse inoculation    | Negative             |
| Urine  | 2               | Do.                  | Do.                  |
| C.S.F. | 2               | Do.                  | Do.                  |
| Serum  | 5               | Do.                  | Do.                  |
|        | 2*              | Serum neutralization | Titres exceeding 1:5 |

\*These were from the same 5 serum samples on which mouse inoculation test was negative.

TABLE 4

*HI antibodies to Influenza A2/Hong Kong/868 and A2/England/42/72*

| Antibody Status | Serum collected (Month and Year) |            |           |            |                                    |            |                |            |              |            |
|-----------------|----------------------------------|------------|-----------|------------|------------------------------------|------------|----------------|------------|--------------|------------|
|                 | May 1973                         |            | June 1973 |            | June 1973<br>(10-15 year old only) |            | June-July 1973 |            | October 1973 |            |
|                 | Incidence                        | Percentage | Incidence | Percentage | Incidence                          | Percentage | Incidence      | Percentage | Incidence    | Percentage |
|                 |                                  |            |           |            |                                    |            |                |            |              |            |
| A               | ..                               | ..         | 10/63     | 16         | 28/56                              | 50         | 11/35          | 31         | 7/34         | 21         |
| B               | 17/31                            | 55         | 14/63     | 22         | 17/56                              | 30         | 6/35           | 17         | 9/34         | 26         |
| C               | 3/31                             | 10         | 27/63     | 43         | 8/56                               | 14         | 15/35          | 43         | 13/34        | 39         |
| D               | 11/31                            | 35         | 9/63      | 14         | 1/56                               | 2          | 3/35           | 9          | 3/34         | 8          |

A Titre equal to or greater than 1 : 40 to both variants : more than 4-fold higher for A2/68.

B Titre equal to or greater than 1 : 40 to both variants : less than 4-fold higher for A2/68.

C Titre equal to or greater than 1 : 40 to A2/68 : less than 1:40 to A2/72.

D Titre less than 1 : 40 for both strains.



## DEPARTMENT OF ZOONOSIS

**Microbial Diseases**

**Plague.**—Plague epidemiological studies in 'D' and 'E' ward areas of Greater Bombay were continued. *R. rattus*, *R. norvegicus* and *B. bengalensis* together were observed to constitute the prevalent rodent fauna. *Mus musculus* and *B. gigantea* were also recovered in good numbers. A study of the prevalence of various types of rodents during different seasons is underway.

In all, 945 rats from 'D' ward area and 602 rats from 'E' ward area were examined. *X. cheopis* showed its predominance, as reported earlier, over other species of fleas recovered viz. *X. astia*, 185 fleas from ward 'D' and 63 fleas from ward 'E' were examined. Other ecto-parasites harboured by these rodents are being studied.

**Serological Studies.**—Erythrocytes, sensitized with fraction I antigen of *Yersinia pestis*, were received from the Middle Asian Research Institute on Plague Control, Zavodskaya under the Ministry of Health of the U.S.S.R. for plague serological studies.

Over 1,000 serum samples of rodents were collected from different parts of Maharashtra and were screened. Out of these, fifteen pools collected from Murud, Dhoki, Barsi, Jalgaon and Malegaon were found to be positive in 1:20, 1:40 and 1:60 dilutions respectively. These findings indicate the likelihood of presence of inapparent foci of plague infection among commensal rodents in these parts.

Apart from these, 240 serum samples of dogs and 100 serum samples of pigs were collected and examined serologically. Fifteen dogs sera samples and 3 pigs sera samples were found to be positive by HA tests. The studies are in progress.

**Laboratory trials with IN-100.**—Laboratory trials were undertaken with N-100—a newly developed rodenticide manufactured by Messrs. Rohm and Haas, Philadelphia. The rodenticide was evaluated against *R. rattus*, *R. norvegicus*, *B. bengalensis* and *M. musculus*. It was found to be effective against all the four species of rodents in the laboratory experiments.

**Susceptibility of rats to anti-coagulant rodenticides.**—Assessment of susceptibility status of various species of rodents to Warfarin and other anti-coagulant rodenticides is in progress.

So far, rodents from 'B', 'C', 'D' and 'E' ward areas have been studied. These studies showed presence of tolerance to Warfarin in *B. bengalensis* population in 'D' and 'E' ward areas.

**Diphtheriae.**—A study has been taken up to evaluate the anti-toxin level in *C. diphtheriae* in immunised, non-immunised and disease positive children below twelve years.

Immune serum was prepared against diphtheriae toxoid in rabbits. A few throat swabs and samples of sera obtained by filter paper method were collected. The work has just been initiated.

**Leptospirosis.**—Leptospirosis is one of the most important zoonotic diseases. This institute has been working on various aspects of leptospirosis for the past

seven years. Fourteen standard strains of leptospira are being maintained in the laboratory. Work carried out during the period under report includes serological examination of 540 samples of sera of both human and animal origin. Serological tests carried out include microscopic agglutination test, sensitised erythrocyte-lysis test, fluorescent antibody test and haemagglutination test. Twenty per cent of the dog sera showed leptospiral agglutinins and the serotype found was *L. canicola*. The other serotypes were found to be *L. pomona*, *L. ballum* and *L. icterohaemorrhagiae*.

*Cholera carrier studies.*—Cholera carrier studies undertaken in Parbhani Town of the State of Maharashtra were concluded. The main object of the study was to investigate the prevalence of human reservoirs, i.e. cholera carriers. Water samples and animal excreta from the selected spots were also studied for their possible role in the maintenance and spread of cholera infection.

The investigations showed that cholera epidemic season in the study area corresponds with the monsoon season. Cholera-like diarrhoea cases occur in an explosive manner in the pre-monsoon and monsoon seasons. The highest incidence was observed in the younger age groups, especially the children.

Ninety-eight strains of *V. cholerae* biotype *El tor* and serotype Ogawa were isolated from 6,104 human faecal samples examined. They belonged to phage types 2 and 4. *V. cholerae* was isolated from 72 index cases, five convalescent and 12 asymptomatic carriers. Non-agglutinable vibrios (NAG) were isolated from six animal faecal samples (pools) and four water samples. The pathogenicity tests showed that 100 per cent isolates from human samples were pathogenic to infant rabbits. Fifty per cent of the NAG strains isolated from animals and water produced cholera-like syndrome in the animals, which was indistinguishable from that produced by *El tor* vibrio strains isolated from clinical cases of cholera in the area. Studies on similar lines are in progress in Virar area of the Thana district. So far, 250 stool samples have been examined.

### Parasitic Diseases

*Filariasis—Epidemiology.*—Epidemiological studies in filariasis in the villages of Murdhe, Rai, Morve and Dongri revealed 96 micro-filaria carriers and 52 filarial disease positive cases in 1,440 people examined. Similar epidemiological study is being carried out at the village Uttan in the Thana District.

*Immunology.*—Skin tests were undertaken with Sawda's FST antigen in the known non-endemic area of Parbhani Town. These tests showed that the people un-exposed to filarial infection do not react to the antigen.

*Drug evaluation.*—Drug trials were undertaken with Mebendazole. Trials were undertaken in forty-nine micro-filaria carriers from the villages of Murdhe, Rai, Morva and Dongri.

*Laboratory studies.*—Maintenance of *L. carinii* culture in cotton rats and albino rats is being continued. One-hundred and twenty-one rodents were infected. *L. carinii* antigen is being prepared for serological studies.

*Transmission studies.*—Infection of *Culex p. fatigans* in the laboratory with *W. bancrofti*, using micro-filaria carriers, is being attempted. Development of *W. bancrofti* in the vector species is being studied. When sufficient number of infective larvae become available, attempts will be made to prepare an antigen for diagnosing Wucheratiasis.

*Dracunculiasis.*—Dracunculiasis studies were carried out in 8 villages of Kolaba District, viz., Tisse, Khuroshi, Madhali-Pugaon, Shihu, Valavli, Rawe, Jite and Durtoli. A population of approximately 4,000 was covered. Prevalence of the disease was noted in 7.2 per cent population, highest being in the age-group 5-18 years. The density of cyclops per litre of water ranged between 12.0 to 15.0. The efficacy of metronidazole in the treatment of dracunculiasis was assessed. Studies are now in progress in Jambivli and Chavne villages.

*Experimental laboratory infections.*—Strains *Nematospiroides dubius* and *H. Nana* are being maintained for carrying out immunological studies and drug evaluations. Attempts are being made to establish *N. americana* culture in hamsters.

*Insecticide resistance and vector control.*—Evaluation of susceptibility status of vectors, viz., fleas, flies, mosquitoes and cockroaches to different insecticides is being continued. As reported in the last year's report, these insects continue to show resistance to DDT and susceptibility to Malathion and Dieldrin.

A survey of microbial pathogens harboured by *Culex p. fatigans* was undertaken. Five-hundred mosquitoes from Kurla area were studied. An array of pathogens was recovered, prominent among these being *Serratia* sp., *E. coli* and *B. anthracis*. Plant juvenile hormones synthesized by the University Department of Chemical Technology, Bombay, are being screened against larval stages of *Musca d. nebulosa*, *Culex p. fatigans* and *P. americana*. The utility of these hormones in vector control will be evaluated later.

*Amoebiasis.*—Mono-axenic culture of *E. histolytica*, a Swiss strain, is being maintained in the laboratory. Attempts are being made to establish axenic culture of *E. histolytica* at this Institute using Diamond's medium.

*Studies in intestinal parasitic infestations.*—In a survey of intestinal parasites in four districts of the Maharashtra State, 2,755 stool samples were examined. It was observed that 1,921 stool samples (69.4 per cent) showed infestation with intestinal parasites. The rate of infection was found to be 45.4 per cent in Bombay city, 90.6 per cent in Thana District and 100 per cent in Kolaba and arbhani Districts.

Prevalence of ascariasis was noted in all the four districts surveyed. Hookworm was found to be prevalent in villages of Thana District (70%). *A. lumbricoides*, *Trichuris trichura*, *Hymenolepis nana* and *E. histolytica* were found to be common intestinal parasites.

#### **enormous Animals**

*Toxicity studies.*—Toxicity of Cobra, Krait, Viper and Echis venoms to various animals is being studied with different dilutions of these venoms. The work is presently being carried out in mice. LD<sub>50</sub> values for different venoms in mice have been worked out.

## PART III

### ROUTINE AND MISCELLANEOUS ACTIVITIES

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#### DEPARTMENT OF ANTITOXINS AND SERA

During the year under review, further changes took place in the department in the matter of distribution of staff and activities.

Demands were rather heavy for anti-snake venom serum as compared to those of the previous years.

The name of this department was changed to Department of Antitoxins and Sera (Production) and the name of the Department of Immunology was retained as a research department.

#### Staff

New additions to staff were made both to research and production sides. Dr. (Smt.) S. A. Kulkarni joined the department as Senior Scientific Officer Class I (Research). Dr. C. B. Hulikavi was promoted to the post of Senior Scientific Officer Class II (Veterinary). Shri V. M. Patankar, M.Sc., joined the department as Junior Scientific Officer Class II. Shri V. R. Bhatkar, Senior Technical Assistant at Pimpri, was posted as Senior Technical Assistant, Department of Antitoxins and Sera at Bombay. Shri V. M. Patki, Senior Scientific Officer Class II, was posted as Officer-in-charge of the Centralised Filling and Packing Section and the Filling and Packing Section of the Immunology Department was amalgamated with the Centralised Filling and Packing Section in December 1973. Shri L. B. Bhavari joined as Farm Supervisor at Pimpri. Dr. A. B. Patil joined the department in April 1973 as a Medical Officer. However, he left the department in January 1974 and Dr. A. R. Phadke took over from him in February 1974, as Medical Officer, Pimpri Farm.

Also, the Immunology Department (Research) was separated and put under the supervision of Dr. (Smt.) S. A. Kulkarni as an independent unit, separated from the production department, viz. Department of Antitoxins and Sera, in September 1973. Bifurcation of these activities resulted in divided responsibilities and smooth working in the two independent units.

Dr. (Smt.) S. R. Kankonkar joined the Immunology Department as Senior Research Fellow in the month of April 1973 and was absorbed in the Immunology Research Unit to work on scorpion venoms. Kum. S. M. Joshi and Kum. S. W. Avchat joined the Research Unit as Research Scholar and Junior Research Fellow respectively.

Collaborative Research work on Anti-Lymphocytic Serum with the K. E. M. Hospital under an I. C. M. R. Scheme was continued. Dr. (Smt.) S. G. Kinare from K. E. M. Hospital and Shri M. E. Kulkarni were the chief collaborators in the Anti-Lymphocytic Serum Scheme. This I.C.M.R. Scheme was closed, owing to shortage of funds, in February 1974.

More collaborative study schemes of an inter-departmental nature were started and persons from the Departments of Immunology and Antitoxins and Sera actively participated in the schemes, such as filariasis, snake venoms, tetanus etc. Shri M. E. Kulkarni and R. C. Kankonkar were the main participants in tetanus and venom schemes and Shri M. E. Kulkarni was the consulting participant in the filariasis scheme.

The construction of two new stables at Pimpri was completed during the year and accommodation capacity of horses increased by 48. Repairs to the old stables blown up by winds will be taken up. However, filling of the ditch near the horses' stable was taken up and will be completed in a couple of months.

A new project of lucern grass for supply to horses was drawn up. Under this project, lucern grass will be produced in the compound of Haffkine Institute Stud Farm at Pimpri. The services of Shri L. B. Bhavari, M.Sc. (Agriculture) and availability of septic tankwater will be taken advantage of for this project. Government sanction for Rs. 80,350 was obtained for the scheme (first phase) and a rapid progress was observed since October 1973. Actual sowing was done in January 1974; However, within 1 1/2 months, there was an average supply of lucerne grass to the extent of 400 kgs./day and since 20th March, it is about 700 kgs./day, which will completely fulfil the requirement of lucern grass for the present strength of horses at Pimpri.

If sufficient water is made available, this department will not be required to purchase any lucerne grass from outside. Moreover, this will result in a considerable saving to the Institute, which could be utilized for development.

The department also produced hay for horses, which will be sufficient for 1 1/2 months. This has resulted in a saving of Rs. 50,000 at Pimpri and Rs. 20,000 at Bombay. Schemes for water supply are under consideration.

Electrification of entrance road to laboratory and few other streets was carried out.

Madras thorn was used as an enclosure to lucerne grass cultivation area and it is now proposed to have this compound along side all the barbed wire compound of the Farm, to avoid intrusion by cattle or people in the area.

The dispensary of Pimpri farm was renovated.

Plans are being prepared for increasing the laboratory working space. Additional bleeding chamber is also being proposed to be constructed to increase the number of bleedings at a time.

Most of the horses at Bombay were sent to Pimpri along with the necessary staff. In Bombay, there are now about 30 horses and the number of horses at Pimpri stood between 245 and 300. No large-scale replenishment in the number of horses was made in order to reduce the maintenance cost. Sufficient stocks of anti-toxic sera have resulted in this economy.

A number of horses were tried for gas gangrene antitoxin. However, only 2 horses responded for a couple of months to titre on border line requirement. Further trials by checking basal immunity will be tried to find out the number of responding horses. The response of even couple of horses for 1 year could meet the present demands. A small batch of gas gangrene antitoxin has been made to cope with partial demands.

Since the supply of horses from military sources started, the general response of horses improved to some extent and their over all life span also seemed to improve to some extent. However, it will take another 2 years to confirm this.

Response in the case of Tetanus seemed to improve and percentage of horses between categories 300-600 IU, 600-900 IU and 900 and above increased as compared to the previous years also 300 IU decreased. There is one horse showing 1500 IU. Response in the case of Diphtheria improved slightly for short periods. However no substantial improvement was noticed.

Venom horses responded regularly, but during the first half of the year only. Response of Echis venom seemed to decrease. Necessary steps were taken and Echis venom response was improved at the cost of Cobra venom response and it took some time to reach the balance. This did not affect the routine production level for the year under review. However, to a certain extent, it may affect the next year's production. The balance will be maintained by transferring more horses for venom immunization.

Demands for anti-toxins (1,500 I.U.) reduced to a large extent, affecting the sales. However, there was increase in therapeutic (20,000 I.U.) tetanus anti toxin from other provinces which balanced the supply to some extent.

Demands for bulk anti-toxins also were received from local firms and this matter is under consideration.

Maximum economy measures were implemented in view of the scarcity conditions during the year. It is expected that the department would be able to balance its expenses with the income.

Following are the highlights of the research unit. Shri P.M. Naik completed his Ph. D. work on "Role of Adjuvants steroids Gonedectomy on Immune response". Dr. D.P. Buduk completed his work of M. Sc. on plasma phoresis in horses. Shri R.P. Vitkar was awarded the M. Sc. degree by the University of Bombay for his thesis on immuno-suppressors. Shri S. V. Gadre was awarded Ph. D. degree by the Bombay University for his work on "Role of phage and Iron in Toxin production by *C. diphtheriae*". Smt. S.S. Sathe was awarded M. Sc. degree by the Bombay University for her work on "Production of diphtheria toxin in casein hydrolysate medium". After being awarded M. Sc. degree, she was transferred to the Department of Pharmacology. Smt. A. A. Gandhi was awarded M.Sc., for her work on "Studies in Tetanus Toxin Production".

Work is also being carried out on snake venoms, tetanus toxins, peptic digest medium, adjuvants for diphtheria toxoid, production of LQ toxins from *Cl. perfringens*, production of tetanus toxin in tryptone medium and efficacy of drug in tetanus and diphtheria.

Work is also being carried out on scorpion venoms.

This year being the year of Platinum Jubilee, most of the staff from both the research and production sections was actively engaged in helping the functions connected with the jubilee activities. The conference on D.P.T. was of great help for further progress in studies and production in case of diphtheria, tetanus and pertussis vaccines and anti-toxins. The W.H.O. workshop on cholera was also useful through its discussions on practical aspects of production and problems of production. Research papers from the department were also discussed.

#### *Production*

| Name of Product       | Quantity |    |    |            |
|-----------------------|----------|----|----|------------|
| Tetanus Anti-toxin    | ..       | .. | .. | 2,474 M.U. |
| Diphtheria Anti-toxin | ..       | .. | .. | 373 M.U.   |
| Antivenine            | ..       | .. | .. | 260 litres |

## DEPARTMENT OF BACTERIOLOGY

During the year, the department continued its usual activity of production of Cholera-El-Tor, T.A.B. and Plague vaccines. Since the sections concerned with the production of the Diphtheria and Tetanus Toxins and Toxoids came under the administrative control of the Department of Bacteriology, in February 1973, the department had to shoulder the additional responsibility of the production of these biologicals.

Due to the prevailing scarcity, there were heavy demands for Cholera-El-Tor vaccine. The department supplied about eight million doses of this vaccine to different places in the Maharashtra State and about five million to other States. The following quantities of Cholera-El-Tor, T.A.B. and Plague Vaccines were produced and supplied during the year:—

| Product                      | Qty. produced<br>(litres) | Qty. supplied<br>(litres) |
|------------------------------|---------------------------|---------------------------|
| Cholera-El-Tor Vaccine .. .. | 14,133·820                | 13,106·147                |
| T.A.B. Vaccine, I. P. .. ..  | 226·553                   | 284·101                   |
| Plague Vaccines, I.P. .. ..  | 37·660                    | 101·360                   |

The department successfully met the exceptionally high demands for Tetanus Toxoid from various scarcity camps from Maharashtra and other places. It also supplied about 2,50,000 doses of Tetanus Toxoid to Egypt.

The following quantities of Toxins and Toxoids were produced during the year :—

| Product                                           | Quantity<br>(Litres) |
|---------------------------------------------------|----------------------|
| <i>Tetanus Toxin and Toxoid</i>                   |                      |
| Peptic-digest medium toxin .. ..                  | 250                  |
| Protein-free medium toxin .. ..                   | 2,558                |
| Toxoid .. ..                                      | 2,157                |
| Tetanus Toxoid, I.P. (in simple solution) .. ..   | 696                  |
| Tetanus vaccine, B.P. (adsorbed) .. ..            | 1,240                |
| <i>Diphtheria Toxin and Toxoid</i>                |                      |
| Papain-digest medium toxin .. ..                  | 185                  |
| Papain-digest medium toxoid .. ..                 | 31                   |
| Protein-free medium toxin .. ..                   | 1,725                |
| Diphtheria-Tetanus vaccine, B.P. (adsorbed) .. .. | 40                   |
| Welchii toxin .. ..                               | 128                  |
| Welchii toxoid .. ..                              | 19                   |

The department examined 17 samples of water for suitability for human consumption, 3 samples of filters for their efficacy as bacterial filters and 3 samples of gelatin for gelatin-liquifying bacterial content.

A research project "Bacteriological analysis of frog legs, with special reference to detection of Salmonella", financially supported by Messrs. Sawant Fisheries Pvt. Ltd., was endorsed by the department from 5th July 1973 to 31st April



1974. About 250 samples of frog legs were examined under this project. Advice was also given regarding proper processing of the frog legs, with a view to avoid contamination with pathogenic organisms.

Dr. Van Hemert visited the department from 26th September to 4th October 1973 as a W. H. O. Expert to assess the possibility of producing a substantial quantity of DPT vaccine on the existing premises and to advise on a possible new set-up for the production of this biological.

As a part of the Platinum Jubilee Celebrations of this Institute, various scientific seminars were organised during the year. The Diphtheria-Pertussis-Tetanus International Seminar, sponsored by the Glaxo Laboratories and the International Cholera Conference, sponsored by the W. H. O. and Haffkine Institute jointly, were of particular significance to the department. An Inter-Regional Workshop in Cholera-Vaccine Production was organised by W. H. O. at the Institute from 4th March 1974 to 12th March 1974. Dr. B. Cvjetanovic, Dr. J. C. Feeley, Dr. Y. Watanabe and Dr. I. Joo conducted this workshop. The course was of immense benefit to all the scientific staff working in the department.

During the period under review, 35 persons, including M. L. T. and short-term trainees, were trained in the production and testing of bacterial vaccines.

## MEDIA SECTION

The Media Section continued the manufacture of culture media, stains and reagents required for the diagnosis of diseases and testing of drugs.

The manufacture of parenteral solutions also continued in full swing. In addition to the above, the department provided general facilities for sterilization, cleaning of glassware, etc.

The Ampoule Cutting Section supplied cut and sterilized ampoules and vials to the Central Filling and Packing Section and other production departments, whenever necessary, for special purposes. The production figures of the different items are presented below.

## CULTURE MEDIA

| Product                                   | Quantity in litres |
|-------------------------------------------|--------------------|
| Casein-hydrolysate medium (cholera) .. .. | 32,332·400         |
| Meat-digest agar (typhoid) .. ..          | 3770·00            |
| Casein-hydrolysate agar (Plague) .. ..    | ....               |
| Casin-hydrolysate medium (plague) .. ..   | ....               |

## STERILITY TEST MEDIA

|                        |           |
|------------------------|-----------|
| Aarobic medium .. ..   | 5,593·600 |
| Anaerobic medium .. .. | 5,749·120 |
| Sabouraud medium .. .. | 4,399·200 |

## PARENTERAL SOLUTIONS

|                                                   |            |
|---------------------------------------------------|------------|
| Dextrose and Sodium Chloride Injection, U.S.P. .. | 42,616·500 |
| Dextrose Injection, I. P., 5 per cent .. ..       | 50,243·500 |
| Sodium Chloride Injection, I. P. .. ..            | 37,272·000 |
| Water for injection .. ..                         | 8,607·000  |
| Dextrose Injection, I. P. 25 per cent .. ..       | 264·000    |
| Sodium lactate, B. P. .. ..                       | 5,559·500  |
| A. C. D. Solution 'A' .. ..                       | 1,296·500  |
| Sodium Chloride Injection, 20 per cent .. ..      | 122·000    |
| Peritoneal solution 'A' .. ..                     | 188·000    |
| Peritoneal solution 'B' .. ..                     | 4·560      |

## OTHER VARIOUS TYPES OF MEDIA AND STAINS AND SOLUTIONS

|                                  |         |
|----------------------------------|---------|
| Acid-digest of meat agar .. ..   | 610·400 |
| Nutrient agar .. ..              | 966·900 |
| MacConkey agar .. ..             | 296·000 |
| Sabouraud agar .. ..             | 73·600  |
| D. E. C. agar .. ..              | 14·000  |
| Löffler's medium .. ..           | 40·120  |
| Lowenstein Jensen's medium .. .. | 13·220  |
| Peptone water .. ..              | 349·500 |
| MacConkey broth .. ..            | 3·000   |
| Ox bile medium .. ..             | 38·300  |
| Hartley's broth .. ..            | 10·788  |
| Koser's citrate .. ..            | 1·500   |

| Product                               | Quantity in litres |
|---------------------------------------|--------------------|
| Glucose phosphate .. .. .             | 9·000              |
| 2 per cent Citrate saline .. .. .     | 12·700             |
| Glucose broth .. .. .                 | 31·900             |
| Nutrient broth .. .. .                | 171·900            |
| Alkaline peptone water .. .. .        | 48·300             |
| Lab-lemco broth .. .. .               | 2·600              |
| Selenite broth .. .. .                | 31·500             |
| Thioglycolate medium .. .. .          | 31·500             |
| Christensen's medium .. .. .          | 3·000              |
| Standard staphylococcal broth .. .. . | 1·000              |
| Litmus milk .. .. .                   | 3·000              |
| T. C. B. S. agar .. .. .              | 25·000             |
| Hoyle's base .. .. .                  | 12·500             |
| Potato agar .. .. .                   | 3·000              |
| Normal saline .. .. .                 | 9,947·000          |
| Muller and Hinton medium .. .. .      | 3·000              |
| Buffer saline .. .. .                 | 23,092·000         |
| Sabouraud broth .. .. .               | 54·000             |

## SUGARS FOR BIOCHEMICAL TESTS

|                   |        |
|-------------------|--------|
| Glucose .. .. .   | 36·200 |
| Lactose .. .. .   | 35·300 |
| Mannitol .. .. .  | 35·800 |
| Sucrose .. .. .   | 35·800 |
| Dulcitol .. .. .  | 12·900 |
| Maltose .. .. .   | 4·200  |
| Arabinose .. .. . | 5·700  |
| Mannose .. .. .   | 1·600  |
| Xylose .. .. .    | 5·700  |

## STAINS AND DIAGNOSTIC REAGENTS

|                                         |         |
|-----------------------------------------|---------|
| Banedit's solution .. .. .              | 960·000 |
| Ziehl Neelson Carbol Fuchsin .. .. .    | 220·000 |
| Methylene blue .. .. .                  | 164·000 |
| Gram stain No. 1 .. .. .                | 28·000  |
| Gram stain No. 2 .. .. .                | 52·000  |
| Albert stain No. 1 .. .. .              | 28·000  |
| Albert stain No. 2 .. .. .              | 24·000  |
| Preservative fluid for W. B. C. .. .. . | 35·000  |
| Preservative fluid for R. B. C. .. .. . | 40·000  |
| Löffler's Methylene blue .. .. .        | 8·000   |
| Saffranine .. .. .                      | 12·000  |
| J. S. B. No. 1 .. .. .                  | 25·000  |
| J. S. B. No. 2 .. .. .                  | 40·000  |
| Indol reagent No. 1 .. .. .             | 0·500   |
| Indol reagent No. 2 .. .. .             | 0·500   |
| Gentin violet .. .. .                   | 12·000  |

## DEPARTMENT OF BIOCHEMISTRY

The department continued to render diagnostic service to city hospitals and private practitioners. The service facilities are particularly useful to the Employee's State Insurance Scheme and M. A. Podar Ayurvedic Hospitals. Increasing number of the staff members of this Institute availed of these facilities. There were more demands for tests like Serum Protein Bound Iodine, Serum Transaminases and Urinary-17 ketosteroids. A total of 7,021 tests were carried out during the year as per Table I appended. A noteworthy feature is that the total number of tests this year is almost one and half times that of the corresponding number in the last year's report.

**Training**

The department continued to take part in the training programme for M.L.T. D.M.L.T. and Short-term Training Courses.

**Staff**

Dr. (Smt.) A. Venkataraman, Assistant Director I/C and Dr. S. D. Ambegaokar, Senior Scientific Officer (Class I), retired during the year. Shri J. K. Kamath, Junior Scientific, officer Smt. A. K. Pandit and Kumari K. S. Korgaokar, Senior Technical Assistants resigned. Kumari N. J. Save was appointed as Senior Technical Assistant on a temporary basis. Shri A. D. Kulkarni, Senior Research Fellow, Government of Maharashtra, Shri A. V. Athavale, Government of India Research Training Scholar and Shri P. B. Godkar, Pool Officer, Government of Maharashtra, joined the department during the year.

**Academic**

Kumari Ranjana Buit and Smt. H. V. Phadnis submitted their thesis to the Bombay University for Ph. D. and M. Sc. degrees in Biochemistry respectively. Shri V. P. Shinde, U. J. Dumaswala and Syed Raziuddin submitted their synopsis for Ph. D. degree in Biochemistry of the Bombay University. Kumari P. H. Jhala also submitted her synopsis for Ph. D. degree in Biochemistry of the Maharaja Sayajirao University of Baroda.

Shri V. P. Shinde, Senior Scientific Officer (Class II), was awarded a research prize for the year 1972-73 for his meritorious work on "Quadruple Cholera Vaccine in Peanut Protein Hydrolystate" under the scheme for Production and Research Awards.

TABLE I  
*Tests carried out during the year 1973-74*

| Name of the test   |    |    |    | No. of tests |
|--------------------|----|----|----|--------------|
| <i>Blood Serum</i> |    |    |    |              |
| Icteric Index      | .. | .. | .. | 88           |
| Van den Bergh      | .. | .. | .. | 98           |
| Amylase            | .. | .. | .. | 13           |
| Bilirubin          | .. | .. | .. | 576          |

| Name of the test               | No. of tests |
|--------------------------------|--------------|
| Calcium .. .. .                | 15           |
| Chlorides .. .. .              | 37           |
| Cholesterol .. .. .            | 116          |
| Creatinine .. .. .             | 3            |
| Non-Protein Nitrogen .. .. .   | 7            |
| Phosphorus (Inorganic) .. .. . | 6            |
| Acid Phosphatase .. .. .       | 10           |
| Alkaline Phosphatase .. .. .   | 336          |
| Proteins—Total .. .. .         | 412          |
| Proteins—Albumin .. .. .       | 352          |
| Proteins—Globulins .. .. .     | 352          |
| Sugar .. .. .                  | 1,159        |
| Thymol Turbidity .. .. .       | 410          |
| Urea Nitrogen .. .. .          | 128          |
| Uric Acid .. .. .              | 26           |
| Fibrinogen .. .. .             | 1            |
| Total ..                       | 4,145        |

*Urine and Stool*

|                                         |     |
|-----------------------------------------|-----|
| Albumin (Urine) .. .. .                 | 2   |
| Bile Salts and Pigments (Urine) .. .. . | 138 |
| Sugar (Urine) .. .. .                   | 149 |
| Calcium (Urine) .. .. .                 | 7   |
| Phosphorus (Urine) .. .. .              | 2   |
| Porphyryne (Urine) .. .. .              | 1   |
| Total ..                                | 299 |

*C.S.F. & Other body fluids*

|                            |    |
|----------------------------|----|
| C. S. F. Chlorides .. .. . | 8  |
| C. S. F. Proteins .. .. .  | 12 |
| C. S. F. Sugar .. .. .     | 8  |
| S. M. Test .. .. .         | 5  |
| Total ..                   | 33 |

*Special Tests (Clinical)*

|                                                   |    |
|---------------------------------------------------|----|
| Faecal Fat .. .. .                                | 1  |
| Gama Globulins (Serum) .. .. .                    | 1  |
| Gastric analysis .. .. .                          | 4  |
| Glucose Tolerance Test .. .. .                    | 32 |
| Lange's Colloidal Gold Curve (C.S.F.) .. .. .     | 67 |
| Potassium (Serum) .. .. .                         | 33 |
| Protein-bound Iodine (Serum) .. .. .              | 35 |
| Proteins by paper electrophoresis (Serum) .. .. . | 20 |
| Sodium (Serum) .. .. .                            | 42 |

| Name of the test                            |    |    |    | No of test |
|---------------------------------------------|----|----|----|------------|
| 17-Ketosteroids (Urine)                     | .. | .. | .. | 29         |
| S. G. O. T.                                 | .. | .. | .. | 662        |
| S. G. P. T.                                 | .. | .. | .. | 676        |
| Urinary Stone                               | .. | .. | .. | 11         |
| Vinyl Mandelic Acid (Urine)                 | .. | .. | .. | 2          |
| Total                                       |    |    |    | 1,615      |
| <i>Special food analysis samples</i>        |    |    |    |            |
| Sukhadi, Banana stuns, Bunn and Bread for : |    |    |    |            |
| Moisture                                    | .. | .. | .. | 60         |
| Proteins                                    | .. | .. | .. | 60         |
| Fat                                         | .. | .. | .. | 60         |
| Ash                                         | .. | .. | .. | 60         |
| Fibre                                       | .. | .. | .. | 2          |
| Carbohydrates                               | .. | .. | .. | 60         |
| Calories                                    | .. | .. | .. | 60         |
| Calcium                                     | .. | .. | .. | 54         |
| Iron                                        | .. | .. | .. | 54         |
| Phosphorus                                  | .. | .. | .. | 54         |
| Vitamin A                                   | .. | .. | .. | 54         |
| Vitamin B1                                  | .. | .. | .. | 54         |
| Total                                       |    |    |    | 632        |
| <i>Filariasis Project samples</i>           |    |    |    |            |
| Total Iodine (Serum)                        | .. | .. | .. | 56         |
| Protein-bound Iodine (Serum)                | .. | .. | .. | 64         |
| Total                                       |    |    |    | 120        |
| <i>Vitamin A Project samples</i>            |    |    |    |            |
| Haemoglobin                                 | .. | .. | .. | 55         |
| Erythrocytes                                | .. | .. | .. | 55         |
| Plasma Vitamin A                            | .. | .. | .. | 46         |
| Plasma Proteins                             | .. | .. | .. | 21         |
| Total                                       |    |    |    | 177        |
| <i>Summary</i>                              |    |    |    |            |
| Blood (Serum)                               | .. | .. | .. | 4,145      |
| Urine and Stool                             | .. | .. | .. | 299        |
| C. S. F. and other body fluids              | .. | .. | .. | 33         |
| Special Tests (clinical)                    | .. | .. | .. | 1,615      |
| Special food analysis samples               | .. | .. | .. | 632        |
| Filariasis Project samples                  | .. | .. | .. | 120        |
| Vitamin A Project samples                   | .. | .. | .. | 177        |
| Grand Total                                 |    |    |    | 7,021      |

## DEPARTMENT OF CHEMOTHERAPY

**General**

The synthesis and studies of newer compounds for their possible use against tuberculosis, leprosy, protozoal infections like amoebiasis, filariasis, helminthic infestation and neoplastic diseases were continued and over 300 new compounds were synthesized, characterized and representative compounds from each of these series were subjected to preliminary screening.

During the year, stress was laid on the synthesis of compounds for their possible anthelmintic, anti-amoebic and anti-filarial activity. Accordingly the synthesis and study on series of benzothiazoles, pyridothiazoles and piperazine derivatives containing imidazole and nitrofurylamido groupings were continued.

In view of the non-availability of diacetyldiamino-diphenylsulphone (DADDS), a derivative of DDS which has been shown to possess a very long "depot" activity (one single injection maintaining the therapeutic level for over 70 days), the department undertook the preparation of DADDS depot injections. This new product has passed all the tests and has been taken up for controlled clinical trials.

In view of the reported advantages of ferroglycine sulphate complex, such as better absorption and higher haemoglobin levels in oral iron therapy, the department undertook a study of ferro glycine sulphate complex. Tablets were prepared from this complex and studied clinically on a limited scale. The preliminary results indicated much higher absorption of iron than with ferrous sulphate.

A new Pilot Plant Committee was appointed to look into the possibility of utilizing the existing pilot plant facilities at the Haffkine Institute for the standardisation of new drugs. Under the direction of this committee, the Department undertook the pilot plant studies of some important drugs and drug intermediates. Laboratory investigations on phenformin (DBI), tetramisole, DADDS, and nicotinamide were undertaken.

Two Senior Research Fellows and two Junior Research Fellows continued to work on their research problems. Similarly, One Government of India Scholar and One Government of Maharashtra Scholar also continued to work on their problems. During the year under review, one student obtained his M.Sc. degree and two candidates were awarded Ph.D. degree in Organic Chemistry of the University of Bombay.

**Routine**

The Micro-analytical Section continued the elemental analysis of all the compounds synthesised in the Department.

## Production

The production and supply of all the products manufactured by the department for the use of Government hospitals, E.S.I.S. and public health bodies was continued. The output was slightly higher than that of the previous year. The list of items and their production are given below :—

| Name of the product             |    |    |    |    | No. of tablets<br>manufactured |
|---------------------------------|----|----|----|----|--------------------------------|
| Vitamin A. D. (P)               | .. | .. | .. | .. | 86,16,800                      |
| Vitamin B. C.                   | .. | .. | .. | .. | 1,95,31,000                    |
| Vitamin B <sub>1</sub>          | .. | .. | .. | .. | 6,61,200                       |
| Vitamin B <sub>2</sub> (5 mgs.) | .. | .. | .. | .. | 4,200                          |
| Vitamin B <sub>2</sub> (Mild)   | .. | .. | .. | .. | 2,92,000                       |
| Vitamin C (50 mgs.)             | .. | .. | .. | .. | 1,18,62,500                    |
| Vitamin C (100 mgs.)            | .. | .. | .. | .. | 26,76,900                      |
| Vitamin C (500 mgs.)            | .. | .. | .. | .. | 7,59,250                       |
| Nicotinic acid                  | .. | .. | .. | .. | 1,49,300                       |
| Vitamin BC <sub>3</sub>         | .. | .. | .. | .. | 13,20,500                      |
| Polyvitamin                     | .. | .. | .. | .. | 1,60,53,400                    |
| Vitamin B-complex               | .. | .. | .. | .. | 1,58,40,000                    |
| Calcium with vitamin D          | .. | .. | .. | .. | 18,87,500                      |
| Iodochlorohydroxyquinoline      | .. | .. | .. | .. | 14,61,100                      |
| Di-iodohydroxyquinoline         | .. | .. | .. | .. | 30,98,300                      |
| Isoniazid                       | .. | .. | .. | .. | 86,33,500                      |
| Thiacetazone and Isoniazid      | .. | .. | .. | .. | 41,85,900                      |
| Sodium aminosalicylate          | .. | .. | .. | .. | 1,01,04,000                    |
| Phthalyl sulphathiazole         | .. | .. | .. | .. | 37,74,300                      |
| Sulphadimidine                  | .. | .. | .. | .. | 97,51,000                      |
| Aspirin                         | .. | .. | .. | .. | 1,70,48,000                    |
| Vitamin K                       | .. | .. | .. | .. | 1,00,900                       |
| Chlorpropamide (250 mgs.)       | .. | .. | .. | .. | 3,24,000                       |
| Chlorpropamide (100 mgs.)       | .. | .. | .. | .. | 7,800                          |
| Berberine                       | .. | .. | .. | .. | 9,95,500                       |
| Analgin                         | .. | .. | .. | .. | 1,52,880                       |
| Tolbutamide                     | .. | .. | .. | .. | 6,90,100                       |
| Vitamin A. D. (Strong)          | .. | .. | .. | .. | 19,77,000                      |
| Grand Total                     |    |    |    |    | 14,19,59,360                   |
| Sodium Pas-Granules             | .. | .. | .. | .. | 12 kgs.                        |
| Pemon                           | .. | .. | .. | .. | 57,500 litres.                 |
| Niacinamide                     | .. | .. | .. | .. | 35,286 kgs.                    |



## DEPARTMENT OF CLINICAL PATHOLOGY

**Staff**

Dr. (Smt.) V. P. Purohit, Medical Officer, M.M.S. Cl. III, Typhoid Unit was temporarily promoted to the post of Medical Officer, M.M.S. Cl. II on 31st December 1972 and appointed in the Haffkine Institute Dispensary *vice* Dr. (Smt.) R. R. Walinjkar.

Dr. (Kum.) S. M. Bhide was appointed as Medical Officer, M.M.S. Cl. III, Typhoid Unit, on 12th February 1974 *vice* Dr. (Smt.) V. P. Purohit promoted to M.M.S. Cl. II post.

Dr. S. Mukerjee was transferred to the Department of Clinical Pathology in the post of S.S.O. Cl. I (Bacteriology) on 8th March 1974.

The Government of Maharashtra Scholarship in Microbiology was awarded to Shri N. T. Joshi who commenced work in the department on 1st September 1973.

Shri Janak B. Dhabuwalla was appointed as a Laboratory Technician in the department on 4th September 1973 in the collaborative project with the International Atomic Energy Agency on the radio-immuno assay of Enteric Fever and Brucellosis.

Shri J. T. Waghmare, Laboratory Assistant, retired on 8th January 1974. Shri H. D. Hatale, Laboratory Attendant, was promoted and appointed as Laboratory Assistant in place of Shri Waghmare.

Shri P. B. Kode, Laboratory Assistant, was transferred to the Immunology Department as a Junior Technical Assistant. Shri V. H. Parkar, Laboratory Attendant from Immunology Department, was promoted and appointed in place of Shri Kode.

Shri A. G. Pawar, Junior Technical Assistant, was transferred to the Zoonosis department on 1st August 1973 and Shri P. A. Mulye, Junior Technical Assistant, was transferred from the Zoonosis Department in place of Shri Pawar.

**Training**

Three additional seats for the D.M.L.T. course were created during the year. Fifteen students were admitted, out of which 12 students completed the course.

Twenty-one short-term trainees completed the training in clinical pathology. In addition, 11 M.L.T. students were given theoretical as well as practical training in clinical pathology.

Shri D. S. Chitnis submitted his synopsis for M.Sc. in Microbiology by research on "Studies on R-factors". Smt. K. D. Walawalkar was registered for M.Sc. in Microbiology.

Dr. S. Mukerjee submitted his thesis for Ph.D. in Microbiology on "A Study of *Bacterium anitratum* and *Bacterium anitratum* like organisms."

### Other Activities

The department participated in the Inter-Laboratory Evaluation Programme conducted by the Central Venereal Diseases Laboratory, Madras Medical College, Madras, for the V.D.R.L. syphilis serology programme.

The department extended co-operation to the various other departments of the Institute in the collection of blood samples. Routine clinical pathological investigations for the staff of the Institute were carried out. Histo-pathological study of the tissues of the experimental animals, received from post-graduates of various departments of the Institute, was also undertaken. Four-hundred and twenty-seven tissues referred by the Pharmacology Department and 925 tissues referred by the Toxicology Unit were studied for drug toxicity reactions.

Dr. (Smt.) J. K. Dave participated in the panel discussion on Cholera Therapy at the Cholera Symposium held at this Institute during the Platinum Jubilee celebrations in March 1974.

Routine investigations carried out during the year are presented below :—

|                                                   |    |    |    | No. of samples |
|---------------------------------------------------|----|----|----|----------------|
| <i>A. Histo-pathology</i>                         |    |    |    |                |
| Specimens received                                | .. | .. | .. | 4,176*         |
| Blocks prepared                                   | .. | .. | .. | 6,249          |
| <i>B. Haematology</i>                             |    |    |    |                |
| Haemoglobin estimation                            | .. | .. | .. | 739            |
| Total R. B. C. Count                              | .. | .. | .. | 708            |
| Total W. B. C. Count                              | .. | .. | .. | 729            |
| Differential W. B. C. Count                       | .. | .. | .. | 725            |
| P. C. V. Estimation                               | .. | .. | .. | 12             |
| E. S. R. Estimation                               | .. | .. | .. | 189            |
| Prothrombin Time                                  | .. | .. | .. | 6              |
| Platelet count                                    | .. | .. | .. | 5              |
| Bleeding Time                                     | .. | .. | .. | 5              |
| Clotting Time                                     | .. | .. | .. | 4              |
| <i>C. Clinical Microscopy</i>                     |    |    |    |                |
| Urine for routine examination                     | .. | .. | .. | 461            |
| Stool for routine examination                     | .. | .. | .. | 347            |
| Semen examination                                 | .. | .. | .. | 16             |
| C. S. F. for cytological examination              | .. | .. | .. | 6              |
| Other puncture fluids for cytological examination | .. | .. | .. | 3              |
| Pregnancy Tests (Qualitative)                     | .. | .. | .. | 1,730          |
| Pregnancy Tests (Quantitative)                    | .. | .. | .. | 228            |
| <i>D. Bacteriology</i>                            |    |    |    |                |
| Blood for cultural examination                    | .. | .. | .. | 25             |
| Blood culture for Salmonellae                     | .. | .. | .. | 897            |
| Urine for cultural examination                    | .. | .. | .. | 297            |
| Urine for enteric organisms                       | .. | .. | .. | 24             |

|                                                             | No. of samples |
|-------------------------------------------------------------|----------------|
| Stool for cultural examination .. .. .                      | 301            |
| Stool culture for Salmonellae .. .. .                       | 204            |
| Throat swabs for diphtheria and other organisms ..          | 212            |
| Antibiotic sensitivity tests .. .. .                        | 1,715          |
| Autovaccines and autolysates .. .. .                        | 67             |
| Culture for tuberculosis .. .. .                            | 100            |
| Guinea-pig inoculation tests for tuberculosis ..            | 129            |
| Smear for A.F.B. (Acid Fast Bacilli) .. .. .                | 129            |
| Material for Tetanus cultural examination .. ..             | 310            |
| Animal inoculation tests for Tetanus (toxin neutralisation) | 104            |
| Smear examination for Gonorrhoea .. .. .                    | 292            |
| Cultures for Gonorrhoea .. .. .                             | 292            |
| Miscellaneous cultural examinations .. .. .                 | 126            |

#### *Mycology*

|                                          |      |
|------------------------------------------|------|
| Cultures for detection of fungus .. .. . | .... |
|------------------------------------------|------|

#### *Serology*

|                                                 |       |
|-------------------------------------------------|-------|
| Widal Tests .. .. .                             | 877   |
| Weil Felix Tests .. .. .                        | 877   |
| Tests for Brucellosis .. .. .                   | 2,291 |
| Paul Bunnell Tests .. .. .                      | 21    |
| V.D.R.L. Tests .. .. .                          | 596   |
| Kahn Tests .. .. .                              | 83    |
| Wassermann Tests .. .. .                        | 7     |
| Cold Agglutination Test .. .. .                 | 1     |
| Rose Waaler Tests .. .. .                       | 130   |
| Latex Fixation Test for Rheumatoid Arthritis .. | 130   |

|                         |               |
|-------------------------|---------------|
| Total investigations .. | <u>22,399</u> |
|-------------------------|---------------|

\*Blocks prepared out of specimens are taken into account while calculating the total number of tests.

The following were the interesting histo-pathology specimens received during the year :—

|                                                       |               |
|-------------------------------------------------------|---------------|
| 1. Angioneurofibroma .. .. .                          | CP/H No.138   |
| 2. T. B. Appendicitis .. .. .                         | CP/H No.1,262 |
| 3. Adenocarcinoma of the Fallopian Tube ..            | CP/H No.2,109 |
| 4. Malignant Schwannoma .. .. .                       | CP/H No.2,759 |
| 5. Heterotopic Gastric Mucosa in the Sigmoid Colon .. | CP/H No.2,922 |

The following table shows regional distribution of 52 primary malignant growths received during the year :—

|                          |    |
|--------------------------|----|
| Skin .. .. .             | 5  |
| Breast .. .. .           | 10 |
| Lymphatic system .. .. . | 1  |
| Buccal cavity .. .. .    | 2  |
| G. I. Tract .. .. .      | 5  |

|                        |    |    |    | No. of samples |
|------------------------|----|----|----|----------------|
| Female Genital organs  | .. | .. | .. | 19             |
| Male Genital organs .. | .. | .. | .. | 6              |
| Nervous Tissue ..      | .. | .. | .. | 2              |
| Bone ..                | .. | .. | .. | 2              |

Ninety-seven specimens of tuberculous lesions were received.

The regional distribution is as follows :—

|                       |    |    |    |    |
|-----------------------|----|----|----|----|
| Female genital organs | .. | .. | .. | 21 |
| Other organs ..       | .. | .. | .. | 76 |

Three-hundred and forty-seven stool samples were examined for parasites during the year. The following table gives the distribution of the parasitic infections in these samples :—

|                                     |    |    |    |    |
|-------------------------------------|----|----|----|----|
| 1. <i>Ascaris lumbricoides</i>      | .. | .. | .. | 85 |
| 2. <i>E. histolytica</i>            | .. | .. | .. | 60 |
| 3. <i>Trichuris trichura</i>        | .. | .. | .. | 53 |
| 4. <i>Giardia lamblia</i> ..        | .. | .. | .. | 37 |
| 5. <i>Ankylostoma duodenale</i>     | .. | .. | .. | 14 |
| 6. <i>Enterobius vermicularis</i>   | .. | .. | .. | 3  |
| 7. <i>Strongyloides stercoralis</i> | .. | .. | .. | 3  |
| 8. <i>Hymenolopis nana</i>          | .. | .. | .. | 2  |
| 9. <i>Taenia solium</i> ..          | .. | .. | .. | 1  |

The Diagnostic Reagents Section produced 74 reagents which include antigen, antisera stains and solutions. Besides, it maintains 84 bacterial strains in stabs or in a freeze-dried condition.

During 1973-74 the sale figures of the products were as follows :—

| Products        |    |    |    | Types | Sale in MI. |
|-----------------|----|----|----|-------|-------------|
| Antigens        | .. | .. | .. | 13    | 5,12,295    |
| Antisera        | .. | .. | .. | 36    | 7,450       |
| Venom solutions | .. | .. | .. | 8     | 1,268       |
| Stains          | .. | .. | .. | 17    | 22,11,882   |
| Total ..        |    |    |    | 74    | 27,32,895   |

These sales exceeded the average sales figures of the last 10 years by 500,000 ml. The increased supply was made despite irregular gas supply during the months of October and November 1973.

Three hundred and forty-two freeze dried and 423 slope cultures were supplied. Since January 1974, the preparation of autovaccine was transferred from Clinical Pathology Department to the Diagnostic Reagents Section in view of the rigid sterile conditions needed for its preparation.

The section prepared new labels conforming to the Drug Control Rules. The Diagnostic Reagents Section was shifted to a new area previously occupied by the Antirabic Vaccine Section. It now occupies about 1,150 sq. ft. area with a provision of 2 sterile rooms.

## DIRECTOR'S LABORATORY

## General

During the year, the Director's laboratory was strengthened by organising few important research projects. The vacant post of Junior Scientific Officer in charge of this laboratory has not yet been filled up. Four Research Fellows worked in the laboratory under the direct supervision of the Director. The laboratory was reorganised to undertake studies of toxins, particularly the tetanus toxin, Diphtheria Toxin and the Pertussis Toxin. Antifertility activity of some of the indigenous drugs was also undertaken.

Studies were initiated to investigate the mechanism of adverse effects of oral contraceptive pills, particularly those concerned with metabolic changes, resulting from chronic intake of oral contraceptive agents.

## Staff

|                    |    |                                                               |
|--------------------|----|---------------------------------------------------------------|
| Shri S. P. Chitnis | .. | UNICHEM Research Fellow                                       |
| Shri S. S. Jadhav  | .. | Junior Research Fellow, Government of Maharashtra.            |
| Shri V. B. Babne   | .. | Junior Research Fellow, Diamond Jubilee Endowment Fellowship. |
| Shri K. T. Shetty  | .. | Do. do.                                                       |

Shri G. R. Fernando, M.Sc., Ph.D., Senior Technical Assistant, resigned and left this Institute for better prospects elsewhere. Shri Khelifat Husein, who was working under Shri Y. S. Nimbkar for his Ph.D. Programme in Microbiology, left this country for his motherland after obtaining the doctorate degree of the Bombay University.

## DEPARTMENT OF IMMUNO-HAEMATOLOGY

During the year under report, the routine production activities of the department, viz. collection of blood, both at the Institute and at the mobile sessions, processing and lyophilisation of blood plasma, fractionation of human plasma and production of blood grouping reagents were continued.

In addition, laboratory testing facilities for specialised laboratory tests like Rh antibody, Australia antigen, red-cell enzyme were offered to the medical profession.

The samples of human sera for export were tested for Custom's authorities. The Government has taken keen interest in promoting the voluntary blood donation movement and has issued instructions to the district authorities to help the mobile blood collection drive. This has helped the Public Relation Officer of this department in expanding his activities further. It is encouraging to note that the number of prisoners donating blood is increasing.

The following figures give the account of the routine activities of the department :—

|                                                                                                     |       |
|-----------------------------------------------------------------------------------------------------|-------|
| Number of institutions/colleges and factories etc. contacted ..                                     | 69    |
| Number of institutions/colleges and factories etc. where blood ..<br>collection sessions were held. | 39    |
| Number of voluntary donors enrolled . . . . .                                                       | 4,840 |

*Blood donors*

|                   |        |
|-------------------|--------|
| Voluntary .. .. . | 3,173  |
| Paid .. .. .      | 3,902  |
| Prisoners .. .. . | 4,937  |
| Hospitals .. .. . | 6,026  |
| Grand Total ..    | 18,038 |

*Production and supply of plasma and plasma products*

| Name of product                 | Packing<br>(ml.) | Produced       | Supplied       |
|---------------------------------|------------------|----------------|----------------|
| Dried plasma .. .. .            | 250              | 1,931 bottles  | 3,752 bottles  |
| Human gamma globulin .. .. .    | 1                | 3,141 ampoules | 3,168 ampoules |
| Human fibrinogen .. .. .        | 150              | 291 bottles    | 304 bottles    |
| Human fibrinogen .. .. .        | 10               | 171 ampoules   | 158 ampoules   |
| Cryoprecipitate .. .. .         | 25               | 68 bottles     | 69 bottles     |
| Fresh Plasma .. .. .            | 250              | 8 bottles      | 8 bottles      |
| Human serum albumin (Powder) .. |                  | 228 gms.       | 228 gms.       |
| Packed cells .. .. .            | 150              | 871 bottles    | 871 bottles    |

*Production and supply of blood grouping reagents*

| Name of product                |    |    | Packing<br>(ml.) | Produced        | Supplied         |
|--------------------------------|----|----|------------------|-----------------|------------------|
| nti-A                          | .. | .. | 1                | 11,312 ampoules | 11,604 ampoules. |
| nti-A                          | .. | .. | 5                | 3,882 ampoules  | 3,928 ampoules.  |
| nti-B                          | .. | .. | 1                | 12,043 ampoules | 11,767 ampoules. |
| nti-B                          | .. | .. | 5                | 3,862 ampoules  | 3,811 ampoules.  |
| ti—A+ B                        | .. | .. | 1                | 1,592 ampoules  | 939 ampoules.    |
| nti—A+ B                       | .. | .. | 5                | 180 ampoules    | 247 ampoules.    |
| oomb's reagent                 | .. | .. | 1                | 525 ampoules    | 643 ampoules.    |
| nti—D                          | .. | .. | 1                | 4,384 ampoules  | 3,999 ampoules.  |
| ovine albumin                  | .. | .. | 1                | 493 ampoules.   | 475 ampoules.    |
| ovine albumin (in powder form) |    |    |                  | 11 gms.         | 11 gms.          |
| nti—A <sub>1</sub> (Lectin)    | .. | .. | 1                | 96 ampoules     | 23 ampoules.     |
| nti—A <sub>1</sub> (Serum)     | .. | .. | 1                | 140 ampoules    | 116 ampoules     |
| nti—H (Lectin)                 | .. | .. | 1                | 241 ampoules    | 147 ampoules.    |

*Tests carried out*

|                                       |    |    |    |     |
|---------------------------------------|----|----|----|-----|
| h tests                               | .. | .. | .. | 104 |
| Paternity tests                       | .. | .. | .. | 1   |
| Coomb's tests                         | .. | .. | .. | 4   |
| ross-matching tests                   | .. | .. | .. | 19  |
| Tests on samples for export           | .. | .. | .. | 41  |
| umber of charges of anti-venine dried | .. | .. | .. | 42  |

## DEPARTMENT OF PHARMACOLOGY

During the year under review, Kum. P. H. Marker, Senior Scientific Officer Cl. II (Microbiology), was transferred to the Director's Laboratory from 24th August 1973. Smt. S.S. Sathe, Senior Technical Assistant, was transferred to this department from Bacteriology Department from 11th January 1974. Shri M.B. Sabnis was transferred to the Accounts (Recovery) Section from 14th April 1973. Kum. S.R. Dhond and Smt. G.V. Aroskar Senior Technical Assistants, resigned from the department from 1st September 1973 and 8th February 1974, respectively. Dr. (Smt.) Noor Jahan Sagheer was appointed as Senior Scientific Officer Class I (Pharmacologist) from 10th December 1973. Kum. S.R. Pathak and Kum. N.G. Wayangankar were appointed as Senior Technical Assistants on purely temporary basis. Shri D.S. Donde was appointed as a Pool Officer under the Government of Maharashtra Pool Officer's Scheme from 8th December 1973. Shri S. Peeran from Sandoz (India) Ltd., was trained in oxytoxic assay for a period of one month.

### Government Analysts' Section

All the sections concerned with Government Analysts were transferred to the Drug Control Laboratory, Bandra and this year, no sample was received from the Drug Inspectors in Maharashtra. Only one sample of Tetanus Antitoxin was received from the State of Gujarat and it was reported as standard.

### Prohibition and Excise Section

The Prohibition and Excise Department sent 481 samples of toddy for detection of adulteration and alcohol content. Out of these, 2 samples were found to be adulterated with chloral hydrate and pyridine bases, while one sample contained high amount of alcohol. Seven-hundred and eighty-five samples of Indian-made foreign liquors were tested for alcohol content only. The department also received 32 samples of Ganja, Bhang etc. for certification. All samples were found to be unfit for human consumption when tested by physical and chemical methods.

### Food Samples

Though the analysis of food samples is not a statutory responsibility of the department, it analysed 48 food samples for the detection of Dhatura alkaloids and for fitness for human consumption. Five samples were free from Dhatura. Eight samples were tested for ergot infestation. Five samples were tested for contamination with sulphur and it was shown that there was no contamination.

### Commercial Samples

Even after the transfer of the biological Section to the Drug Control Laboratory, Bandra, the department continued to receive samples from commercial firms. The total number of samples received during the year was 2,271. The samples were mainly received for biological testing, for tests requiring infra-red Spectroscopy and chromatographic techniques.



### **bioavailability Studies**

During the year, the department turned out to be one of the largest centres for carrying out bioavailability studies. Single dose or multiple dose studies on chloramphenicol palmitate, chloramphenicol, tetracycline, oxytetracycline, erythromycin, ampicillin, cloxacillin and cycloserine have already been reported. Studies on diazepam, phenyl butazone, oxyphenyl butazone and thiamine hydrochloride are in progress.

### **Toxicology**

A study on percutaneous toxicity of hexachlorophene from talc powder was carried out in Rhesus monkeys. One gram of the powder was applied for five days a week to the abdomen up to 6 months. The hexachlorophene contents in the samples were 3 percent and 10 percent. No toxicity could be observed when the gross behaviour, haemogram, liver function tests and kidney function tests were performed on the monkeys. At necropsy, no abnormal changes were noted. The department of Clinical Pathology assisted in examining the tissues microscopically. No abnormality was observed.

### **Scientific Seminars, Symposia etc.**

Western Zone Scientific Seminar on Indigenous Systems of Medicines was held at the Durbar Hall, Haffkine Institute, on 11th and 12th August 1973. It was inaugurated by the Honourable Minister for Education, Prof. A.N. Namjoshi. The seminar was attended by scientists in Yoga, Ayurveda, Modern Medicine and Basic Sciences like botany, chemistry and pharmacology from States of Gujarat, Maharashtra, Andhra and Karnatak. Twenty-six scientific papers were presented in various disciplines of Indigenous Systems of Medicine. The seminar was followed by Yogic demonstrations.

### **Journal club and Departmental Seminars**

The department consists of scientists of different disciplines, such as pharmacology, chemistry, Microbiology etc. To enable them to keep pace with the recent trends in the respective fields and to encourage the habit of critical evaluation, a Journal Club was started, which meets twice a month and seminar is arranged once a month.

Dr. M.B. Bhide was invited to participate in the Clinical Pharmacology Seminar organised by the Association of Medical Advisers to the Pharmaceutical Industry (AMAPI) on September 8, 1973 at New Delhi and a seminar on Practical Aspects of Drug Therapy organised by the Bulletin of the Indian Medical Association and Association of Medical Advisers to the Pharmaceutical Industry (BIMA-AMAPI) on 24th February 1974 at Bombay. He also participated in the Laboratory Workshop on Animals held at Haffkine Institute from 7th May 1973 to 17th May 1973.

### **Training**

The department participated in the training programmes of the Institute. The Assistant Director I/c Department of Pharmacology acted as Honorary Secretary for the certificate Course in Medical Laboratory Technology.

### **Post-graduate teaching programme**

The department also participated in the post-graduate lecture series programme arranged by the Institute for the benefit of the post-graduate students.

### **Post-graduate degrees**

Five members of the staff of this department were admitted for M.Sc., degree by research. Shri D.V. Pradhan was awarded M. Sc., degree in Biochemistry of the Bombay University.

## QUALITY CONTROL DEPARTMENT

The department continued to perform its varied functions of great responsibility of maintaining stringent quality control on products of the Institute, examining and certifying them with regard to their purity, strength and quality, in order to fulfil the drug control obligations and maintain high standards of quality control.

During the period under review, the department continued to function mainly on the second floor, but the 3 animal rooms with test/experimental animals and the sterility testing area were located on the ground floor. Unification of all the sections of the department at a single site was not possible owing to lack of adequate space. However, it has succeeded in getting a dark room constructed in the chemical section as also a fuming chamber. New electric connections and fans and lights were provided in the animal rooms. Owing to financial stringency, the renovation of the sterility testing area had to be deferred. So also, setting up of a microbiology room, bioassay room, sample storage room etc., had to be postponed for the same reason.

In addition to testing and certification of finished products, the department keeps a scrupulous check on the raw materials that go into the manufacture of the drugs. Intermediaries of a number of products are also tested.

Besides the heavy routine, the department participated in various constructive activities of the Institute, e. g.

(i) The department collaborated with the Department of Bacteriology in the functioning of the 'Pertussis Cell' with the participation of the Director. This involved a lot of experimental work, biological standardisation, analysis of data, contribution of constructive ideas and modification in the production methodology. The dauntless pursuit to attain good results culminated in the heartening success of preparation of two batches of Triple Vaccine that passed the N. I. H. test and were found fit for issue. Further work is afoot and more success is envisaged.

(ii) In addition to being recognised in Microbiology, Dr. F. L. Saldanha, Assistant Director in charge of the department, was also recognised by the University of Bombay as a post-graduate guide in Applied Biology. Six members of the staff are working for their Master's degree by research and some more are involved in scientific investigations. One candidate from the department secured her M. Sc. degree during the period under review. Dr. F. L. Saldanha presented two papers at the Annual Conference of the Association of Microbiologists of India and one paper was read at the Cholera Seminar sponsored by W. H. O. and Haffkine Institute.

(iii) The department participated in the 'Cholera Workshop' jointly sponsored by W. H. O. and Haffkine Institute. The senior personnel of the department had an opportunity to discuss on different aspects of biological standardisation of drugs. The contacts built up during the different international seminars proved fruitful in solving the difficulties encountered and y controversial issues could be referred to the participants for opinion.

(iv) Many members of the staff of the department took part in the organisational and managerial aspects of the varied activities connected with the Platinum Jubilee Celebrations.

(v) The department imparted technical training to the following post graduate candidates, viz.

Candidates sponsored by—

|                  |    |    |    |    |    |
|------------------|----|----|----|----|----|
| Private firms    | .. | .. | .. | .. | 2  |
| Private students | .. | .. | .. | .. | 18 |
| M.L.T. trainees  | .. | .. | .. | .. | 10 |
| Total            |    |    |    |    | 30 |

Shri D. V. Rajadyaksha, S.S.O. Class II, was appointed to the post of S.S.O. Class I (Bio-assay). The other post of S.S.O. Class I (Chemistry), sanctioned more than 3 years back, remained vacant. Shri B. C. Mehta, S.S.O. Class II, left the Institute to join the State Drug Control Laboratory (F.D.A.). Two Senior Technical Assistants, Kum. V. G. Bapat and Smt. M. N. Shah, resigned. One old vacant post of Junior Technical Assistant was filled up.

The number of different tests and the samples analysed by the department during the period under review are enumerated below :

| Products                                  |    |    |    |    | No. of<br>samples tested |
|-------------------------------------------|----|----|----|----|--------------------------|
| <i>Sterility Tests</i>                    |    |    |    |    |                          |
| Bacterial Vaccines                        | .. | .. | .. | .. | 7,928                    |
| Antirabic Vaccine                         | .. | .. | .. | .. | 4,528                    |
| Parenteral bulk solutions                 | .. | .. | .. | .. | 5,008                    |
| Antitoxins, Sera, etc.                    | .. | .. | .. | .. | 6,014                    |
| Miscellaneous                             | .. | .. | .. | .. | 838                      |
| <i>Pyrogen Tests</i>                      |    |    |    |    |                          |
| Parenteral bulk solutions                 | .. | .. | .. | .. | 1,061                    |
| Blood products                            | .. | .. | .. | .. | 2                        |
| <i>Chemical and Physical Examinations</i> |    |    |    |    |                          |
| Parenteral bulk solutions                 | .. | .. | .. | .. | 1,130                    |
| Vitamin preparations                      | .. | .. | .. | .. | 343                      |
| Sulpha Drugs                              | .. | .. | .. | .. | 110                      |
| Miscellaneous                             | .. | .. | .. | .. | 261                      |
| <i>Toxicity Tests</i>                     |    |    |    |    |                          |
| Antitoxins, Toxoids Vaccines, etc.        | .. | .. | .. | .. | 592                      |

| Products                                                                    | No. of<br>samples tested |
|-----------------------------------------------------------------------------|--------------------------|
| <i>Tests for absence of infective virus</i>                                 |                          |
| Viral Vaccines                                                              | 300                      |
| <i>Potency Tests</i>                                                        |                          |
| Antitoxins                                                                  | 13                       |
| Antivenine                                                                  | 44                       |
| Toxoids                                                                     | 38                       |
| Triple antigen                                                              | 13                       |
| <i>Preservatives</i>                                                        |                          |
| Vaccine and antitoxins                                                      | 352                      |
| <i>Free formaldehyde Tests</i>                                              |                          |
| Bacterial vaccines and toxoids                                              | 178                      |
| <i>Microbiological assays</i>                                               |                          |
| Pemon                                                                       | 142                      |
| Vitamin preparations                                                        | 163                      |
| <i>Tests on raw materials</i>                                               |                          |
| Vitamins                                                                    | 206                      |
| Sulpha Drugs                                                                | 42                       |
| Miscellaneous, fine chemicals, antiseptics, pharmaceutical necessities etc. | 743                      |
| Grand Total                                                                 | 30,049                   |

## RADIATION BIOLOGY UNIT

### General

The Radiation Biology Unit started functioning during the year in the interest of public service and advancement of medical research. In the current progress of Nuclear Science and in an era of peaceful use of atoms, the unit was a necessity for a research institution like the Haffkine Institute.

Presently, radioactive nucleides have assumed a high priority in the diagnosis and prognosis of diseases. Their use simplifies many elaborate procedures, brings in more accuracy and also serves the purpose of treatment of diseases with their direct use in Radiopharmaceuticals or through their irradiations.

The Unit is headed by the Director and is staffed with one Senior Scientific Officer, Cl. II (Dr. N. G. Chanderkar, who was transferred from the Department of Zoonosis) as an officer in-charge of the Unit and one Research Scholar (Shri S. C. Talwalkar) financed by the Unichem Laboratories. Dr. (Kum.) S. Mavinkurve, who returned from U. K. after getting her Doctorate in Radiobiology, worked as a C. S. I. R. Pool Officer. A scheme for the conversion of this Unit into a full-fledged department is under consideration.

The Unit is engaged in carrying out thyroid function tests on patients referred by private practitioners and hospitals, at very reasonable charges. This has helped in bringing the most sophisticated thyroid function tests within the reach of a common man.

The thyroid function tests presently carried out here are as follows :—

- (a) Thyroidal uptake of Radio Iodine
- (b) Thyroxine suppression test
- (c) Radio-iodinated tri-iodo-thyronine uptake by red blood cells.
- (d) Protein-bound radio-iodine in plasma.

The Unit is equipped with Medical Spectrometer, Gamma Scintillation Well-type Counter and Gamma Scintillator Probe for thyroid tests. Recently, an irradiation source (Cobalt-60 Gamma Chamber) has been installed for radiation sterilisation of medical products.

### Routine

Thyroid function studies on cases referred to the Unit were carried out, which helped in the diagnosis of the disease. Details of the results of diagnosis of cases are as follows :—

|                  |    |    |    |    |
|------------------|----|----|----|----|
| (i) Hyperthyroid | .. | .. | .. | 24 |
| (ii) Hypothyroid | .. | .. | .. | 8  |
| (iii) Euthyroid  | .. | .. | .. | 29 |
| Total ..         |    |    |    | 61 |

## TOXICOLOGY AND HUMAN PHARMACOLOGY UNIT

The Toxicology and Human Pharmacology Unit was organised during this year with a view to study in detail the toxicity and human pharmacodynamics of synthetic compounds as well as biological agents prepared in this Institution. This unit is probably the first of its kind in India.

The unit also served to test acute, sub-acute and chronic toxicity of drugs and chemicals referred to it by various industrial concerns. Most drugs were of therapeutic value, but some were also of toxicological significance.

The unit works directly under the supervision of the Director. It consists of one Senior Research Fellow, Shri Phatak, and one Junior Research Fellow, Shri S. S. Jadhav.

The Central Council for Research in Indian Medicine and Homoeopathy also sanctioned a toxicological unit to work under Dr. B. B. Gaitonde for undertaking toxicity studies of some of the compounds investigated under the auspices of that Council. This unit consisted of one Assistant Research Officer, one Laboratory Assistant and an Animal Attendant. However, due to financial stringency, the post of Assistant Research Officer could not be filled. This unit, therefore, joined hands to work with the unit of the Institute. The activities of the unit are increasing rapidly and it is proposed to strengthen this unit of Toxicology and Human Pharmacology and to ultimately develop it into a full-fledged department.

The unit undertook toxicity studies of the compounds referred to it by the Central Council for Research in Indian Medicine and Homoeopathy under the code letter "Ayush". Thus, four different compounds were studied for their acute toxicity, out of which two were studied for their analeptic potentiality also. The unit also undertook extensive toxicity studies of a number of compounds referred to it by commercial firms (Refer Appendix I).

### APPENDIX I

#### *Routine Tests*

| Serial No. | Test                                           | Drugs                                         |
|------------|------------------------------------------------|-----------------------------------------------|
| 1          | Comparison of local intramuscular tolerance .. | Reverine and Terramycin.                      |
| 2          | Acute toxicity .. ..                           | Vallium injections.                           |
| 3          | Local irritancy studies .. ..                  | Testosterone Ca-Levulinate                    |
| 4          | Local irritancy studies .. ..                  | Vitamin B injections.                         |
| 5          | Sub-acute toxicity .. ..                       | Vallium ampoules                              |
| 6          | Acute toxicity .. ..                           | Vitamin B injections— I. M. and I. V. routes. |
| 7          | Oral acute toxicity .. ..                      | Pollyster wood finish.                        |
| 8          | Oral acute toxicity .. ..                      | Household spray                               |
| 9          | Eye irritation study .. ..                     | Chloramson eye drops                          |
| 10         | Undue toxicity .. ..                           | Various commercial samples.                   |
| 11         | Test for depressor substances .. ..            | 29 commercial samples.                        |

## DEPARTMENT OF VIRAL VACCINES

**General**

Dr. K. H. Dave, Assistant Director, was deputed to U.S.A. and Japan under a W. H. O. fellowship to study recent advances in the production and control of Oral Polio Vaccine.

Shri S. V. Pandit, Junior Scientific Officer, was awarded the degree of M.Sc. (Microbiology) for his thesis entitled "Isolation and characterization of rat viruses".

A team of three W. H. O. Consultants visited the Oral Polio Vaccine Production Unit. The team has made several recommendations before a regular production programme is undertaken. The Government of Maharashtra has been approached for permission to carry out the recommended structural alterations. The W. H. O. would supply the seed virus for production of Oral Polio Vaccine on completion of the recommended alterations.

**Production**

(a) *Processing of Oral Polio Vaccine.*—During the year under review, 21,50,720 doses of Trivalent Oral Polio Vaccine were distributed.

(b) *Production of Antirabic Vaccine.*—During the period under review, 36,38,548 ml. of Antirabic Vaccine for human use and 27,288 ml. of Canin Prophylactic Vaccine were produced.



## DEPARTMENT OF VIROLOGY

**General**

Dr. M. V. N. Shirodkar, Consultant Virologist, was appointed Head of the Department and Assistant Director In charge of the Department of Virology. He joined the Institute on December 17, 1973.

Dr. A. N. D. Nanavati was relieved on leave preparatory to voluntary retirement from November 1st 1973 to November 1, 1974.

Dr. S. M. Desai, Junior Scientific Officer, went on study leave for a period of one year from October 10, 1972 to the Argonne Cancer Research Hospital, Chicago, Illinois, U.S.A. He applied for extension of this leave for a further period of one year.

Kum. M. P. Kakalia, who was appointed to officiate as Junior Scientific Officer *vice* Dr. S. M. Desai, worked as Junior Scientific Officer from April 14, 1973 to July 4, 1973. Thereafter, she proceeded on leave on medical grounds and continued to be on leave during the period under review.

Dr. S. V. Gadre continued to work as a Government of Maharashtra Senior Research Fellow.

Dr. (Kum.) C.D.Kulkarni joined the department on July 20, 1973 as Medical Officer. M. M. S. Class III.

**Routine****I. HOST-VIRUS INTERACTION-APPLIED STUDIES : DIAGNOSIS****A. Rabies**

*Microscopy and mouse inoculation test.*—In all, 21 specimens were received by the Department for purposes of diagnosis or confirmation of rabies. Of these, 16 were post-mortem specimens; their distribution being : one dog salivary gland ; 5 dog brains ; 9 human brains and 1 cat brain.

The brain samples were examined both by microscopy of smears following staining by Seller's method and by intracerebral mouse inoculation. The results are presented in Table 1.

*Fluorescent antibody test.*—In order to minimise time, funds and effort and to maximize accuracy and efficiency in diagnosis and research, the fluorescence microscope has been speedily commissioned.

Although quite some time will undoubtedly elapse before this method becomes entirely a matter of routine, not only for the diagnosis of rabies, but for other viruses as well, active efforts are being made to standardize the technique or the ultimate purposes mentioned. Hyperimmune horse antirabies antiserum has been used for the preparation of the conjugate and smears from infected and uninfected brains are being examined to standardize the conjugate.

## B. Rubella

*Preparation of tissue cultures.*—Routine work during the year consisted mainly of preparation and maintenance of primary human amnion (PHA), VERO and BHK-21 cell cultures for use in rabies and rubella host virus studies at the cellular and molecular levels.

## C. Variola

*Virus isolation.*—Specimens of pustular material or of whole blood from a total of 14 cases suspected to be suffering from variola infection were referred to the Department by the Kasturba Infectious Diseases Hospital Laboratory, Bombay.

From 11 of these cases, the materials yielded pocks on the chick embryonic chorioallantoic membrane.

## II. Immunisation Clinic

As a service for the staff of the Institute and for the public, an Immunisation Clinic is conducted every working day between 11-00 and 13-00 hours by the Department of Virology. The following vaccines are administered :

- Oral polio vaccine (OPV)
- Antirabic vaccine (ARV)
- Antirabic horse hyperimmune serum (AHHS)
- Cholera (El Tor) vaccine
- Anti-typhoid-paratyphoid vaccine (TABC)
- Tetanus toxoid (TT)
- Triple antigen
- Diphtheria-tetanus antigen

The number of patients treated at the Clinic, at the time of writing this report, is 1120. Of these, 701 belonged to the Institute and 419 were from outside.

A total of 251 doses of OPV was dispensed, most of the patients having attended the Clinic on due dates. In all, 461 doses of ARV were administered and it was observed that those for whom a course of three injections was prescribed attended regularly. Some patients did not present themselves after the completion of seven injections, whilst a few discontinued the treatment after 12 inoculations. In 8 cases, minor local side reactions, such as erythema and pruritus were noted. Only in one case, following 10 injections subsequent to scratches received from a cat, there was inguinal lymphadenopathy. The treatment was discontinued after 12 injections in this particular case.

As far as triple antigen injections were concerned, a total of 74 doses was administered, most of the patients having observed regular attendance. Sixty-nine doses of TT were given and it was noted that few patients came for the

second and third immunising doses. Six doses of diphtheria-tetanus antigen were administered, the patients being regular in their attendance. Two hundred and fifty-nine doses of TABC vaccine were administered. On 25th August 1973, typhoid and cholera immunisation was carried out at the Haffkine Chawls. Most of the patients avoided the two-dose schedule. In two cases, side reactions consisting of pustules periorally and headache with pyrexia were noted.

TABLE 1  
*Diagnosis of rabies during the year*

| Species and specimens |    |    | Microscopy—ve<br>Inoculation—ve | Microscopy—ve<br>Inoculation+ ve | Microscopy+ ve<br>Inoculation+ ve |
|-----------------------|----|----|---------------------------------|----------------------------------|-----------------------------------|
| dog brains            | .. | .. | 2                               | 2                                | 1a                                |
| dog salivary gland    | .. | .. | 0                               | 0                                | 1<br>(Microscopy not applicable)  |
| cat brain             | .. | .. | 1                               | 0                                | 0                                 |
| Human brains          | .. | .. | 6                               | 1                                | 2a                                |

a—Virus not isolable either due to illness in normal mice or unsuitability of specimens

## DEPARTMENT OF ZOONOSIS

Cholera carrier studies initiated in Kadrabad area of Parbhani Town were concluded. Recently, these studies were initiated in Virar area of Thana District. Epidemiological studies in Dracunculiasis in Kolaba District were continued.

Epidemiological studies in plague were continued in 'D' and 'E' ward areas of Bombay City. A state-wide serological survey was undertaken by collecting rodent samples through various municipalities for the detection of inapparent foci of *Y. pestis* among commensal rodents. Epidemiological studies in filariasis were carried out in Uttan Village of Thana District.

Attempts to establish axenic culture of *E. histolytica* were continued.

Trainees from D.M.L.T. course were demonstrated different parasitological and entomological techniques. Two short-term trainees attended training course in Advanced Entomology and Parasitology.

Dr. V. H. Apte joined this department as Senior Scientific Officer, Class II (Parasitology) in October 1973. Shri B. D. Sathe and Kum. J. Y. Pradhan joined as Pool Officers.

Mr. Hussein Khalifet was awarded Ph.D. Degree in Microbiology. Smt. L. V. Sarma submitted her thesis for Ph.D. Degree in Microbiology.

Dr. M. V. Sant continued to guide Sarvashri D. S. Chitnis and A. K. Patil for M.Sc. Degree and Sarvashri R. S. Karbhari, K. K. Das, T. U. K. Menon R. P. Bhamaria, P. R. Dichwalkar, Smt. S. Kumar, Smt. S. Cherian and Kum. M. S. Kulkarni for Ph.D. degree.

Dr. M. V. Sant, Dy. Director (Research), attended International Atomic Energy Agency meeting at Austria, Vienna. He also attended a meeting of the I.C.M.R. Expert Committee on Cholera as its member. Dr. Sant attended the meeting of Indian Association of Pathologists at Madras, Association of Microbiologists of India at Poona and the Third National Conference on Infectious Diseases at Bombay. He also actively participated in the symposia sponsored by the Haffkine Institute to commemorate the Platinum Jubilee Celebrations.

Dr. D. M. Renapurkar and Dr. N. E. Vad were recognised by the Bombay University as post-graduate teachers for M.Sc. degree in Zoology. Dr. N. E. Vad delivered lectures on *snake prevention* and first-aid in snake bite at Messrs. Larson and Toubro at Lonavla and Messrs. Eagle Vacuum Glass Factory, Talegaon.

The department continued to provide expert guidance in respect of identification and control of insect and rodent pests of public health importance as also venomous reptiles. The table below shows the number of snakes and other items received for identification and advice.

TABLE 1

| Material received                  | Number |
|------------------------------------|--------|
| Snakes                             | 50     |
| Rodents, Insects and other queries | 8      |
| Rodent and insect control queries  | 3      |

Besides attending to the above queries, this department also supplied insects, snakes, rodents and micro-slides of insects to various research and educational organisations. Table 2 below lists the number of various items supplied to various institutions.

TABLE 2

| Material supplied                     |    |    |    |    | Number |
|---------------------------------------|----|----|----|----|--------|
| Dead Cobra                            | .. | .. | .. | .. | 5      |
| Dead Russell's Viper                  | .. | .. | .. | .. | 3      |
| Dead Krait                            | .. | .. | .. | .. | 5      |
| Dead <i>Echis carinatus</i>           | .. | .. | .. | .. | 4      |
| Dead non-poisonous snakes             | .. | .. | .. | .. | 4      |
| Dead Scorpions                        | .. | .. | .. | .. | 4      |
| Snake skins                           | .. | .. | .. | .. | 8      |
| <i>X. cheopis</i> (fleas)             | .. | .. | .. | .. | 12     |
| <i>M. domestica</i> (flies)           | .. | .. | .. | .. | 12     |
| <i>Culex P. fatigans</i> (mosquitoes) | .. | .. | .. | .. | 12     |
| <i>P. americana</i> (cockroaches)     | .. | .. | .. | .. | 3      |

Colonies of fleas (*X. cheopis* and *X. astia*), flies (*Musca domestica nebulosa*), Cockroaches (*P. americana*), mosquitoes (*Culex P. fatigans*) and rodents (*Tatera indica*) were continued to be maintained for insecticide and plague studies. Peel-Grady chamber was erected, for the first time, for the valuation of insecticide aerosol formulations. Presently, evaluation of two aerosol insecticide formulations D-1 and D-2 is underway.

### visitors

Visitors from various walks of life and from different parts were demonstrated the actual venom extraction technique and were given detailed information on identification of snakes and first-aid treatment of snake-bite cases etc. The total number of visiting teams and distinguished visitors, who visited this department, is shown in the following table:—

TABLE 3

| Details                               |    |    |    |    | No. of teams and demonstrations |
|---------------------------------------|----|----|----|----|---------------------------------|
| Students from different colleges      | .. | .. | .. | .. | 5,037                           |
| visitors from scientific institutions | .. | .. | .. | .. | 2,595                           |
| distinguished Visitors                | .. | .. | .. | .. | 405                             |

About 8,037 people saw the venom extraction demonstration.

## Production

During the period under report, 207·31 gms. of snake venom was processed and 372·81 gms. of venom was supplied for the manufacture of antivenion and research purposes as shown in the tables below :—

TABLE 4

| Snake venom details |    |    |    | Quantity of venom in gms. |          |
|---------------------|----|----|----|---------------------------|----------|
|                     |    |    |    | Processed                 | Supplied |
| Krait               | .. | .. | .. | 44·29                     | 66·19    |
| Cobra               | .. | .. | .. | 77·65                     | 111·03   |
| Russell's Viper     | .. | .. | .. | 50·10                     | 92·02    |
| Echis               | .. | .. | .. | 35·27                     | 58·57    |
| Total               | .. |    |    | 207·31                    | 327·81   |

The quantity of scorpion venom processed and supplied is shown in the Table 5 below :—

TABLE 5

| Scorpion venom                                  |    | Quantity of venom in gms. |          |
|-------------------------------------------------|----|---------------------------|----------|
|                                                 |    | Processed                 | Supplied |
| Black scorpion ( <i>Palamaneus gravimanus</i> ) | .. | 11·50                     | 10·42    |
| Red Scorpion ( <i>Buthus tamulus</i> )          | .. | 11·01                     | 5·38     |
| Total                                           | .. | 22·51                     | 15·80    |

The number of snakes and scorpions purchased during the year is shown in the Table 6 below :—

TABLE 6

| Snake species        |    |    |    | Total number |
|----------------------|----|----|----|--------------|
| Cobra                | .. | .. | .. | 117          |
| Krait                | .. | .. | .. | 644          |
| Russell's Viper      | .. | .. | .. | 133          |
| Echis                | .. | .. | .. | 814          |
| Non-poisonous snakes | .. | .. | .. | 235          |
| Red scorpions        | .. | .. | .. | 4,674        |
| Black scorpions      | .. | .. | .. | 9,305        |

## ANIMAL FARM

The breeding and supply of different species of laboratory animals were satisfactory. The farm met the requirements of the departments during the year under report.

Twelve students of Diploma in Medical Laboratory Technology (D.M.L.T.) and 11 students of Medical Laboratory Technicians' Training Course (M.L.T.) were trained in general handling and management of laboratory animals. Sixteen trainees of Laboratory Animals Information Service of the Indian Council of Medical Research were provided facilities for practical work in connection with their course. Dr. C. Williams, Shri Gulabchand Kanojia and Kum. L. R. Iyer attended short-term training course in breeding, maintenance and management of laboratory animals during the year.

Facilities were provided by the Institute and the Animal House Section for the Workshop conducted on behalf of the University Grants Commission. It was attended by representatives of many Universities receiving information on care, breeding and management of laboratory animals.

The figures mentioned below will show the number of different species of laboratory-bred animals in the Animal Farm and supplied to the departments of this Institute and sold to other laboratories during the year.

| Species of animals |    |    |    | Production | Supplied to departments | Sold to other laboratories |
|--------------------|----|----|----|------------|-------------------------|----------------------------|
| White mice         | .. | .. | .. | 2,38,705   | 1,78,947                | 7,519                      |
| Albino rats        | .. | .. | .. | 16,674     | 7,673                   | 5,063                      |
| Rabbits            | .. | .. | .. | 2,412      | 1,607                   | 533                        |
| Guineapigs         | .. | .. | .. | 5,351      | 3,948                   | 1,023                      |
| Hamsters           | .. | .. | .. | 3,098      | 792                     | 126                        |
| Cotton rats        | .. | .. | .. | 115        | 3                       | ..                         |
| Total              |    |    |    | 2,66,355   | 1,92,970                | 14,264                     |

In addition to the breeding of the abovementioned species of animals, sheep, monkeys, cats and fowls were maintained as per the requirements of different departments of this Institute.

## LIBRARY

**Reorganization**

The efforts to make available a Xerox copying machine to this library were successful this year and a machine has recently been purchased. This was a long felt need of the Haffkine Institute Library. The machine is also provided with an attachment with the help of which microfilmed documents can be enlarged and made available on paper in a readable size. This facility has been kept open for outsiders also at a nominal charge on "No profit no loss" basis. This facility will really prove to be a boon to the research workers from this Institute as well from outside.

The work of collecting references and getting reprints, wherever possible on Cholera, in respect of papers published during last five years, was taken up last year as a first step towards establishment of a reprint library in communicable diseases like Cholera, Plague, Poliomyelitis, Typhoid, etc. This work has almost been completed and this section has been able to collect over 1,300 references from different sources like Index Medicus, Tropical Diseases Bulletin, Current Contents etc. and to get about 200 reprints from authors and other sources. A new division viz., "Haffkine Biography Division", has, been started in the library from the Platinum Jubilee year. This division will collect and preserve documents on the biography of Haffkine and his work. This work is in progress.

**General**

During the year, two library committee meetings were held and the reorganization and other related matters were discussed. Useful suggestions were made by the members of the library committee to improve the library services. The library continued to participate in the international exchange programme sponsored by the W.H.O. and could procure about 30 issues of different journals to complete the sets of the journals. During the year, 51 books were donated to this library by CIBA Foundation, W.H.O. Dr. (Mrs.) Lutzkar and many others.

The library continued to participate in the inter-library loan service. The extension of working hours up to 7-00 p.m. was continued during the year.

**Library Working**

The following figures indicate the working of the library during the year :—

|                                                                |       |
|----------------------------------------------------------------|-------|
| Number of outside readers visiting the library ..              | 4,728 |
| Number of books and periodicals issued to our staff ..         | 1,540 |
| Number of periodicals issued under inter-library loan service. | 260   |

Books and periodicals obtained for our staff from other libraries. 56

**Additions to Library**

|                                                          |     |
|----------------------------------------------------------|-----|
| Number of books added during the year ..                 | 230 |
| Number of volumes bound .. ..                            | 436 |
| Number of current periodicals subscribed ..              | 183 |
| Current periodicals received free of charge ..           | 115 |
| Number of reports of scientific Institutions received .. | 32  |



## LIST OF PAPERS PUBLISHED DURING THE YEAR 1973-74

Bapat, J. P. and Baxi, A. J. :

Quantitative levels of Glucose-6-phosphate dehydrogenase, Pyruvate Kinase and glutathione in normal and G-6-PD deficient individuals (1973).

*Indian Journal of Medical Research*, 61, 1031—35

Bapat, J. P., Baxi, A. J. and Kulkarni, K. V. :

A rapid Counter immunoelectrophoretic technique for the detection of Australia antigen and Alpha-fetoprotein on the same slide (1973).

*Indian Journal of Medical Research*, 61, 1036—38

Chatterjee, P. K., Rao, S. S. and Dutta, N. K. :

Studies on the culture filtrates of some NAG Strains (1973).

*Indian Journal of Medical Research*, 61, 1744—50

Chatterjee, P. K., Rao, S. S. and Dutta, N. K. :

Studies on the nature and antigenicity of the toxins produced by *V. cholerae* (1973).

*Indian Journal of Medical Research*, 61, 1051—57

Desai, S. M., Sehgal, P. B., Nanavati, A. N. D. and Shirodkar, M. V. :

A Rabbits-induced Serum Factor inhibiting Rous Sarcoma Virus in Chicks (1973).

*Journal of General Virology*, 19, 285—93

Gaitonde, B. B. :

Impact of legislation on therapeutics (1973)

*Indian Journal of Pharmacology*, 5, 80—83

Gaitonde, B. B., Dattani, K. and Morwani, K. :

Antipyretic Activity of Ibuprofen (Brufen). (1973).

*Journal of the Association of Physicians of India*, 21, 579—84

Kankonkar (Smt.), S., Gatlwar, W. N. and Sant, M. V. :

Diagnosis of Filariasis by indirect Haemagglutination Test (1973)

*Indian Journal of Medical Research*, 61, 1413—15

Nanavati, A.N.D. :

Rabies : A review of current problems and trends (1973)

*Indian Journal of Medical Sciences*, 29, 649—55

Rumao, L. M. and Sant, M. V. :

Seriological Typing of Beta-Hemolytic streptococci Group-A (1973).

*Journal of Post-Graduate Medicine*, 19, 63—67

# LIST OF PAPERS SENT FOR PUBLICATION DURING THE YEAR 1973-74

Apte, V. H. :

Estrus Cycle (Vaginal smear examination)

*The Proceedings of Workshop on Laboratory Animals*

Bhide, M. B. :

Biological equivalence and Non-equivalence of generically identical drug products—My experience.

## BIMA

Bhide, M. B. and Naik, P. Y. :

Mechanism of action of thalidomide in leprosy patients suffering from reactions.

*Indian College of Allergy and Applied Immunology*

Bhide, M. B., Naik, P. Y., Mahajani, S. S. ; Goohi, R. B. and Joshi, R. S. :

(1) Pharmacological evaluation of Vasicinone as an anti-asthmatic drug.

(2) Studies on the anti-asthmatic effects of *Tylophora Indica*.

*Bulletin of Haffkine Institute*

Bhide, M. B., Nikam, S. T. and Chavan, S. R. :

Bioavailability of Nitrofurantoin from commercially available nitrofurantoin tablets.

*Bulletin of Haffkine Institute*

Bhide, M. B., Pilankar, P. D., and Dholam, S. B. :

Comparative Bioavailability of seven brands of ampicillin.

*Bulletin of Haffkine Institute*

Chavan, S. R., Nikam, S. T., Pradhan, D. V., Bhide, M. B. and Gaitonde B. B. :

Bio-availability of INH in liquid dosage form.

*Indian Journal of Physiology and Pharmacology*

Dave (Smt.), J. K. and Fernandes (Smt.), Y. :

Mammary Carcinoma with Cartilaginous Metaplasia.

*Indian Journal of Cancer*

Divakaran, P., Rao, N. R. and Venkatraman (Smt.), A. :

Serum cholesterol levels in a typical urban group of population using coconut oil as the chief dietary fat

*Bulletin of Haffkine Institute*

Farrow, M. M., Dighe, P. Y. and Nanavati, A.N.D. :

An Investigation for possible rabies carriers among dogs in Bombay.

*Indian Journal of Medical Research*

aitonde, B. B. and Jetmalani, M. H. :

Effects of cholinergic blocking agents on response of isolated rat urinary ladder.

*Indian Journal of Pharmacology*

aitonde, B. B., Kulkarni, H. J., Nabar, S. D. and Jogalekar, S. N.,  
Diuretic activity of Punarnava (*Boerhavia Diffusa*, Linn).

*Bulletin of Haffkine Institute*

apadia (Kum.), B. K., Shah, M. H. and Deliwala, C. V. :  
New Hydantoin derivatives as anti-convulsants.

*Bulletin of Haffkine Institute*

Kini, R. P., Patel (Smt.) , M. R. and Shah, M. H. :

Chemotherapy of tuberculosis—Synthesis and study of some ketone thioisocarbazoncs.

*Bulletin of Haffkine Institute*

alvankar, T. S., Sabnis, S. S., Patel (Smt.), M. R. and Deliwala, C. V. :  
Chemotherapy of bacterial infections.

*Bulletin of Haffkine Institute*

imbkar, A. Y. and Gaitonde, B. B. :

Selective action of tetrodotoxin on the responses of isolated stomach fundal strip of rat induced by field stimulation.

*Bulletin of Haffkine Institute*

Nimkar, Y. S. , Karbhari, R. S., Renapurkar, D. M. and Sant , M. V. :

Susceptibility of rats to plague and presence of antibodies to plague in rat sera.

*Bulletin of Haffkine Institute*

Patel, H. V., Shah , M. H. and Deliwala, C. V. :

New B-Unsaturated ketones as possible diuretics.

*Bulletin of Haffkine Institute*

Patil, A. K., Saldhana, F. L. and Sant, M. V. :

Studies on *Vibrio parahaemolyticus* in fish in the Coast of Bombay : Part I.

*Bulletin of Haffkine Institute*

Renapurkar, D. M., Menon, T.U.K., Bhat (Kum.), V. M. and Sant, M. V. :

Tolerance in *Bandicota bengalensis* to Warfarin .

*Bulletin of Haffkine Institute*

Renapurkar, D. M. and Sant, M. V. :

Changing ecology and plague.

*Bulletin of Haffkine Institute*

Saldhana, F. L., Sant, M. V., Jhala, H. I. and Nimbkar, Y. S. :

Studies on ecology and symbiosis of *V. cholerae* and *E. coli*.

*Bulletin of Haffkine Institute*

Sant, M. V. :

Some aspects of zoonosis.

*The Proceedings of Workshop on Laboratory Animals*

Shekawat, D. R., Sabnis, S. S. and Deliwala, C. V. :

Potential anti-cancer agents V-Schiff basis from P-(3-azaspiro-[5.5] undec-3-y) benzaldehydes.

*Bulletin of Haffkine Institute*

Soni, H. K. and Pradhan, D. V. :

Stability of Chloramphenicol Palmitate Suspension

*Bulletin of Haffkine Institute*

Venkataraman (Smt.) , A. :

A critical study of the conditions for the colorimetric estimation of serum transminases : Part II.

*Bulletin of Haffkine Institute*

Venkataraman (Smt.) , A. :

The nucleotide composition of the ribonucleic acids from the *Salmonella* organisms.

*Bulletin of Haffkine Institute*

## MAINTENANCE ENGINEERING SECTION

The year under review kept the Section fully busy with varied activities due to the over all increase in the activities of the Institute.

The Engineers and Technicians of the Section carried out a number of fabrication and execution jobs and routine maintenance work. Constant attention to Desivac Plant of the Blood Bank Department has resulted in the smooth working of that department.

It is gratifying to note that the Mechanical Section carried out a number of jobs, such as designing, dies and punches to cut 4" diameter aluminium foil and preparing about 3,000 such pieces which helped the Media Section in the use of saline bottle capping. In addition to this, a large number of additions and alterations to the various pharmaceutical machineries were carried out as per the requirements of the departments. Such types of jobs are highly noted for by outside agencies. This has also resulted in saving loss of production hours.

The Electrical Section played a major role in the repair and maintenance work. The staff undertook a number of electrical connections to various machineries and also maintained ovens, incubation rooms, centrifuge machines, compressors, vacuum pumps, shaking machines, stirrers and different types of electrical motors have been maintained by this section regularly. In addition to this, a separate cell was created in the Engineering Section for maintaining and servicing various electronic instruments, such as pH metres, colorimeters, power-pack, spectrophotometers, etc., which were being so far maintained and serviced by outside agencies.

All this was made possible as a result of the training of the Electrical Supervisor in handling electronic instruments etc. The Refrigeration Unit also played a significant role in the field of production activities. A large number of refrigeration plants were maintained, i.e. deep-freeze, refrigerators, cold rooms, air conditioners, bottle coolers, walk-in-coolers, water coolers etc. It is gratifying to note that this year, the unit has installed 5 walk-in-coolers departmentally for the use of the various departments, thus providing increased cold room facilities to the departments of the Institute as a whole.

The Carpentry Section also contributed substantially to carpentry jobs undertaken at the Institute, both for production and research departments and also for other services and administrative departments.

During the year, an additional activity of Mobile Maintenance Unit, which was formerly under the control of the Director of Health Services, was entrusted to the Maintenance Engineering Section of this Institute.

The staff of this Unit was stationed at the Institute under the immediate supervision of the Assistant Engineer (Maintenance). This Unit undertook repairs and maintenance of vehicles belonging to officers of the Health Department in Greater Bombay. This has resulted in quick servicing and maintenance of Government vehicles and has also effected considerable saving in time and money.

In short, this Section has played quite a significant role in the total output of the Institute.

## BULLETIN OF HAFFKINE INSTITUTE

The Institute has started the publication of a tri-annual scientific journal called 'Bulletin of Haffkine Institute' scheduled to be out in April, August and December. The first issue, 'The Platinum Jubilee Number', was released on 10th August 1973—the day the Platinum Jubilee Year began. The Bulletin is devoted to Biomedical Sciences and project researches carried out at the Institute. Every issue contains a review article by an eminent scientist. The Bulletin of Haffkine Institute is edited and managed by Dr. F. L. Saldanha, Assistant Director in charge of Quality Control Department. The Editorial Board comprises of distinguished scientists with the Director Dr. B. B. Gaitonde, as the Chairman.

### **Platinum Jubilee Commemoration Volume**

A Platinum Jubilee Commemoration Volume, with a glimpse into the history, achievements, functions and future plans of the Institute, compiled and edited by Dr. F. L. Saldanha, was released on the 14th January 1974 the Inauguration Day of the Platinum Jubilee Celebrations.

## CRITICS' CLUB

The Club continued its activities under the Secretaryship of Dr. H. J. Man-sharamani. A total of 21 meetings were held during the year under review. Seven meetings were addressed by Guest Scientists from outside the Institute, while the remaining 14 were addressed by scientists from within the Institute.

The activities of the Critics' Club had to be suspended from December 1973 to March 1974 to allow members of the staff to participate in the preparations for the Platinum Jubilee celebrations beginning in January 1974.

The details of the scientific meetings are given below :—

## INSTITUTE SPEAKERS

| <i>Speaker</i>         |    | <i>Subject</i>                                                                                            |
|------------------------|----|-----------------------------------------------------------------------------------------------------------|
| Kum. R. S. Buit        | .. | .. Cyclic A. M. P. (A Review)                                                                             |
| Shri D. V. Pradhan     | .. | .. Studies on the use of <i>Schizosaccharomyces pombe</i> in the assay of pantothenic acid and its salts. |
| Kum. J. M. Kikani      | .. | .. Use of labelled glucose for sterility testing.                                                         |
| Shri J. P. Bapat       | .. | .. Red-cell enzyme activities as an index of preservation in various anti-coagulants.                     |
| Shri B. G. Khadse      | .. | .. A Review on Anthelmintic                                                                               |
| Dr. D. M. Renapurkar   | .. | .. Epidemiological studies in Dracunculosis.                                                              |
| Dr. K. H. Dave         | .. | .. Studies on Simian intestinal viruses.                                                                  |
| Smt. A. A. Gandhi      | .. | .. Studies in tetanus toxin production using peptic digest of meat (PDM) medium.                          |
| Shri V. P. Shinde      | .. | .. Quadruple cholera vaccine in peanut protein hydrolysate.                                               |
| Shri R. B. Ghooi       | .. | .. Indigenous drugs in the treatment of bronchial asthma.                                                 |
| Dr. A. N. D. Nanavati  | .. | .. Freedom and responsibility in research.                                                                |
| Shri Husein Ali Mutlag | .. | .. Effect of phenylmercuric nitrate on <i>B. subtilis</i> spores.                                         |
| Dr. John Lopes         | .. | .. Plasma membranes of human blood cells and lymphocytes.                                                 |
| Dr. K. V. Kulkarni     | .. | .. Radio-immune assay for Australia antigen (HBAG) a comparative study.                                   |

## GUEST LECTURES

- Dr. A. Myasnyenko, Iuanowski Institute of Virology, Moscow. Studies on transport and transmission of arboviruses by migratory birds.
- Dr. G. S. Mutalik, Dean, Sassoon Hospital, Poona. Human Genetics.
- Dr. (Smt.) Shanta S. Rao, Dy. Director and Administrative Head, Institute for Research in Reproduction (ICMR), Parel, Bombay. Current trends in fertility control carried out in the country.
- Dr. Charles H. Campbell, Associate Professor, Tropical Medicine, The School of Public Health and Tropical Medicine, the University of Sydney, N.S.W, 2006, Australia. The clinical effects of Australasian snake bite on the nervous system.
- Dr. Warren Levinson, Associate Professor, Department of Microbiology, University of California, San Francisco, California 94122. RNA-dependent DNA polymerase of Rous sarcoma virus.
- Dr. V. R. Mody, Professor of Medicine Chief, Division of Infectious Diseases, Howard Medical School, Washington, U. S. A. Anaerobic infections.
- Dr. Keerti V. Shah, Professor of Pathobiology, Johns Hopkins University, School of Hygiene and Public Health, Baltimore, U.S.A. New human Papovaviruses : possible role in disease.



## DISTINGUISHED VISITORS

|                        |    |    |                                                                                                                   |
|------------------------|----|----|-------------------------------------------------------------------------------------------------------------------|
| Dr. A. Myasnyenko      | .. | .. | Epidemiologist, Institute of Virology named by Ivanovski.                                                         |
| Dr. B. Obukhova        | .. | .. | Virologist, Institute of Virology named by Ivanovski.                                                             |
| D. G. Sidozova         | .. | .. | Medical Zoologist, Institute of Virology named by Ivanovski.                                                      |
| Dr. S. Nesski          | .. | .. | Parasitologist, Institute of Natural Foci of Infection.                                                           |
| Dr. Joseph Szita       | .. | .. | National Institute, of Public Health, Budapest, Hungary.                                                          |
| Dr. (Mrs.) Agnex Ecker | .. | .. | National Institute of Public Health, Budapest, Hungary.                                                           |
| Mr. Anne Fiqrtoft      | .. | .. | Editor, Norwagian Broadcasting Corporation, Osle 3, Norway.                                                       |
| Mr. Bengt Jonson       | .. | .. | Setora Film Company, Sweden.                                                                                      |
| Dr. Popa Serban        | .. | .. | Ministry of Health, Rumania, Bucarest.                                                                            |
| Dr. Dragomirescu Mihai | .. | .. | Professor in Institute of Medicine, Timisora, Rumania.                                                            |
| Dr. H. W. Perera       | .. | .. | Associate Port Health Officer, Colombo, Sri Lanka.                                                                |
| Dr. J. Warwick Bucklev | .. | .. | Research Department, The Boots Company, U. K.                                                                     |
| Shri B. V. Laud        | .. | .. | Labour Commissioner, Bombay.                                                                                      |
| Dr. Allen G. Noble     | .. | .. | University of Akron, Ohio, U.S.A.                                                                                 |
| Dr. Khin Mawang Tin    | .. | .. | Head, Division of Experimental Medicine, Department of Medical Research, Ministry of Health, Government of Burma. |
| Dr. S. S. Gotheskar    | .. | .. | Deputy Drug Controller (India).                                                                                   |
| Shri S. N. Khatri      | .. | .. | Assistant Director (Law), National Police Academy, Abu.                                                           |
| Dr. Maung Mg Gale      | .. | .. | Deputy Director, Central Research Organization, Rangoon, Burma.                                                   |
| Dr. Mai Aung           | .. | .. | Deputy Director, Central Research Organization, Rangoon, Burma.                                                   |
| Shri R. K. Khadilkar   | .. | .. | Union Health Minister, New Delhi.                                                                                 |
| Dr. Rafiq Zakaria      | .. | .. | Minister, Public Health, Urban Development and Aukaf, Government of Maharashtra.                                  |
| Shri A. N. Namjoshi    | .. | .. | Minister, Education and Sports (Except Indian Games), Government of Maharashtra.                                  |

|                              |    |                                                                                                                                  |
|------------------------------|----|----------------------------------------------------------------------------------------------------------------------------------|
| Shri Pandit Shiv Sharma      | .. | Chairman, Scientific Advisory Board, Central Council for Research in Indian Medicine and Homoeopathy.                            |
| Brig. P. V. Ramachandran     | .. | Assistant Director of Medical Services, Headquarters, Maharashtra and Gujarat Area, Colaba, Bombay.                              |
| Dr. Leela Ramchandran        | .. | C/o Brig P. V. Ramachandran, Assistant Director of Medical Services, Headquarters, Maharashtra and Gujarat Area, Colaba, Bombay. |
| Major A. G. Gokaran          | .. | C/o Assistant Director of Medical Services, Headquarters, Maharashtra and Gujarat Area, Colaba, Bombay.                          |
| Dr. C. P. Canaw              | .. | Guyana Rice Corporation, Guyana.                                                                                                 |
| Shri M. S. Pawar             | .. | Vice-Chancellor, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar.                                                            |
| Dr. and Mrs. William Jackson | .. | Bowling Green State University, Bowling Green, Ohio, U.S.A.                                                                      |
| Dr. C. H. Campbell           | .. | School of Public Health and Tropical Medicine, University of Sydney, Australia.                                                  |
| Dr. Ramane Rubinelli         | .. | Lakeside Laboratories, Milwaukee, Wisconsin, U.S.A.                                                                              |
| Professor Frederick Coulston | .. | Institute of Experimental Pathology and Toxicology, Albany Medical College of Union University, New York.                        |
| Shri Budhadeo D. Singh       | .. | State Health Minister, Government of Bihar.                                                                                      |
| Dr. A. Ramchandra Rao        | .. | Honorary Consultant, Department of Health, Municipal Corporation of Madras.                                                      |
| Dr. L. B. T. Jayasundara     | .. | Deputy Director, Laboratory Services, Sri Lanka.                                                                                 |
| Dr. Warren Levinson          | .. | Associate Professor, Department of Microbiology, University of California.                                                       |
| Dr. Van Hemert               | .. | Head of the Vaccine Department, Netherlands.                                                                                     |
| Dr. Francis Lothe            | .. | Medical Officer, Health Laboratory Services Unit, World Health Organisation, Geneva (Switzerland).                               |
| Dr. Vinod R. Mody            | .. | Chief, Division of Infectious Diseases, Howard University, Washington, U.S.A.                                                    |

|                            |    |    |                                                                                                                      |
|----------------------------|----|----|----------------------------------------------------------------------------------------------------------------------|
| Dr. C. A. Franklin         | .. | .. | Police Surgeon, Bombay.                                                                                              |
| Dr. W. Chas Cockburn       |    | .. | Chief Medical Officer, Virus Diseases,<br>World Health Organization, Geneva,<br>Switzerland.                         |
| Dr. Paul D. Parkman        | .. | .. | Bureau of Biological Food and Drug<br>Administration, New York.                                                      |
| Dr. I. Archetti            | .. | .. | Chief, Virus Department, Laboratory<br>of Microbiology, Institute of<br>Hygiene, Rome.                               |
| Dr. R. Hlavaty             | .. | .. | Trade Commissioner, Trade Repre-<br>sentation of the Czechoslovak<br>Socialist Republic, Bombay.                     |
| Dr. N. W. Preston          | .. | .. | Reader in Bacteriology, Department<br>of Bacteriology and Virology<br>University of Manchester,<br>Manchester, U. K. |
| Shri Ali Yawar Jung        | .. | .. | Governor of Maharashtra.                                                                                             |
| Shri V. P. Naik            | .. | .. | Chief Minister of Maharashtra                                                                                        |
| Shri G. S. Sarnayak        | .. | .. | Minister of State for Health, Maha-<br>rashtra.                                                                      |
| Shri Sudhir Joshi          | .. | .. | Mayor of Bombay                                                                                                      |
| Dr. Habibuz Zaman          | .. | .. | Regional Medical Officer, Fellowships,<br>World Health Organization, New<br>Delhi.                                   |
| Dr. Sidney Hamilton        | .. | .. | Medical Advisor, British High Com-<br>mission, New Delhi.                                                            |
| Dr. B. Kuppuswamy          | .. | .. | Parasitologist, Mysore Serum Institute,<br>Habbal, Bangalore (Karnataka<br>State.)                                   |
| Dr. A. Megoutchi           | .. | .. | Medical Officer, World Health Organi-<br>zation, New Delhi.                                                          |
| Dr. (Smt.) S. M. Vachha    |    | .. | Assistant Director of Health Services,<br>In-Charge State Public Health<br>Laboratory, Poona.                        |
| Dr. (Smt.) Barbara Serafin |    | .. | Professor, Technical University,<br>Warsaw, Poland.                                                                  |
| Dr. Alexandru Popascu      |    | .. | Institute Cantacuzino, Bucharest,<br>Rumania.                                                                        |
| Dr. Ion Cherciu            | .. | .. | Institute Cantacuzino, Bucharest,<br>Rumania.                                                                        |
| Dr. George Nicolau         | .. | .. | Hospital of Communicable Diseases,<br>"COLENTINA", Bucharest,<br>Rumania.                                            |

**VISITING SCIENTISTS AND OTHER DIGNITARIES VISITING  
HAFFKINE INSTITUTE DURING THE PLATINUM JUBILEE  
YEAR**

**Indian Scientists**

|                           |    |    |                                                                                                                                         |
|---------------------------|----|----|-----------------------------------------------------------------------------------------------------------------------------------------|
| Dr. R. K. N. Sanders      | .. | .. | Medical Superintendent, The Duncan Hospital, Raxaul, Champaran District, Bihar.                                                         |
| Dr. K. C. Agarwal         | .. | .. | Associate Professor of Microbiology, Department of Microbiology, Post-Graduate Institute of Medical Education and Research, Chandigarh. |
| Dr. (Smt.) V. R. Parvathy |    | .. | Assistant Professor of Pediatrics and Medical Officer, Institute of Child Health and Hospital for Children, Egmore, Madras.             |
| Dr. N. S. Deodhar         | .. | .. | Professor of Preventive and Social Medicine, B. J. Medical College, Poona.                                                              |
| Dr. S. S. Jolly           | .. | .. | Professor and Head, Department of Medicine, Medical College, Patiala.                                                                   |
| Dr. G. S. Sainani         | .. | .. | Head of the Department of Medicine, B. J. Medical College, Poona.                                                                       |
| Dr. Hari Vaishnava        | .. | .. | Professor of Medicine, Maulana Azad Medical College, New Delhi.                                                                         |
| Dr. V. B. Athavale        | .. | .. | 5, Shiv Mahal, Opposite Sion Hospital, 26, Sion Main Road, Bombay.                                                                      |
| Dr. D. D. Banker          | .. | .. | Bacteriology Department, Glaxo Laboratories (I) Ltd., Worli, Bombay.                                                                    |
| Dr. J. A. D'Sa            | .. | .. | Bacteriology Department, Glaxo Laboratories (I) Ltd., Worli, Bombay.                                                                    |
| Dr. L. Marquis            | .. | .. | Bacteriology Department, Glaxo Laboratories (I) Ltd., Worli, Bombay.                                                                    |
| Dr. F. D. Dastur          | .. | .. | Flat 12, Block 4, Tata Mills Housing Colony, Elphinstone Road, Bombay.                                                                  |
| Dr. K. G. Nair            | .. | .. | Director-Professor, Department of Medicine, Seth G. S. Medical College and K. E. M. Hospital, Bombay.                                   |
| Dr. S. M. Merchant        | .. | .. | 101, Walkeshwar Road, Bombay.                                                                                                           |
| Dr. S. S. Rao             | .. | .. | 37, Citizen's Co-operative Housing Society Ltd., 26, S. V. Savarkar Marg, Bombay.                                                       |
| Dr. N. S. Tibrewala       | .. | .. | Rungta House, 68-A, Nepean Sea Road, Bombay.                                                                                            |
| Dr. P. M. Udani           | .. | .. | Al Jabereya Court, Netaji Subhash Road, Bombay.                                                                                         |
| Dr. B. J. Vakil           | .. | .. | Colaba Mills Flats, Sorab Bharucha Road, Bombay.                                                                                        |

|                      |    |    |                                                                                                                               |
|----------------------|----|----|-------------------------------------------------------------------------------------------------------------------------------|
| Dr. M. M. Wagle      | .. | .. | Moti Mahal, Jamshedji Tata Road, Bombay.                                                                                      |
| Dr. V. N. Rao        | .. | .. | Director of Health Services, Government of Maharashtra, Bombay.                                                               |
| Dr. G. K. Karandikar | .. | .. | Director of Medical Education and Research, St. George's Hospital Compound, Bombay.                                           |
| r. G. S. Mutalik     | .. | .. | Joint Director of Medical Education and Research, St. George's Hospital Compound, Government Dental College Building, Bombay. |
| Dr. T. R. Rindani    | .. | .. | Joint Director, Jaslok Hospital, Bombay.                                                                                      |
| r. F. J. Mendonca    | .. | .. | Ex-Director of Health Services, Government Quarters, Narayan Dabholkar Road, Bombay.                                          |
| r. M. S. Kekre       | .. | .. | Dean, Topiwala National Medical College and B. Y. L. Nair Charitable Hospital, Bombay.                                        |
| Dr. R. K. Gadgil     | .. | .. | Dean, J. J. Group of Hospitals and Grant Medical College, Bombay.                                                             |
| Dr. C. K. Deshpande  | .. | .. | Dean, K. E. M. Hospital and Seth G. S. Medical College, Bombay.                                                               |
| r. H. I. Jhala       | .. | .. | Medical Superintendent, Sir Harkinsondas Narottamdas Hospital, Charni Road, Bombay.                                           |
| rofessor T. K. Tope  | .. | .. | Vice-Chancellor, University of Bombay.                                                                                        |
| r. M. Paul Anand     | .. | .. | Medical Director, Glaxo Laboratories (I) Ltd., Worli, Bombay.                                                                 |
| r. D. K. Dastur      | .. | .. | Professor of Neuropathology, Grant Medical College and J. J. Group of Hospitals Bombay.                                       |
| Dr. M. D. Deshmukh   | .. | .. | Honorary Professor of Tuberculosis, Grant Medical College and J. J. Group of Hospitals, Bombay.                               |
| Dr. Arthur E. De Sa  | .. | .. | Consulting Surgeon, Bombay Hospital, Bombay.                                                                                  |
| Dr. C. G. Pandit     | .. | .. | Ex-Director, Indian Council of Medical Research, Poona.                                                                       |
| Dr. F. P. Antia      | .. | .. | 534, Sandhurst Bridge, Bombay.                                                                                                |
| Dr. B. Bhandari      | .. | .. | 90 Road "L", Bhupalpura, Udaipur, Rajasthan.                                                                                  |
| Dr. N. K. Channapa   | .. | .. | Professor and Head of the Department of Medicine, Karnatak Medical College, Hubli (Karnatak State).                           |
| Dr. A. B. Chowdhary  | .. | .. | Director, Calcutta School of Tropical Medicine, Chittaranjan Avenue, Calcutta.                                                |

|                          |      |                                                                                                                                                                  |
|--------------------------|------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Dr. (Smt.) S. C. Johnson | ..   | Department of Medicine, Christian Medical College, Vellore, Tamil Nadu.                                                                                          |
| Dr. R. D. Kulkarni       | ..   | Professor and Head of the Department of Pharmacology, Grant Medical College, J. J. Group of Hospitals, Bombay.                                                   |
| Dr. N. Madangopalan      | ..   | Physician and Gastroenterologist, Government General Hospital, Madras and Additional Professor of Medicine and Gastroenterology, Madras Medical College, Madras. |
| Dr. D. N. Pai            | ..   | Professor of Preventive and Social Medicine, B. Y. L. Nair Charitable Hospital and T. N. Medical College, Bombay.                                                |
| Dr. P. Raghavan          | ..   | Emeritus Professor of Medicine, Seth G. S. Medical College, Bombay.                                                                                              |
| Dr. A. K. Saraya         | ..   | Assistant Professor of Pathology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi.                                                              |
| Dr. U. K. Sheth          | ..   | Professor of Pharmacology, Seth G. S. Medical College, Parel, Bombay-12                                                                                          |
| Dr. B. N. Tandon         | ..   | Associate Professor of Medicine, Department of Gastroenterology, All India Institute of Medical Science, New Delhi.                                              |
| Dr. F. P. Udwadia        | ..   | Beach Candy Hospital and Nursing Home, Bombay.                                                                                                                   |
| Dr. (Smt.) S. Vaishnava  | ..   | Department of Pediatrics, Safdarjung Hospital, New Delhi.                                                                                                        |
| Dr. K. Bhaskaran         | .... | Scientist, Department of Microbiology, Central Drug Research Institute, Lucknow.                                                                                 |
| Dr. N. K. Dutta          | ..   | Special Director, Vaccine Institute, Baroda (Gujarat State).                                                                                                     |
| Dr. S. C. Pal            | ..   | Director, Cholera Research Centre, Calcutta.                                                                                                                     |
| Dr. S. Basu              | ..   | Assistant Director, Central Research Institute, Kasuali.                                                                                                         |
| Dr. K. V. Murthy         | ..   | Assistant Director, King Institute, Guindy, Madras                                                                                                               |
| Dr. K. Rajyalakshmi      | ..   | Director, Institute of Preventive Medicine, Narayanaguda, Hyderabad.                                                                                             |

VISITOR-SCIENTISTS AND OTHER DIGNITARIES VISITING  
HAFFKINE INSTITUTE DURING THE PLATINUM  
JUBILEE YEAR

**Foreign Scientists**

|                            |    |    |                                                                                                                                 |
|----------------------------|----|----|---------------------------------------------------------------------------------------------------------------------------------|
| Dr. G. Edsell              | .. | .. | Professor of Microbiology and Head of the Department, London School of Hygiene and Tropical Medicine, London.                   |
| Dr. Noel W. Preston        | .. | .. | Reader in Bacteriology, University of Manchester, Manchester.                                                                   |
| Dr. Eric Snell             | .. | .. | Medical Director, Glaxo Laboratories Ltd., England.                                                                             |
| Dr. D. R. Laurence         | .. | .. | Professor of Pharmacology and Therapeutics, University College Hospital Medical School, London.                                 |
| Dr. G. Kryzhanowsky        |    | .. | Corresponding member of Academy of Medical Sciences of USSR, Institute of Normal and Pathological Physiology, Moscow, U.S.S.R.  |
| Dr. J. W. C. Smith         | .. | .. | Deputy Director, Epidemiological Research Laboratory, Central Public Health Laboratory, London.                                 |
| Professor Kevin M. Cahill  |    | .. | Director, Tropical Disease Centre, Lenex Hill Hospital, New York, U.S.A.                                                        |
| Dr. Dan C. Cavanaugh       |    | .. | Walter Reed Army Medical Centre, Washington.                                                                                    |
| Professor M.J.J. Colbourne |    | .. | Reader, Department of Tropical Hygiene, London School of Hygiene and Tropical Medicine, and Assistant Director, Ross Institute. |
| Professor W.R.F. Collis    |    | .. | Tropical Disease Centre, Lenex Hill Hospital, New York, U.S.A.                                                                  |
| Dr. Edward F. Elslager     |    | .. | Section Director, Research Division of Parke Davis and Company, Michigan (U.S.A.)                                               |
| Dr. R. <sup>A</sup> Foster | .. | .. | Scientific and Administration Manager, European Clinical Research Group, Pfizer Central Research, Belgium.                      |
| Dr. Frank Hawking          | .. | .. | Clinical Research Centre, Harrow, London.                                                                                       |
| Dr. P.A.J. Janssen         | .. | .. | Director, Janssen Pharmaceutical Research Laboratories, Belgium.                                                                |
| Dr. E. Jucker              | .. | .. | Editor, "Progress in Drug Research," Basle, Switzerland.                                                                        |

|                           |                                                                                                                                                       |
|---------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|
| Professor B. H. Kean ..   | .. Professor of Clinical Medicine, Cornell University Medical College, and Director, Parasitology Laboratory, The New York Hospital, New York, U.S.A. |
| Dr. Friderich Kradolfer   | .. Head of Infectious Diseases Research, Biological Research Laboratories, Pharmaceutical Division, CIBA-GEIGY, Switzerland.                          |
| Professor Max J. Miller   | .. Professor and Chairman, Department of Tropical Medicine and Parasitology, Tulane University, New Orleans (U.S.A.)                                  |
| Professor Rudolf Rett     | .. Institute of Virology and Veterinary Medicine, Justus Liebig University, Giessen, Federal Republic of Germany.                                     |
| Professor Manabu Sasa     | .. Professor of Parasitology, Institute of Medical Science, University of Tokyo, Japan.                                                               |
| Dr. C. E. Gordon Smith    | .. Dean, London School of Hygiene and Tropical Medicine, London.                                                                                      |
| Dr. Francisco Biagi ..    | .. Faculty of Medicine, Department of Parasitology, University of Mexico, Mexico.                                                                     |
| Dr. David R. Botero ..    | .. School of Medicine, University of Antioquia, Medellin, Columbia.                                                                                   |
| Dr. D. Duwel ..           | .. Chemotherapeutic Department, Farbwerke Hoechst A. G., Frankfurt, West Germany.                                                                     |
| Dr. Harinasuta Tranakchit | .. University of Medical Sciences, Faculty of Tropical Medicine, Bangkok, Thailand.                                                                   |
| Dr. T. Sawada ..          | .. Department of Parasitology, School of Medicine, Gunma University, Maebashi, Japan.                                                                 |
| Dr. O. D. Standen ..      | .. Director, Wellcome Research Laboratories, U. K.                                                                                                    |
| Dr. Richard B. Hornick    | .. Division of Infectious Diseases, University of Maryland, School of Medicine, Baltimore, Maryland, U.S.A.                                           |
| Dr. A. L. Furniss ..      | .. Director, Public Health Laboratory Service, Preston Hill Hospital, Kaidstone, Kent, U. K.                                                          |
| Dr. David R. Nalin ..     | .. Cholera Research Laboratory, Institute of Public Health, Dacca, Bangla Desh.                                                                       |



|                              |    |    |                                                                                                                               |
|------------------------------|----|----|-------------------------------------------------------------------------------------------------------------------------------|
| Dr. William Burrows          | .. | .. | Professor Emeritus, University of Chicago, U. S. A.                                                                           |
| Dr. George Curline           | .. | .. | Interim Director, Cholera Research Laboratory, Institute of Public Health, Dacca, Bangla Desh.                                |
| Dr. Norbert Hirschhorn       | .. | .. | Management Sciences for Health, Massachusetts, U.S.A.                                                                         |
| Dr. I. Joo                   | .. | .. | ' HUMAN ' Institute for Serobacteriological Production and Research, Hungary, Budapest.                                       |
| Dr. B. Cvjetanovic           | .. | .. | Chief Medical Officer, Bacterial Diseases, Division of Communicable Diseases, World Health Organization, Geneva, Switzerland. |
| Dr. Y. Watanabe              | .. | .. | Bacterial Diseases, Division of Communicable Diseases, World Health Organization, Geneva, Switzerland.                        |
| Dr. J. C. Feeley             | .. | .. | Bacterial Immunology Section, Bacterial Branch, Centre for Disease Control, Atlanta, U.S.A.                                   |
| Dr. W. F. Verwey             | .. | .. | Chairman, Department of Microbiology, University of Texas, Medical Branch, Galveston, Texas, U.S.A.                           |
| Dr. Bosman Pohan             | .. | .. | Laboratorim Keschatan Pusat, Jalan Percetakan Nagara, Djakarta, Indonesia.                                                    |
| Dr. Sutas Guptarak           | .. | .. | Chief, Biological Division, Government Pharmaceutical Organisation. Bangkok, Thailand.                                        |
| Dr. O. Da Rocha- Afodu       | .. | .. | 5, Ilu Drive Ikoyi, Lagos, Nigeria.                                                                                           |
| Mr. George Mwagae Mzera      | .. | .. | National Public Health Laboratory Services, Nairobi, Kenya.                                                                   |
| Dr. Hossein Mirchasmy        | .. | .. | Razi State Serum Institute, Teheran, Iran.                                                                                    |
| Dr. Ekbal Karim Mohamed Amin | .. | .. | 20, KL Koroom, Dooky, Cairo, Egypt.                                                                                           |
| Dr. Turgust Tulga            | .. | .. | 29. Azik Sokak Street, Ellika-Asagiegence, Ankara, Turkey.                                                                    |
| Dr. Lim Teong Wah            | .. | .. | Senior Virus Research Officer, Institute for Medical Research, Kuala Lumpur, Malaysia.                                        |
| Dr. Alejandro A. Jose        | .. | .. | Serum and Vaccine Laboratories, Alabang, Rizal, Philippines.                                                                  |
| Dr. G. Leonardi              | .. | .. | Head of Medical Department, Civil Hospital, Venice, Italy.                                                                    |
| Dr. (Mrs.) G. Leonardi       | .. | .. | C/o. Dr. G. Leonardi, Head of Department, Civil Hospital, Venice, Italy.                                                      |
| Dr. Stanley C. Browne        | .. | .. | Director, Leprosy Study Centre, London.                                                                                       |

## ACADEMIC COURSES AND TRAINING OF HEALTH PERSONNEL

The Institute is affiliated to the University of Bombay for post-graduate training courses in respect of the following degrees and diplomas :

### Post-graduate degrees

M.Sc. .. Microbiology, Biochemistry, Zoology, Organic Chemistry and Applied Biology, Pharmacology.

|          |    |                  |                |            |
|----------|----|------------------|----------------|------------|
| Ph.D.    | .. | Do.              | Do.            | Do.        |
| M. Pharm | .. | Pharmacology and | Pharmaceutical | Chemistry. |

### Diploma

D.M.L.T. .. Diploma in Medical Laboratory Technology.

The following Post-graduate Teachers are recognised by the University of Bombay :—

|                       |                            |
|-----------------------|----------------------------|
| Dr. B. B. Gaitonde    | Dr. C. V. Deliwala         |
| Dr. M. V. Sant        | Shri Y. S. Nimbkar         |
| Dr. A. N. D. Nanavati | Dr. (Smt.) A. Venkataraman |
| Dr. K. V. Kulkarni    | Dr. M. H. Shah             |
| Dr. S. D. Ambegaonkar | Dr. P. Y. Dighe            |
| Dr. M. B. Bhide       | Dr. K. H. Dave             |
| Dr. F. L. Saldanha    | Dr. S. S. Sabnis           |

and Dr. A. J. Baxi.

During the year, the following two more post-graduate teachers were recognised by the University of Bombay :

Dr. D. M. Renapurkar and Dr. N. E. Vad

Dr. M. V. Sant and Dr. (Smt.) J. K. Dave of this Institute are recognised by the College of Physicians and Surgeons of Bombay for training candidates for :  
(a) F. C. P. S. Examinations in Pathology, Bacteriology and Pharmacology  
and (b) D. P. B. (Diploma in Pathology and Bacteriology).

The Institute is also affiliated to the University of Poona for post-graduate training courses.

It is also affiliated to the University of Baroda for post-graduate training courses in respect of the following subjects :—

Organic Chemistry, Pharmacology, Pathology, Bacteriology, Parasitology, Microbiology, Entomology (Zoology) and Biochemistry.

During the year, the Institute was also recognised by the National Council of Educational Research and Training, New Delhi, for guiding National Talent Scholars for M. Sc. studies. The Nagpur University has also recognised this Institute as a place of research in Biochemistry in the Faculty of Science.

*Number of Post-graduate students on roll during the year*

|                   |    |        |    |    |
|-------------------|----|--------|----|----|
| Organic Chemistry | .. | M.Sc.  | .. | 11 |
|                   |    | Ph. D. | .. | 7  |
| Microbiology      | .. | M.Sc.  | .. | 20 |
|                   |    | Ph. D. | .. | 9  |
| Biochemistry      | .. | M.Sc.  | .. | 17 |
|                   |    | Ph. D. |    | 11 |
| Applied Biology   | .. | M.Sc.  | .. | 6  |
|                   |    | Ph. D. |    | 10 |
| Zoology           | .. | M.Sc.  | .. | 1  |
| Pharmacology      | .. | Ph.D.  | .. | 1  |

## DEGREE AWARDS DURING THE YEAR

| Name of candidate                  | Subject                       | Topics                                                                                                                                            |
|------------------------------------|-------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
| Shri D. V. Pradhan                 | .. M.Sc.(Biochemistry)        | Studies on the use of "Schizosaccharomyces pombe in the assay of pantothenic acid and its salts".                                                 |
| Smt. M. N. Shah                    | .. Do.                        | "Stability of vitamins in light".                                                                                                                 |
| Shri V. D. Rane                    | .. Do.                        | "Lipid metabolism in relation to dietary carbohydrates and fats".                                                                                 |
| Shri S. V. Pandit                  | .. M.Sc.(Microbiology)        | "Isolation and characterization of rat viruses".                                                                                                  |
| Smt. S. S. Sathe                   | .. Do.                        | "Studies in the nutritional requirements of <i>Corynebacterium diphtheriae</i> in acid hydrolysate of casein medium.                              |
| Shri R. P. Vitkar                  | .. Do.                        | "Studies in the use of adjuvants and hormones in immunization of guineapigs with diphtheria toxoid".                                              |
| Smt. A. A. Gandhi                  | .. Do.                        | "Studies in tetanus toxin production".                                                                                                            |
| Shri S. V. Gadre                   | .. Ph.D.(Microbiology)        | "Studies in <i>Corynebacterium diphtheriae</i> and its phages".                                                                                   |
| Shri P. K. Chatterjee              | .. Do.                        | "Studies in cholera toxin".                                                                                                                       |
| Shri Husein Ali Mutlag<br>Khleifat | .. Do.                        | "Studies on Dynamics of Disinfection".                                                                                                            |
| Shri R. P. Kini                    | .. M. Sc. (Organic Chemistry) | "Synthesis of organic compounds of possible therapeutic value, with special reference to antitubercular activity".                                |
| Shri G. V. Joshi                   | .. Do.                        | "Synthesis of thiazole derivatives as possible antitubercular agents".                                                                            |
| Shri D. P. Buduk                   | .. M.Sc. (Applied Biology)    | "Studies in haematological aspects of hyper-immunized horses under bleeding, with special reference to effects of adjuvants and plasma phoresis". |
| Kum. V. M. Barsivala               | .. Do.                        | "Hormonal levels of women taking oral contraceptives".                                                                                            |

# FELLOWSHIPS AND SCHOLARSHIPS ATTACHED TO THE INSTITUTE DURING THE YEAR

| Name                       | Fellowship or Scholarship                                | Subject           | Title of project                                                                                      |
|----------------------------|----------------------------------------------------------|-------------------|-------------------------------------------------------------------------------------------------------|
| Shri K. Y. Mahajan         | .. Government of India Research Training Scholarship.    | Organic Chemistry | Synthesis of Pyrimidine compounds of possible Biological and Medicinal interest.                      |
| Shri A. V. Athawale        | .. Government of India Research Training Scholarship.    | Biochemistry      | Amoebiasis                                                                                            |
| Kum. R. M. Baride          | .. Government of India Research Training Scholarship.    | Biochemistry      | A study on biochemical changes in experimental rabies.                                                |
| Kum. P. H. Jhala           | .. Government of India Research Training Scholarship.    | Biochemistry      | Lipid patterns in Health and Disease.                                                                 |
| Shri B. G. Khadse          | .. Government of Maharashtra Senior Research Fellowship. | Organic Chemistry | Synthesis of Heterocyclic compound as possible Anthelmintic Agents.                                   |
| Shri D. T. Chaudhari       | .. Government of Maharashtra Senior Research Fellowship. | Organic Chemistry | "Chemotherapy of Cancer".                                                                             |
| Shri M. G. Phatak          | .. Government of Maharashtra Senior Research Fellowship. | Pharmacology      | Toxicity studies of Indigenous Drugs and commercial products.                                         |
| Dr. S. V. Gadre            | .. Government of Maharashtra Senior Research Fellowship. | Microbiology      | Diphtheria Toxin Production in surface and sub-merged culture.                                        |
| Shri A. D. Kulkarni        | .. Government of Maharashtra Senior Research Fellowship. | Biochemistry      | Studies on Immunological aspects of Helminthic Infections.                                            |
| Dr. (Smt.) S. R. Kankonkar | .. Government of Maharashtra Senior Research Fellowship. | Biochemistry      | (1) Cobra Venom toxoid<br>(2) Biochemical and Immunological studies of scorpion venom (Black and Red) |
| Shri P. P. Dalvi           | .. Government of Maharashtra Junior Research Fellowship. | Organic Chemistry | Synthesis of and development of Anthelmintic compounds.                                               |
| Shri S. S. Jadhav          | .. Government of Maharashtra Junior Research Fellowship. | Pharmacology      | Pharmacology of Pertussis and Diphtheria Toxins.                                                      |

|                      |                                                             |                   |                                                                                                                                        |
|----------------------|-------------------------------------------------------------|-------------------|----------------------------------------------------------------------------------------------------------------------------------------|
| Shri S. V. Kekatpure | Government of Maharashtra<br>Junior Research Fellowship     | Organic Chemistry | Biopharmaceutics and Development of<br>injectables.                                                                                    |
| Kum. S. W. Awachat   | .. Government of Maharashtra<br>Junior Research Fellowship  | Biochemistry      | Immunological studies on Leprosy.                                                                                                      |
| Shri H. S. Ved       | .. Government of Maharashtra<br>Senior Research Scholarship | Biochemistry      | Studies on Rickets.                                                                                                                    |
| Shri B. R. Colah     | .. Government of Maharashtra<br>Research Scholarship        | Organic Chemistry | Synthesis of organic compounds of<br>possible therapeutic value.                                                                       |
| Kum. S. M. Joshi     | .. Government of Maharashtra<br>Research Scholarship        | Biochemistry      | Biochemical studies on the venom of<br><i>Echis carinata</i>                                                                           |
| Shri N. T. Joshi     | .. Government of Maharashtra<br>Research Scholarship        | Microbiology      | (1) Standardizing the techniques for<br>anaerobic bacteriology.<br>(2) Sensitivity of <i>N. gonorrhoea</i> to<br>antimicrobial agents. |
| Shri S. P. Chitnis   | .. Unichem Research Fellowship                              | Pharmacology      | Pharmacology of scorpion venom.                                                                                                        |
| Shri S. C. Talwalkar | .. Unichem Research Fellowship                              | Biochemistry      | Studies on malnutrition stress effects<br>on adrenal function.                                                                         |
| Shri R. S. Joshi     | .. Unichem Research Fellowship                              | Pharmacology      | Pharmacological studies on the venoms<br>of Indian snakes elapid.                                                                      |
| Shri G. S. Chhatwal  | .. Diamond Jubilee Research<br>Fellowship.                  | Biochemistry      | Biochemical studies on the venom of<br>Indian elapid snakes.                                                                           |
| Shri T. K. Shetty    | .. Diamond Jubilee Research<br>Fellowship.                  | Biochemistry      | Effect of Oral Contraceptives on Brain<br>Neurohumoral pattern in experi-<br>mental animals.                                           |
| Shri V. B. Babne     | .. Diamond Jubilee Research<br>Fellowship.                  | Biochemistry      | Antifertility agents of plant origin.                                                                                                  |

**Trainees admitted to**

|                                                    |       |    |
|----------------------------------------------------|-------|----|
| Medical Laboratory Technology Course (M.L.T.)      | ..    | 11 |
| Diploma in Medical Laboratory Technology (D.M.L.T) | ..    | 12 |
| Short-term Training Course                         | .. .. | 67 |

**Number of Research Fellowships and Scholarships**

|                                                        |    |   |
|--------------------------------------------------------|----|---|
| Government of India Research Training Scholarships     | .. | 4 |
| Government of Maharashtra Senior Research Fellowships  | .. | 6 |
| Government of Maharashtra Junior Research Fellowships  | .. | 5 |
| Government of Maharashtra Senior Research Scholarship  | .. | 1 |
| Government of Maharashtra Research Scholarships        | .. | 3 |
| affkine Institute Diamond Jubilee Research Fellowships | .. | 3 |

The Internal Audit Section normally audits accounts for at least one month in each quarter. Special audit of cash book was taken up since September 1970 onwards. In addition, audit of various departments of the Institute was carried out as per the programme approved by the Director.

## FINANCIAL SUMMARY

During the year under review, the Institute continued its traditional services of production, research and other activities in the cause of public health of the State and the country. As compared to the sales of Rs. 121.85 lakhs, inclusive of sales worth Rs. 11.71 lakhs to the Employees' State Insurance Scheme during the last year, those during the year under review amounted to Rs. 123.95 lakhs, inclusive of sales of Rs. 29.62 lakhs to the Employees' State Insurance Scheme.

In spite of the difficulties encountered in procurement of raw materials and the over all increase in the cost of raw materials, packing materials etc., the Institute has been able to increase its sales during the year under review by Rs. 2.10 lakhs.

The trifurcated accounts, started in 1966-67, were drawn up for the year under review as well. The preliminary outstandings against sales as on 31st March 1974 stood at Rs. 46.77 lakhs as against the final figure of Rs. 33.94 lakhs for the last year.

The financial picture as on 31st March 1974 is reproduced below :—

*Our position as on 31st March 1873**What we have (Assets)—*

|                                                               | 1972-73     | 1971-72     |
|---------------------------------------------------------------|-------------|-------------|
|                                                               | Rs.         | Rs.         |
| Land and buildings.. ..                                       | 1,13,45,166 | 1,06,22,995 |
| Plant and Machinery .. ..                                     | 57,55,105   | 55,79,983   |
| Furniture and Fixture and Equipments ..                       | 16,06,181   | 15,64,697   |
| Motor Vehicles .. ..                                          | 1,74,720    | 1,74,720    |
| Library Books .. ..                                           | 10,86,498   | 8,90,843    |
| Live Stock (excluding gifts) .. ..                            | 3,97,750    | 4,95,421    |
| Stocks and Stores .. ..                                       | 94,52,531   | 72,48,196   |
| Amount to be received .. ..                                   | 32,30,465   | 24,59,697   |
| Cash, transit remittances etc. .. ..                          | 13,39,723   | 19,23,412   |
| H. I. Depreciation Fund (Investment Account with Government). | 19,00,000   | 16,50,000   |

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|             |             |
|-------------|-------------|
| 3,62,88,139 | 3,26,09,964 |
|-------------|-------------|

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*What we owe (Liabilities)—*

|                                            |           |           |
|--------------------------------------------|-----------|-----------|
| Depreciation Fund .. ..                    | 34,60,571 | 32,05,935 |
| Amount to be paid .. ..                    | 14,86,240 | 16,44,554 |
| Miscellaneous deposits .. ..               | 2,55,175  | 1,73,638  |
| H. I. Depreciation Fund with Government .. | 19,00,000 | 16,50,000 |

---

|           |           |
|-----------|-----------|
| 71,01,986 | 66,74,127 |
|-----------|-----------|

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*Net Surplus/losses—*

|                                         |           |           |
|-----------------------------------------|-----------|-----------|
| Production (Net surplus) .. ..          | 4,20,331  | 10,65,757 |
| Research and Training (Net loss) .. ..  | 16,12,589 | 19,53,274 |
| Public Health Services (Net loss) .. .. | 5,82,544  | 3,34,844  |

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|           |           |
|-----------|-----------|
| 17,74,802 | 12,22,361 |
|-----------|-----------|

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|                                                |       | 1972-73<br>Rs.           | 1971-72<br>Rs.           |
|------------------------------------------------|-------|--------------------------|--------------------------|
| <i>hat remains (Assets minus)—</i>             |       |                          |                          |
| Liabilities and surplus revenue                | ..    | 2,74,11,351              | 2,48,70,080              |
| <i>hat we earned (Income)—</i>                 |       |                          |                          |
| Sale of biologicals and drugs                  | .. .. | 1,08,81,269              | 1,24,60,494**            |
| Fees for analytical tests                      | .. .. | 6,99,710                 | 5,78,953                 |
| Hostel fees                                    | .. .. | 19,415                   | 19,110                   |
|                                                |       | <hr/>                    | <hr/>                    |
| Value of closing stocks                        | .. .. | 1,16,00,394<br>94,52,531 | 1,30,58,557<br>72,48,196 |
|                                                |       | <hr/>                    | <hr/>                    |
| Grand Total                                    | ..    | 2,10,52,925              | 2,08,06,753              |
| <hr/>                                          |       |                          |                          |
| <i>What we spent (Expenditure)—</i>            |       |                          |                          |
| On employees                                   | .. .. | 50,99,756                | 54,57,938                |
| On raw materials                               | .. .. | 65,30,855                | 53,83,464                |
| On purchases and services                      | .. .. | 13,40,158                | 19,06,862                |
| On provision for pension                       | .. .. | 3,56,596                 | 3,63,885                 |
| On provision for depreciation                  | .. .. | 5,26,272                 | 5,03,105                 |
| On provision for interest on mean capital      | .. .. | 17,57,594                | 15,57,594                |
|                                                |       | <hr/>                    | <hr/>                    |
| Total                                          | ..    | 1,55,79,531              | 1,51,72,848              |
|                                                |       | <hr/>                    | <hr/>                    |
| Value of opening stocks                        | .. .. | 72,48,196                | 63,56,266                |
|                                                |       | <hr/>                    | <hr/>                    |
| Grand Total                                    | ..    | 2,28,27,727              | 2,15,29,114              |
| <hr/>                                          |       |                          |                          |
| Net Surplus (After providing for all expenses) | ..    | (—)17,74,802             | (—)12,22,361             |
| <i>Break-up of net surplus and net loss—</i>   |       |                          |                          |
| Production                                     | .. .. | (+ )4,20,331             | (+ )10,65,757            |
| Research and Training                          | .. .. | (—)16,12,589             | (—)19,53,274             |
| Public Health Services                         | .. .. | (—) 5,82,544             | (—)3,34,844              |
|                                                |       | <hr/>                    | <hr/>                    |
|                                                |       | (—)17,74,802             | (—)12,22,361             |
| <hr/>                                          |       |                          |                          |

The production and sale values (exclusive of sales tax and packing charges, but inclusive of excise duty) for the year 1973-74 is as under :—

| Production<br>Rs. | Sale<br>Rs. |
|-------------------|-------------|
| 1,21,21,310       | 1,23,95,081 |

\*\*This is the gross sales figure including sales tax and packing charges. The figure shown here in last year's report is Rs. 1,16,07,458 was the net sales figure excluding sales tax and packing charges.

## WELFARE ACTIVITIES

## General

Welfare activities at the Institute were not only maintained, but also given further impetus during the period under report. Though the main activities of the Institute are research, training and production of drugs and vitamins, the welfare activities of the workers received attention of the management increasingly and, as such, the Haffkine Institute Sports Club, which was established in the year 1958, has made a significant progress in providing sports facilities to all its members.

## Sports Club

The Sports Club has facilities for all the Indian and foreign games like Kabaddi, Cricket, Table Tennis, Badmington, Foot-ball, Volley-ball and Lawn Tennis.

It participated in all the local and inter-departmental tournaments.

The Club arranged inter-departmental tournaments as also friendly matches with its counterparts in other organisations.

The activities of the Sports Club are also extended to the workers of the Pimpri Stud Farm, Poona. Indoor and outdoor games like Carrom, Chess, Ring Tennis etc., were played regularly and these employees evinced great interest in these games.

The Sports Club arranged display of cinema slides and cultural programmes.

The Haffkine Institute Sports Club has published three issues of the chronicle 'Haffkine Vrutta' during the year under report. This activity and some other activities have now been transferred to Labour Welfare Section from the Sports Club.

The chronicle is distributed *gratis* to all the employees both at Bombay and Pimpri. It serves as a news item covering all aspects of our employees, viz., social and cultural life, including their office life, such as promotions, transfers, retirements etc. The chronicle publishes social and semi-scientific articles contributed by the staff members and it is expected that this will develop the latent writing faculties present among various classes of employees.

It is gratifying to mention here that the following employees of this Institute were deputed to participate in the All-India Civil Services Foot-ball tournaments held at Shillong from 25th August 1973 to 3rd September 1973.

|                     |                      |                             |                          |                          |
|---------------------|----------------------|-----------------------------|--------------------------|--------------------------|
| Shri Salim Abbas    | ..                   | Office Assistant, Grade-III | ..                       | Pay Group Section.       |
| Shri K. S. Tambe    | ..                   | Machine Operator            | ..                       | Chemotherapy Department. |
| Shri D. M. Waghmare | Laboratory Attendant | ..                          | Chemotherapy Department. |                          |
| Shri M. K. Kadam    | ..                   | Laboratory Attendant        | ..                       | Chemotherapy Department. |
| Shri S. N. Tandel   | ..                   | Laboratory Attendant        | ..                       | Media Section.           |

Shri K. S. Tambe, Machine Operator, working in the Department of Chemotherapy of this Institute, was deputed to participate in the Eighth All-India Civil Services Table Tennis Tournament held at Bhopal from 19th November 1973 to 27th November 1973.

The following three members of this Institute were also deputed to participate in the Seventh All-India Civil Services Kabaddi Tournaments held at Meerut (U. P.), from 2nd December 1973 to 4th December 1973.

|                     |                         |                                     |
|---------------------|-------------------------|-------------------------------------|
| Shri M. N. Kadam .. | Laboratory Assistant .. | Immunology Department.              |
| Shri T. S. Kadam .. | Laboratory Attendant .. | Immunology Department.              |
| Shri M. B. Raut ..  | Assistant Welder ..     | Maintenance Engineering<br>Section. |

### Library Service

The Sports club also conducts a library for its members. It has a stock of about 2,500 books of eminent authors and many more new books are added from time to time. The library facilities are offered free of charge to the members.

The ' Book Bank Section ', started in the year 1970, is now becoming increasingly popular and more than 60 employees of this Institute regularly avail of the facilities for college books, S. S. C. magazines, guides, etc.

### Training

(a) *Civil Defence Unit*.—The third batch of Civil Defence Course of fire fighting commenced from 12th September 1973 to 21st September 1973 and 35 employees of this Institute attended the classes and all of them were declared successful. At a function specially arranged on 28th February 1974, the Director gave away the Civil Defence Certificates to the successful candidates.

Shri A. S. Kulye, Office Assistant, Grade III, Costing Section, has undergone training under the Civil Defence Warden Course No. 40 sponsored by the Civil Defence Staff College, Bombay, held from 20th November 1973 to 30th November 1973. Shri Kulye obtained grading as ' High Average ' in the said examination.

(b) *Worker Teachers' Training Course (held at Bandra)*.—Shri P. G. Patil, Office Assistant, Grade III, Costing Section, was deputed to the Worker Teachers' Training Course held at Bandra for a period of three months from 1st December 1973 to 28th February 1974.

### Other Activities

(a) *Chawl Committee*.—There is a Chawl Committee, headed by Dr. K. H. Dave, Assistant Director I/c Department of Viral Vaccines, as President and Shri V. G. Bavaskar, Laboratory Attendant, Animal House, as its Secretary, to look after the various complaints and problems of about 325 families of the employees residing in the existing chawls in the Institute compound.

Class IV Quarters in the ' D ' chawl were allotted to nearly 56 members of the staff of this Institute during the year under report.

(b) *Union activities* —During the period under report, three regular meetings of Class III Employees' Union and two meetings of the Class IV Employees' Union were convened for discussing their demands with Director, besides other meetings with the office bearers of both the unions on specific subjects. The demand of the Unions regarding revised pay scales was considered by the Director. The Director recommended to the Government the appointment of a Pay Commission for revision of pay scales of the staff of Haffkine Institute.

Discussions were arranged with the Minister for Public Health and the State Minister for Public Health. Final orders in this respect are still awaited from Government.

### **Labour Welfare Committee**

In April 1972, the Director constituted a Labour Welfare Committee to deal with the cultural and social activities that the management may like to undertake and evolve, from time to time, labour welfare programmes ; to dispose of various matters such as individual complaints and other day-to-day problems, in consultation with the nominated representatives on such matters as the Director may desire and to obtain advice from the Presidents of both the Unions etc. The Committee consists of the Director as Chairman and the Deputy Director (Production), Deputy Director (Research), Assistant Director (Administration), Presidents of the Class III and Class IV Employees' Unions, Exhibition Officer, Maintenance Engineer, Deputy Engineer, Buildings and Communications Department as members and the Labour Welfare Officer as the Convener. During the year under report, two meetings of this Committee were held. This has also fostered a sort of family relationship between the management and the employees. Further, prompt and timely action to redress the grievances right at the initial stages by the Labour Welfare Section and the management has helped maintain and develop a harmonious relationship between the management and all employees and the Unions concerned.

As proposed by the Labour Welfare Committee, a Sewing Class was started from 15th August 1973. The class is held three days in a week (i.e. on Mondays, Wednesdays and Fridays, between 2.00 p.m. and 4.00 p.m.) in the Gymkhana Hall. About 25 lady members related to the employees of this Institute took advantage of this class. Two "Merritt" sewing machines were provided for use of the class.

"Balvadi" has also been started from 15th August 1973 during week days between 11.00 a.m. to 2.00 p.m. Nearly 50 children of the employees of this Institute attend this class.

### **Miscellaneous**

1. The representatives from the office of the Director of Small Savings Scheme approached the employees requesting them to open C. T. D. accounts. In response to this, 90 employees have opened C. T. D. accounts in the month of January 1974.

2. "Ambedkar Jayanti", "Buddha Jayanti" and "Shiv Jayanti" were celebrated by the Haffkine Institute Chawl Committee. "Ganesh Chaturthi" festival was also started by the employees of this Institute during the year. All employees of this Institute participated in these festivals with great enthusiasm.

3. Collection for the Flag Day on 7th December 1973 was arranged for the benefit of the Ex-Servicemen and their families.

4. Collection for the Flag Day on 13th September 1973, in aid of the blind, was also arranged.

5. The Director gave T. V. set for the Labour Welfare Centre of this Institute during the year under report.

6. All the employees participated in the Platinum Jubilee Celebrations of the Institute held on 14th, 15th and 16th January 1974. Cultural programmes and the Marathi drama 'Bhaubandki', staged during this festival, were enjoyed by everyone.

# HAFFKINE INSTITUTE CO-OPERATIVE CREDIT SOCIETY LTD., BOMBAY-12

The Society continued its progress satisfactorily during the year under review. Following is the position as on 30th June 1973 :

|                        |                 |        |
|------------------------|-----------------|--------|
| Membership .. .. .     | ..              | 1,008. |
| Loans advanced .. .. . | Rs. 9,25,907.80 |        |
| Share Capital .. .. .  | Rs. 7,56,691.41 |        |
| Net Profit .. .. .     | Rs. 59,003.34   |        |

The Society is doing the following types of business :

- (i) Advancing loans to members for various purposes, including insurance premia.
- (ii) Running of a staff canteen.
- (iii) Running of a grain shop.

## DISPENSARY

The Institute dispensary continued to serve the needs of its staff and their dependents as hithertofore.

New premises were provided, which are more spacious and better suited than the old premises.

A total of about 37,328 patients attended the dispensary for treatment.

About 260 cases of pulmonary tuberculosis, including the staff and their family members, were treated at the dispensary. Out of these, some were new patients. The old cases were sent for regular check-ups.

Mass miniature radio-graphy of all the staff members of Institute was carried out in the month of May, 1973.

|                   |    |    |    |    |       |
|-------------------|----|----|----|----|-------|
| Total No. X-rayed | .. | .. | .. | .. | 1,112 |
| Positive cases    | .. | .. | .. | .. | 82    |
| Old cases         | .. | .. | .. | .. | 71    |
| New cases         | .. | .. | .. | .. | 11    |

All the cases detected were referred to the T. B. Clinic at J. J. Group of Hospitals for consultation and were treated at this dispensary. Many patients did not take treatment regularly.

Family planning programme has been started with the administration of contraceptive tablets. About 100 women are taking these tablets regularly.

About 400 children were immunised with polio and triple antigen.

Cases requiring expert guidance were referred to the teaching hospitals.

The Departments of Immunohaematology and Clinical Pathology co-operated in rendering facilities for routine investigation tests.

## A BRIEF REPORT OF THE HAFFKINE INSTITUTE PLATINUM JUBILEE CELEBRATIONS 1899—1974

The Haffkine Institute, founded on 10th August 1899, completed 75 years of its eventful service in the cause of suffering humanity. The platinum jubilee was celebrated in a befitting manner.

A committee, under the Chairmanship of Prof. P. N. Wahi, Director-General, Indian Council of Medical Research, was set up. This committee formulated guidelines for the proposed celebrations. Based on these guidelines, the Government of Maharashtra constituted an 'Executive Committee for the Platinum Jubilee Celebrations of the Haffkine Institute' under the Chairmanship of Dr. Rafiq Zakaria, Minister for Public Health. Other members of the Committee were :

Shri G. S. Sarnayak, Shri L. M. Nadkarni, Shri S. E. Sukthankar, Shri P. D. Kasbekar, Shri Rajni Patel, Dr. L. H. Hiranandani, Shri S. V. Pillai, Dr. J. N. Banerjee, Shri A. V. Mody, Mr. G. J. Medley, Dr. J. B. Shrivastav, Dr. J. B. Mody, Dr. H. I. Jhala, Shri S. S. Hate, Shri M. K. Rangnekar, Prof. P. N. Wahi, Shri Nusli N. Wadia and Dr. B. B. Gaitonde.

The Executive Committee for Platinum Jubilee Celebrations met on several occasions and a programme for the jubilee celebrations was chalked out.

In order to collect donations for the Institute's expansion schemes, formulated as given in Appendix I, a Finance Committee, with Shri Nusli N. Wadia, Managing Director, Bombay Dyeing and Manufacturing Co. as the Chairman, was formed. Members of this Committee were :

Shri Nusli N. Wadia—Chairman, Shri Vasant Rao Vaidya, Shri Ismail M. Kanga, Shri S. R. Damani, Shri M. L. Apte, Shri Ram K. Batre, Shri Ratan Tata, H. H. Maharaja Madhav Rao Scindia of Gwalior, Shri Vinay K. Shah, Shri Shivax Vakil, Shri S. V. Pillai, Shri K. G. Anantanarayanan.

A Publicity/Souvenir/Programme Committee was also set up under the chairmanship of Shri Khushwant Singh with Shri Percy D'Souza and Smt. Neela D'Souza as members.

The Platinum Jubilee celebrations were spread over a period of one year starting from 10th August 1973. The following programmes were successfully performed :

1. *Inauguration of the Platinum Jubilee Year and release of the First Number of the "Bulletin of Haffkine Institute".*—On 10th August 1973, Shri R. K. Hadilkar, Hon'ble Minister for Health, Government of India, inaugurated the Platinum Jubilee Year and released the first number of "Scientific Bulletin" of the Institute, started on the occasion of the Platinum Jubilee.

During the last 75 years, a number of new departments have been added. The Institute's scientists have contributed several papers to the national and international scientific journals. On an average, the Institute has been publishing annually about 20-30 papers in the various branches of biological sciences.

For a long time, it was felt that the Institute should have a scientific publication of its own. This would naturally give an opportunity for its 300 and odd scientists to publish their preliminary research findings as soon as possible. This long-felt need has thus been fulfilled, whereby scientists working in the Institute will publish their scientific papers to project to the world their scientific achievements through the pages of the Bulletin of Haffkine Institute. The Bulletin is a valuable addition to the existing scientific journals.

On this day, the Institute accepted the motto :

“ रोगापहारी विज्ञानं लोककल्याण साधनम् । ”

“ Science that alleviates human suffering and conquers disease is the best means of achieving the welfare of the people ”

On 11th January 1974, Hon'ble Minister for Public Health, Dr. Rafiq Zakaria, held a Press Conference at Taj Mahal Hotel. He outlined the object of the Platinum Jubilee and the future expansion plans of the Institute.

The inaugural function of the platinum jubilee celebrations was held on 10th January 1974. The Governor of Maharashtra, Shri Ali Yawar Jung, inaugurated the function on behalf of Shrimati Indira Gandhi, the Prime Minister, as she could not come owing to sudden developments and unavoidable circumstances. In her message, the Prime Minister paid high tributes to late Dr. Haffkine and late Major-General Sokhey and commended the work done by the Institute in the alleviation of human suffering. A plaque commemorating the services rendered by the Institute was also unveiled on the occasion. Important personalities, such as the Mayor, the Sheriff, distinguished scientists from all parts of the world and citizens from all strata of society attended the function.

## SEMINARS

A number of scientific seminars were organised in collaboration with other institutions/agencies.

1. *Seminar on Indigenous Systems of Medicine (including 'Yoga')*.—This seminar was held in the Durbar Hall of the Institute on 11th, 12th and 13th August 1973. It was arranged under the auspices of the Central Council for Research in Indian Medicine and Homoeopathy, New Delhi, and was inaugurated by Prof. A. N. Namjoshi, Hon'ble Minister for Education, Government of Maharashtra. About 28 Indian scientists participated in the seminar. Pandit Shiv Sharma, Chairman of the Scientific Advisory Board of Central Council for Research in Indian Medicine and Homoeopathy, delivered the opening speech. He referred to the rich heritage of the Indian system of medicine—the Ayurveda—and desired that more efforts should be put in for a systematic research in Ayurveda. He said that a centre is being developed with the help of the World Health Organisation at Melbourne, Australia, for research in different systems of medicine including Ayurveda. He further added that the volumes of Sushruta have been translated word for word in Japan.



Professor A. N. Namjoshi, in his inaugural speech, stated that it is necessary to understand the concepts of Ayurvedic theory. Research in Ayurveda is not synonymous with research in indigenous system of drugs. The system has to be taken as a whole, whereby a symbiosis of Ayurvedic research can possibly be explained in modern scientific views. He further added that Yoga, which is also a part of Ayurveda, needs further detailed studies and expressed his satisfaction at the researches in Ayurveda and Yoga that are being conducted on scientific lines in our country.

The inauguration was followed by discourses on "Yoga" and Basic Sciences and yogic demonstrations.

2. *Seminar on Diphtheria, Pertussis and Tetanus.*—This seminar was organised on 13th, 14th and 15th January 1974 under the joint auspices of the Haffkine Institute and Messrs. Glaxo Laboratories (India) Pvt. Ltd., Forty-four Indian and seven foreign scientists participated in the seminar.

Eminent scientists from abroad like Dr. Noel W. Preston, Dr. J. W. G. Smith, Dr. D. R. Laurence, Dr. G. Edsall, Dr. E. Snell from U. K., Dr. G. Kryzhanovsky from U. S. S. R., Dr. G. Leonardi from Italy, Dr. G. Veronesi from Brazil, attended the seminar.

3. *Seminar on guidelines for research in communicable diseases.*—This seminar was held on 14th, 15th and 16th January 1974, under the joint auspices of the Institute and Messrs. Sandoz (India) Limited.

Fourteen Indian and sixteen foreign scientists participated in the seminar. Dr. E. Jucker, Director of Sandoz, Basle (Switzerland) led the team of foreign scientists and organised the symposium. Eminent scientists from abroad, who attended the seminar, were Dr. Frank Hawking, Dr. C. E. Gordon Smith from U. K., Dr. Friedrich Kradolfer from Switzerland, Dr. P. A. J. Janssen from Belgium, Dr. Max J. Miller and Dr. B. H. Kean from U.S.A., Dr. Rudolf Rott from Germany and Dr. Manabu Sasa from Japan. Notable amongst the Indian scientists were Dr. C. G. Pandit and Dr. V. N. Rao.

4. *Seminar and workshop on cholera.*—Cholera workshop (a practical laboratory course) was organised from 4th to 12th March 1974, under the auspices of the World Health Organisation. Participants from various countries were sponsored by the W. H. O. Dr. Y. Watanabe, Dr. I. Joo, Dr. B. Cvjetanovic and Dr. J. C. Feeley who acted as Faculty Members guided the various discourses, and conducted the laboratory practicals. This was followed by a three-day conference on cholera from 13th to 15th March 1974. Here also, 11 Indian and 3 foreign scientists participated.

The proceedings of these seminars are going to be published in the Volumes nos. 17 and 18 of "Progress in Drug Research" edited by Dr. Jucker, Director, Sandoz, Basle.

5. *Workshop on ' Rabies '.*—It is proposed to organise a workshop on Rabies sometime in September next year.

6. *Programme arranged on All-India Radio.*—(A) A panel discussion of doctors in the programme ' Doctors Talk It Over ' was broadcasted from the All-India Radio Station, Bombay, at 9-30 p.m. on 8th September 1973. The subject of discussion was ' Immunization in Communicable Diseases '. Dr. B. B. Gaitonde acted as the Moderator and Dr. A. N. D. Nanavati, Shri Y. S. Nimbkar, Dr. (Smt.) J. K. Dave, Dr. M. M. Wagle and Dr. H. D. Daftary participated in the discussion.

(B) Programme entitled ' Focus ' was broadcasted on 14th October 1973. In this programme, the participants were interviewed on various subjects. Dr. Gaitonde gave a brief resume on the Institute, its major contributions in the service of nation, other aspects of production and research developments and future expansion schemes. Shri Y. S. Nimbkar replied to question on vaccine production. Dr. (Smt.) J. K. Dave outlined the scope of diagnostic services and allied subjects. Dr. N. E. Wad gave an interesting account of zoonotic diseases and Dr. K. H. Dave presented the problems in viral diseases including polio.

(C) Shri Y. V. Thakre, Assistant Director (Administration), gave a talk in Marathi entitled ' Haflkine Institute in the Service of Nation ' on 14th January 1974.

(D) A panel discussion in the programme ' Doctors Talk It Over ' on the subject of Helminthiasis was broadcast on February 1974.

(E) All the programmes of the various seminars and the Platinum Jubilee functions were covered by the All India Radio.

7. *Television Programmes.*—(A) All the jubilee programmes were televised by the T. V. Centre, Bombay.

(B) An interview programme was televised in January 1974 showing Dr. Gaitonde discussing with Dr. E. Jucker, Director of Sandoz, Basle, Switzerland, on the subject of guidelines for Research in Communicable Diseases.

7. *Cultural Programmes.*—Cultural programmes were arranged on 14th, 15th and 16th January 1974. These included classical dances, mimicry and a Marathi drama " Bhaubandaki " staged by eminent actors like Padmashree Daji Bhatwadekar, Smt. Ashalata Wabagaonkar and others.

The untiring efforts put in by all the staff members made the Jubilee Function a grand success,

## APPENDIX I

## HAFFKINE INSTITUTE PLATINUM JUBILEE EXPANSION SCHEMES

## New Departments

1. *Toxicology and Human Pharmacology*.—Newer drugs are being developed now in this country and detailed toxicological studies and evaluation of the good and bad effects of the new medicines is a condition by law. Such laboratories exist nowhere in this country.

A new drug has to be studied in detail for its harmful effects before it is introduced for use in patients. Similarly, many foods and industrial effluents may be contaminated or contain very toxic substances injurious to health. Well-equipped laboratories to undertake studies of this type exist in all advanced countries. However, there are no such laboratories in our country. Haffkine Institute has made a beginning by starting a Toxicology Unit. The Haffkine Institute is the only institute which is well-equipped for such an undertaking, and possesses the necessary ancillary facilities and trained scientists.

The Development of the Toxicology Unit into a full-fledged department is expected to require about Rs. 5 lacs (Rupees Five Lacs). The department can be named after the donor.

2. *Epidemiology Unit*.—The communicable diseases still form a major problem in our country. There are various complicated aspects, and surveillance activities and epidemiological research throughout the State is absolutely necessary. Deeper studies have to be done in the spread of communicable diseases like filariasis (elephantiasis), dracunculosis (guinea worm diseases—known in Marathi as Naru), cholera, typhoid etc.

This Institute had done a pioneering work in the field of epidemiology of plague and cholera. There are many more communicable diseases, which need to be studied, especially in rural areas. The prevalence and incidence rates, the nature, environmental factors, conditions, nutritional factors, vector population responsible for spread, form a multifaceted study of a disease for its total eradication.

The Institute scientists are working in the fields in close collaboration with the public health authorities for such studies on filariasis, worm infestation, guinea worm diseases etc.

Expenditure on the expansion of this scheme is expected to be about Rs. 2.50 lacs (Rupees two and half lacs). The new Unit of Epidemiology can be named after the donor.

3. *Department of Radiobiology and Radiopharmaceutics*.—Nuclear energy has been used for peaceful purposes such as diagnosis and treatment of diseases. The radio-isotopes are useful in the development of bacterial vaccines and for studying fundamental aspects of immunology.

The equipment for this unit is sophisticated and expensive. A small unit has been started and it needs to be expanded into a full-fledged department.

The estimated expenditure for expansion of the present small unit is about Rs. 4 lacs (Rupees four lacs). The Department can be named after the donor.

4. *Parasitology*.—Malaria, filaria, guinea worm and a host of other similar diseases caused by parasites are prevalent on a large scale. Such parasitic diseases are responsible for widespread sickness and therefore heavy morbidity. Infestation with parasites leads to multiple nutritional disorders. The study of parasitic diseases is therefore of paramount significance to our country. There are but only one or two parasitology departments in this country. It is proposed to organise a Department of Parasitology for study of parasitic diseases in all its aspects. The initial expenditure for starting such a unit will be about Rs. 2.50 lacs (Rupees two and half lacs). The department can be named after the donor.

5. *Testing of Antitubercular drugs in laboratory animals*.—Tuberculosis eradication is a national health programme. It still forms a major health hazard in this country. In spite of advances in drug treatment, there are limitations in their use. There is need to study the disease in all its aspects and also seek newer drugs.

Newer drugs have got to be tested in laboratory animals infected with tuberculosis, to prove their efficacy. This hazardous task requires a specialised unit. It is therefore proposed to reorganise the present facilities. The expenditure involved will be Rs. 2.50 lacs (Rupees two and half lacs). The entire T. B. testing laboratory can be named after the donor.

6. *Division of Clinical Research*.—A well-equipped 75-bedded hospital with at least 4 wards with attached laboratory and investigational facilities is expected to be built. The total cost of the Hospital has been worked out to be about Rs. 25 lacs. Each ward could be separately named after the donor who contributes the cost of one ward, which may be approximately Rs. One lac.

### Research

*Chair For Research—Endowment*.—Heads of Departments or eminent scientists will be invited to spend their sabbaticals at the Institute, initiating or strengthening research projects.

### Scholarships. Prizes.

1. *Dr. Haffkine Memorial Oration—Endowment*.—To be given once a year by a renowned scientist from India or abroad.—Rs. 1,00,000-00.

2. *Platinum Jubilee Scholarships—Endowment*.—6 Senior and 4 Junior, in various branches to be awarded to scientists from all over India to work in the Institute. Rs. 8,00,000-00

### Documentation Centre

The present library facilities the oldest medical library in India with about 18,000 volumes and about 200 journals in Indian and foreign languages to be expanded and built up into a Documentation Centre, which will serve as a Reference Library on communicable diseases. Rs. 2,00,000-00.

*For details, please write to :*

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