



सत्यमेव जयते

GOVERNMENT OF MAHARASHTRA

ANNUAL REPORT
OF THE
HAFFKINE INSTITUTE
FOR THE YEAR 1972-73



1975

**PRINTED AT THE GOVERNMENT CENTRAL PRESS
BOMBAY**

STAFF OF THE INSTITUTE

Director

- Dr. C. V. Deliwala, M.Sc. (Bom.) Ph.D. (Bom.) (up to 9-4-1972).
Dr. B. B. Gaitonde, M.D., M.Sc. (Med.) (from 10-4-1972).

Deputy Directors

- Dr. C. V. Deliwala, M.Sc. (Bom.) Ph.D. (Bom.) (up to 9-4-1972).
Division of Pharmacology and Pharmaceutical Chemistry.
Dr. M. V. Sant, M.D. (Bom.), F.C.P.S. (Bom.), D.E. (Prague), Division of Microbiology.
Shri Y. S. Nimbkar, M. Sc. (Bom.), Dip. (Bact.) (Manchester), Production Division.

Assistant Directors

- Dr. A. N. D. Nanavati, M.D. (Bom.).
Department of Virology.
Dr. (Smt.) Alamelu Venkataraman, B.A. (Madras), M.Sc. (Madras), Ph.D. (Madras).
Department of Biochemistry.
Dr. S.S. Rao, M.Sc. (Banaras), Ph.D. (Toronto).
Department of Immunology (up to 28-9-1972).
Dr. K. V. Kulkarni, M.B.B.S., (Bom.).
Department of Immuno-Haematology.
Dr. F. L. Saldhanha, Ph.D. (Bom.).
Department of Quality Control.
Dr. M. B. Bhide, M.D. (Poona).
Department of Pharmacology.
Dr. K. H. Dave, M.D. (Bom.).
Department of Viral Vaccines.
Shri M. E. Kulkarni, M.Sc. Dip. (Bact.).
Department of Bacteriology (from 31-7-1971 to 31-5-1972) and Department of Immunology from 1-6-1972 onwards.
Dr. (Smt.) J. K. Dave, M.D. (Bom.).
Department of Clinical Pathology.
Shri R. S. Karbhari, M.Sc. (Bom.).
Department of Bacteriology from 1-6-1972 as additional charge and Department of Bacterial Vaccine from 1-12-1972.
Shri Y. V. Thakre, M.A., LL.B. (Nagpur).
Department of Administration.

Heads of Sections

- Dr. A. E. Kulkarni, G.B.V.C. (Bom.).
Senior Scientific Officer (Class I), I/c Animal House.

GENERAL REVIEW

Haffkine Institute is one of the oldest research institutes in this part of the world. Right from its inception in 1896, the Institute has been involved in the advancement of research in communicable diseases. This Institute was established initially as a Bacteriological Research Laboratory under the Directorship of Late Dr. W. M. Haffkine. After the discovery of plague vaccine by Dr. Haffkine, the laboratory was shifted into the present premises on 10th August 1899. Thus, in the year 1973, the Institute will be entering into the seventy-fifth year of its existence. It is, therefore, proposed to celebrate the Platinum Jubilee of the Institute in the most befitting manner. Steps have already been taken to chalk out a programme for the celebration of the Platinum Jubilee in the year 1974. The Institute has grown from time to time since its inception. At present, it has two main divisions—a division of production and a division of research. The divisions of production and research are intimately linked up with each other, so as to enable a collaborative programme of work. The Institute has 10 research departments in addition to a library with a very rich collection of books. It has a large workshop manned by competent engineering staff and a big animal house. The Institute has, in its employment, about 1,400 persons, which include over 200 junior and senior scientists.

The Institute performs three important functions in addition to rendering public health service. It is primarily involved in carrying out extensive research in all aspects of communicable diseases. Right from its inception, it has also been called upon to produce life-saving drugs, especially biologicals. In fact, at the time when the technology in this field did not exist in this country, the Institute was pioneer in developing such important processes as the preparation of vaccines and antisera on a large scale, so that these agents could be made available throughout the country for use in the epidemics of preventable diseases. The Institute has always risen to occasions of national emergencies and provided drugs, both biologicals and non-biologicals, required for urgent use in the country. During the Second World War, the Institute was called upon to take up research in chemotherapeutic agents and it thus set itself to manufacture a number of pharmaceuticals, such as vitamins, antimalarials and sulphonamides, specifically required in the country due to scarcity created by the exigencies of war. The Institute has, thus, played a historic role in developing import substitutions for many such drugs.

It is evident that although research is the primary function of this Institute, it is also involved in a large-scale production. The annual turn over is in the vicinity of Rs. 120 lakhs which consists of biologicals, such as blood products, diagnostic re-agents (both bacterial and viral), toxoids, antisera against tetanus, diphtheria, snake venoms, gas gangrene and also non-biologicals such as chemotherapeutic agents and vitamin supplements.

All these are produced on "No profit no loss" basis and supplied to the State Government and the Armed Forces and such other official agencies or institutions as may indent for the same. Besides, the Institute supplies to research institutions and medical colleges in Maharashtra as well as in other parts of the country, diagnostic reagents of the required standard and quality and laboratory-bred animals under the best or standard conditions.

The Institute also renders public health laboratory service. It has a close liaison with the Public Health Section of the Bombay Municipal Corporation as also with the Public Health Department of the Maharashtra State. In this year, it has also started an immunisation clinic and revived the immunisation programme where the public at large are given advice on various immunisation regimens.

For all these years, the Institute has been run as a Department of the Government of Maharashtra, totally financed by the State Government. During this year, a proposal was initiated to make the Institute autonomous, so that the bottlenecks experienced by virtue of the Institute's present administrative set-up, could be minimised. This proposal was accepted by the Cabinet Sub-committee specially constituted by the Government of Maharashtra. The committee has now proposed to the Government to take steps to reorganise the Institute, so that it could become an autonomous body.

During this year, the State of Maharashtra was faced with an unprecedented calamity of drought due to failure of rains in three successive years. There was an acute shortage of power supply. There was also a concomitant scarcity of raw materials needed for production. The State was called upon to tackle the problems arising out of such acute scarcity conditions in almost three-fourth of the State. The Institute was called upon to gear up its activities and to accept the challenges arising out of this great national calamity. In the scarcity areas, more than 40 lakhs of people were uprooted from homes and huts and were engaged in scarcity works. The Institute geared up its production activities to meet the challenges posed as a result of this calamity and, in the later part of the year, in spite of acute shortage of water and power supply and scarcity of raw materials, the production activity was geared to its pitch in order to be able to meet the demands of the scarcity areas.

In the first part of the year, there was a drop in the production which, as stated above, was due to numerous difficult conditions created by the drought. However, in the second part of the year, the situation improved remarkably and it is gratifying that due to the efforts put in by the scientists and the employees of the Institute, the production was geared up and supplies maintained throughout the State.

The production wing of the Institute was inspected by the Food and Drugs Administration. The Commissioner, Food and Drugs Administration, suggested a number of changes in the production units and these were effected. The Commissioner, Food and Drugs Administration has rendered.

valuable advice, from time to time to help maintain the highest standards in production. Further, during the scarcity conditions, when there was a difficulty in obtaining raw materials, the Commissioner, Food and Drugs Administration and his officers rendered every possible help to procure raw materials to keep up production.

The administrative set-up of the Institute was stream-lined and a number of reforms introduced in order to increase the efficiency and productivity. The installed production capacity was assessed and efforts were made to utilise the installed capacity to the fullest extent. The Finished Products Section was re-organised and the procedures were stream-lined so as to minimise delays and to expedite the supply of goods to the indentors.

The Institute is affiliated to the University of Bombay and has been involved in training a number of scientists in various disciplines of Science. It has, on its roll, 86 students, all working for their Master's or Doctorate programmes in various branches, such as Microbiology, Biochemistry, Pharmacology, Applied Biology, Organic Chemistry and Zoology. During this year, the number of seats at this Institute was increased from 86 to 99 and the Institute was recognised by the University of Bombay for the M. Pharm. Course in Pharmacology and Pharmaceutical Chemistry.

One of the dire necessities in a developing country, which is rapidly expanding its health services, is to provide trained technicians in various medical and auxiliary fields. The Institute was the first in this part of the country to start a diploma course in Medical Laboratory Technology offered by the University of Bombay. In addition, the Institute also conducts its own certificate course in Medical Laboratory Technology, principally to train young scientists in the techniques of bio-assay and drug analysis.

Since this is a teaching Institute, the programme was stream-lined that each of the research students, numbering about 100 was required to present a progress report on his research work to the Director every 3 months. It was also reorganised, so that each of the research students could be directly supervised intimately and intensively by his post-graduate teacher. In order to provide theoretical background to students, a lecture course was organised and the Assistant Director, Quality Control Department, was put in charge of organising the lecture series in consultation with the Director. A total of 140 lecturers in various disciplines, such as bacteriology, immunology, virology, pharmacology, bio-assay, organic chemistry, herpatology, instrumentation, biochemistry, animal technology and biostatistics were organised throughout the year. The lecture courses were also open to scientists of all other teaching institutions and medical colleges affiliated to the University of Bombay.

The production of the Bacteriology Department was not only kept up but was geared up to the maximum. The department was re-organised. Those sections concerned with the production of bacterial toxins and

toxoids, which were previously under the control of the Immunology Department, were brought under the control of the Bacteriology Department. The acetone killed and dried T.A.B. Vaccine preparation was standardised and it would soon be possible to produce large quantities of A.K.D. T.A.B. Vaccine. Creation of a registry for identification of bacteria and maintenance of strains in a lyophilised condition was organised by forming a Cell in the Bacteriology Department.

The Institute had been facing difficulties with respect to the production and standardisation of triple pertussis vaccine. A task force was, therefore, formed with the Director as the Chairman, Deputy Director (Production), Assistant Director I/c Department of Bacteriology and Assistant Director I/c Department of Quality Control as members, to immediately take up the problem of pertussis vaccine production. During the year, the World Health Organisation was approached to provide the services of a Consultant to help in manufacture and standardisation of pertussis vaccine. Accordingly, Dr. A. F. B. Standfast, Director of the Lister Institute, spent about six weeks here and worked actively with the Task Force to standardise the production of the pertussis vaccine. It has now become possible to undertake a regular programme of production of pertussis vaccine from 1st January and it is expected that the triple vaccine (DPT) would be made available from 10th January 1974 in fairly large quantities.

The Infusion Section of the Institute was geared up in its activities. In addition to the usual infusions manufactured, two additional products were taken up for manufacture, i.e. 20 per cent. Sodium Chloride Solution and Peritoneal Dialysis Fluid so very urgently needed as a life-saving drug in the treatment of renal failure etc.

The Immuno-haematology Department continued its vigorous blood collection and research activities. Thanks to the fine organisation and the efforts of the Public Relations Officer of that Department, there were almost 4,000 and above voluntary donors from about 54 institutions all over the State and in all, blood from over 13,000 paid and voluntary donors was collected.

The Institute has a pilot plant which was erected almost 20 years back through the efforts of Late Major-General Sokhey. The plant was initially used for the manufacture of sulphathiazole and it was used from time to time. During the year, a Pilot Plant Committee was formed under the Chairmanship of the Director. The Commissioner and the Assistant Commissioner, Food and Drugs Administration, were invited to be members of this Committee, along with the Head of the Department of Chemotherapy and one of the officers of the department acted as the Convenor. The object of the committee was to formulate a policy with respect to utilisation of the facilities of the pilot plant. Several new projects were initiated. The pilot plant produced Nicotinic Acid and Nicotinamide which were urgently needed for the manufacture of vitamin tablets required for the scarcity areas.

The Pilot Plant Committee also took up projects involving import substitutions. The research wing of the Chemotherapy Department took up synthesis of a number of new compounds, such as D. A. D. D. S. a long-acting derivative of D. D. S. required for the treatment of leprosy, tetrazimazole a new derivative of Imidazole useful in the treatment of helminthiasis and complex of iron and glycine as a substitute for ferrous sulphate. The department has also taken up the synthesis of DBI and Clofazimine. Thus, the objective was not only to utilise fully the facilities of pilot plant for the benefit of the Institute but also (1) to aim at import substitution and (2) to provide facilities to University students to carry their laboratory-scale synthetic work on to a larger pilot plant scale. In years to come, it has been proposed to reorganise the entire plant, so that it can be fully modernized to provide pilot plant facilities not only to the University departments and the research scientists in the University, but also to a small-scale industry which may not be in a position to have such facilities of its own.

One of the important features of the public health service is a surveillance of the enteric fever and also of plague in the city and suburbs of Bombay. The Institute maintains a typhoid epidemiology unit manned by experts. The unit is sent to any part of the city or suburbs for on the spot, investigations. Its experts collect blood from any fever patient and examine it for serological as well as clot culture for diagnosis of typhoid and related infectious diseases and send the reports to the attending physicians with a copy to the public health authorities. This is all done free of charge. This serves to keep the health authorities informed of the nature and extent of enteric fever in the city.

The Immunology Department has continued its production activities and has also been able to step it up. The production of Gas-gangrene Antitoxin has been initiated during the year. The Institute faced a constant problem of horses all these years. Personal contacts were, therefore, established with the military authorities and thanks to the active co-operation offered by them, it has been possible to obtain a large number of horses from the Defence Services in order to step up the production of antisera. During this year, a decision was taken to transfer a large number of horses from the present premises of the Haffkine Institute to Pimpri Stud Farm. Immediate arrangements were made at Pimpri and by the end of March, most of the horses have been transferred. It is also gratifying to note that the total expenditure on the purchase of horses from outside agencies has been completely eliminated.

The Institute was called upon to start a Laboratory to provide facilities to the pharmaceutical industry to carry on drug standardisation and analytical work. The Assistant Director in charge of the Pharmacology Department was designated as the Government Analyst. After a full-fledged development of the Food and Drugs Administration, headed by a Commissioner, the work has not been shifted to the office of the Commissioner

of Food and Drugs Administration. However, the Institute, which has been pioneer in drug standardisation and analytical work, still continues to receive samples for drug assay and analysis, especially biologicals, from various pharmaceutical firms in the country.

The Department of Pharmacology continued its programme of drug testing and analysis. During the year under review, the entire Drug Analysis and Biological Testing Sections, functioning on behalf of the Food and Drugs Administration, but located at this Institute owing to shortage of space, were transferred to the parent department. This department, therefore, continued only with the drug testing activity on commercial basis. During the year, the department was called upon to undertake analysis of food samples suspected to be contaminated with *Datura* seeds as well as analysis of milo flour. The departmental scientists did an excellent job and were able to report on the contaminations at a very short notice.

The Quality Control Department continued its vigorous activities to exercise strict quality control over the products manufactured by the Institute. It was also called upon to test a number of raw materials that go into production of biologicals. In addition, the Quality Control Department was involved in international collaboration with the World Health Organisation for study on the replacement of the present international standards for diphtheria toxoid. The study was completed within the specified time and the result were made available to the World Health Organisation.

The Department of Viral Vaccines was completely reorganised. The production of rabies vaccine was separated from the Department of Virology and attached to this department so as to streamline the production of all types of viral vaccines.

There was a lot of difficulty with respect to initiation of the production of Oral Polio Vaccine programme. In spite of the fact that the facilities and technical know-how existed in the Institute for the last over six years, the problem had got stuck up due to many administrative difficulties. These were completely sorted out with the active co-operation and advice of the Director-General of Health Services, the Drugs Controller, Government of India, and the Commissioner, Food and Drugs Administration, Government of Maharashtra. It is very gratifying to note that the Oral Polio Vaccine production has since been initiated; type I virus strain of about 17 litres has already been collected, production of type II seed virus fluid and seed virus type III is in process. It is expected that, if everything goes all right, it will be possible to produce indigenously at this Institute fairly large quantities of Oral Polio Vaccine by the beginning of the next year.

An Enterovirus Seminar was held at the Institute which was inaugurated by the Minister of Public Health and presided over by Dr. P. N. Wahi, Director-General, Indian Council of Medical Research. The pathogenesis and epidemiology of poliomyelitis and the efficacy of the polio vaccine were discussed.

The Institute, in collaboration with the Municipal Corporation, takes up a surveillance over the epizootic infection of plague. Wild rats collected from the various wards of the city are examined for the presence of plague infection. A statistical data of the different species of rats is also maintained for further study.

During this year, the Institute has strengthened its activities in the field of epidemiological research. A very close liaison was established between the Epidemiology Unit of this Institute and the Directorate of Public Health. The Institute planned epidemiological studies in the State of Maharashtra in the field of gastro-enteritis, filariasis and guinea worm infestations.

The number of scientists and dignitaries who visited this Institute during the year was 78, the notable amongst whom are :

Dr. Julius Axelord, Nobel Laureate in Medicine, U.S.A.; Mr. H. J. Higgins, President, International Veterinary Students' Association, Royal Veterinary College, England; Shri Ajit Panja, Minister for Health, Government of West Bengal, Shri D. N. Saraf, Divisional Commissioner for Handicraft, Ministry of Foreign Trade, Government of India, New Delhi; Prof. Louis S. Diamond National Institute of Preventive Medicine, London; Shri G. S. Sarnayak, Lister Institute of Preventive Medicine, London; Shri G. S. Sarnayak, Minister of State for Urban Development, Public Health and Buildings and Communications, Government of Maharashtra; Prof. Stanley A. Plotkin, Associate Member, Wistar Institute, and Assistant Professor of Paediatrics, University of Pennsylvania, U.S.A.

The Critics Club activity was pursued vigorously. About 41 scientific meetings were organised during the year, at which 18 outside scientists were invited to deliver guest lectures.

The Institute has one of the finest libraries having more than 18,000 volumes of books and a number of monographs. In fact, the library is a Reference Library not only for the scientists of this Institute but is kept open upto late hours in the evening to provide facilities of reference work to scientists from the city. Since the library specialises mainly in the field of communicable diseases, it was decided during the year to compile references on each of the communicable diseases. Steps were, therefore, taken to create a Reference Cell on Cholera to begin with. The Institute's scientists published in all about 21 scientific papers during the year and 17 more scientific papers were under publication.

General Sahib Singh Sokhey Memorial Oration was delivered by Dr. V. Ramalingaswami, Director, All-India Institute of Medical Sciences, New Delhi, on "Geographic Pathology of Liver Disease".

During the year under review, the following officers retired :---

1. Dr. M. Y. Mhasalkar, Senior Scientific Officer, Class-I, Department of Chemotherapy. July 1972.
2. Dr. S. S. Rao, Assistant Director I/c Department of Immunology. September 1972.
3. Shri H. K. Mhatre, Senior Scientific Officer, Class-II, Department of Immunology. October 1972.
4. Dr. R. A. Bellare, Senior Scientific Officer, Class-I, Department of Chemotherapy. January 1973.

The airconditioning of major departments was accomplished. Additions and alterations to the Immunohaematology Department were also completed. Various changes suggested by the Commissioner, Food and Drugs Administration, after his inspection of the production wings, were accomplished.

The construction of Class IV quarters at the Haffkine Institute, Bombay, has been completed and as many as 140 families have been allotted quarters and they have already moved in.

One of the important aspects of efficient working of the Institute is an excellent Maintenance Engineering Section. During this year, the Section not only kept up its activities but, in fact, put in a lot of efforts to maintain the various apparatus and equipment required for production and research. During the year, a special Electronic Repair Cell has been organised and one of the staff members of the Section has been specially trained in the repairs and maintenance of important electronic apparatus, at the Electronic Research Division of the Bhabha Atomic Research Centre, Bombay.

In order to create a feeling of fraternity and comradeship, a Home Bulletin called the "Haffkine Vritta" was started. It is a quarterly publication of the Sports Club and serves to promote harmony and brings about a feeling of family relationship. The Sports Club also started a free reading room and a library as well as other similar social welfare activities.

A Labour Welfare Committee was constituted for the first time in order to initiate labour welfare activities.

Incentive award schemes were formulated by the Director and some members of the staff were awarded prizes and certifications.

The Institute's employees contributed generously to the Chief Minister's Scarcity Relief Fund.

There were several meetings with the Unions in order to bring about a good labour management relationship. Unions were provided with office accommodation.

The dispensary was completely reorganised and shifted into new premises. The family planning programme was actively taken up.

The Haffkine Institute Co-operative Credit Society, Limited, continued to make steady progress. Its membership has shown a steady increase from 924 in 1971 to 962 in 1972.

Two new cells were established, viz. a Department of Radiobiology and a Unit of Toxicology.

The Cell in Radiobiology was initiated to act as a nucleus or radiation medicine with particular reference to aspects of diagnostic procedure and is proposed to be converted into a Department of Radiology. A thyroid clinic was started. It has taken up other activities concerning the use of radioisotopes for diagnostic purposes.

The Toxicology Unit has been organised to undertake detailed toxicological studies of compounds synthesized in the Institute or referred to it by the pharmaceutical industry. It is proposed to develop this Unit into a full-fledged Department of Toxicology and Human Pharmacology.

Both these are directly under the administrative control of the Director.

Research being the primary function of the Institute, the research activity was reorganised. It was decided to take up certain research programmes as collaborative research schemes so as to bring together the scientists specializing in various disciplines, to tackle various aspects of the research problems. With this end in view, collaborative research cells were formed with Director as the Chairman. These cells meet every month to discuss the progress of research. Collaborative researches organised during this year are in the fields of (a) filariasis, (b) amoebiasis, (c) venoms.

In the field of filariasis, extensive epidemiological work was carried out, both in the urban and the rural areas. Epidemiology of filarial infections in animals was also studied with a view to find out a relation, if any, between filariasis in man and animals as a zoonotic problem. Experimental infection in rats was established and an *in vitro* method for testing of drugs in filariasis was accomplished both by maintaining filaria and microfilaria in laboratory animals and tissue culture. A method of Vero cell line culture was initiated so as to be able to maintain human microfilaria in the cell line.

In the field of amoebiasis, the studies include epidemiology, development of axenic culture, experimental amoebiasis and evaluation of anti-amoebic compounds.

In the field of venoms, the entire approach was to standardise procedures of venom extraction, to study the various fractions of venoms obtained from some of the species of poisonous snakes, to carry out a detailed study of their biological activity and the immunological characteristics. In addition to this, a detailed study of the effect of venom on the blood clotting mechanism was also carried out and has yielded very interesting results of clinical significance.

From time to time, deaths due to scorpion bites have been reported from the various areas of Maharashtra. In order to elucidate the mechanism of action of venom and to find out the protective methods, studies were initiated in scorpion venoms under the direct supervision of the Director. These have yielded some significant data. The snake venom was irradiated in order to find out whether a toxoid could be prepared.

The venom has been shown to lose its toxicity. Its antigenicity is being tested.

During the year, emphasis was also laid on developing collaborative research with other research institutes and medical colleges. Thus, a research group was formed with the Pediatrics Department of the J. J. Group of Hospitals to undertake extensive studies on the problem of rickets, with particular reference to the study of vitamin D metabolism. Similarly, other collaborative research schemes were undertaken.

A collaborative research has been started with the Radiation Medicine Centre of the Bhabha Atomic Research Centre. Labelled metabolites required for the growth of micro-organisms are incorporated in the culture media in order to assess whether a very quick method for sterility testing of biologicals can be developed. The results are extremely encouraging. It has been established that the bacterial contamination at various stages in the production of biologicals can be detected within 24 hours as against a period of almost a week that elapses before the results can be assessed. The project is being pursued further and is expected to yield substantial results which will go to save a number of production hours that are lost in sterility testing.

In addition to this, the individual departments have continued to pursue their own research interests. The Department of Bacteriology has further perfected a fermentation technology for preparation of bacterial vaccines and it has reached a take off stage for adopting this technology for the production of different bacterial vaccines. Diphtheria and plague projects, which have been started a few years back, have been continued. A synthetic medium has been developed for the growth of diphtheria organisms and this has been found to increase the yields of diphtheria toxin. The plague surveillance programme was continued to find out the lingering foci of plague. Presence of high flea index was noted in a number of areas in Greater Bombay and the presence of tolerance to Warfarin in rats, especially *B. bengalensis* and *Rattus, rattus*, was further established. The strains of plague referred from various areas and especially from the W. H. O., were identified on the basis of presence of Fraction I antigen. A number of strains of plague were studied for Pesticin I producing activity and all of them showed this activity. The fluorescent antibody technique has been standardised for the study of specificity of the plague strain.

The entire Bio-pharmaceutical Division was activated to undertake a collaborative study between the Departments of Chemotherapy, Biochemistry and Pharmacology. Two derivatives have been synthesized and actively

screened for anti-parasitic activity in the Pharmacology Department. A series of mannich bases prepared by 5-Nitro-furfuryl-2-carboxamide and N-substituted piperazine have been prepared and are being tested for antibacterial, anti-parasitic and antihelminthic activity.

Studies on the resistance factor of Enterobacteraceae and their transfer have been continued in the Department of Clinical Pathology. Two hundred and ten strains of *Salmonella typhi* have been tested for sensitivity against a large range of antibiotics. Eighteen cultures of *S. typhi* and 10 *Salmonella para typhi* were found to be resistant to Chloramphenicol. The resistance was found to be transient and was lost on sub-culture.

Extensive studies on Gonococci have been undertaken. Studies on Brucella, both with respect to its epidemiology and zoonotic problems, were continued. It was established that prevalence of Brucellosis in dairy and pig workers is about 12 per cent. and 9 per cent. respectively, while the animal prevalence ranged anywhere between 6.6 per cent. and 70 per cent. *

During this year, studies on Australia antigen were continued. Similarly, in professional donors as well as in various liver disorders, an attempt was made to produce antibody to Australia antigen. Other studies in immunoglobulins in the Department of Immunohaematology included alpha Fetoproteins, especially in liver malignancy, studies on immunoglobulins, especially M-haemoglobin in rheumatoid arthritis, haptoglobins and alpha-2 haemoglobin binding proteins in neo-natal anaemias and neo-natal jaundice. Specific immunoglobulins, especially against tetanus, as a programme have been taken up for study on a very large scale. Similarly, red cell enzymes, especially G-6 PD, PK and GSH, are being studied in various haemolytic anaemias.

The Department of Pharmacology was reorganised to undertake a collaborative research activity with the Biochemistry and Chemotherapy Department to produce antibody to Australia antigen. Other studies in immunosensitive pharmacological screening programme, so that the compounds synthesized in the Chemotherapy Department could be effectively and expeditiously evaluated pharmacologically. The department has specialised in the studies on leprosy. Shepard's mouse foot pad method for the growth of leprosy bacilli was standardised and the growth curve on lepra bacilli in thymectomised and anti-lymphocytic serum (A. L. S.) treated mice was studied. Further, drugs were screened in experimental amoebiasis and filarial infections. Extensive study was undertaken in *Giardia lamblia* infections. A number of drugs were screened for their *in vitro* activity against *G. lamblia*.

The Institute has a very large centre for studies on enteroviruses, financed by the Indian Council of Medical Research. As stated previously, it organised an Enterovirus Seminar. The Assistant Director in charge of Department of Viral Vaccines has been designated as the leader of a team of Enterovirus Units all over the country, which are financed by the Indian Council of Medical Research. The centre is mainly involved in carrying out extensive surveillance programme in the field of poliomyelitis in the

cities and the rural areas. It recorded 1,338 cases of paralytic poliomyelitis from January to December 1972 in Bombay. Progressive rise in endemicity of polio in Bombay and a rise in the incidence of paralytic poliomyelitis in Bombay were noted. Sixty per cent of the paralytic polio cases were seen to occur between the months of June and September and the vulnerable population was found to be below 3 years of age. Sixty-five per cent of the paralytic polio cases showed excretion of polio virus. Out of these, almost 70 per cent were found to excrete polio virus type I. Out of the 1,338 cases of paralytic polio, 98 cases had received 3 doses of oral polio vaccine. Studies on response to polio vaccine were pursued vigorously. Typing of the virus excretion and seroconversion have brought out some interesting features. Sero-conversion rates after 3 doses of the vaccine were 53.3 per cent for Type I, 90 per cent for Type II and 76 per cent for Type III. Incidence of paralytic polio in the entire child population of Bombay was studied. One in 250 children seemed to get paralysed if unvaccinated, while 1 in 4000 was the incidence of paralytic polio in the vaccinated age group of 0-2 years. This clearly brings out the relative efficacy of the vaccine.

The Department of Virology continued its interest in the field of rabies, small-pox, measles, influenza surveillance programme and epidemiological studies on Rubella infection. During this year, it was decided to undertake a joint collaborative programme with Dr. Plotkin of the University of Pennsylvania, U.S.A., to study the efficacy of the new human diploid cell grown antirabic vaccine. This study has been initiated and is in progress.

The Department of Zoonosis continued its activity in the field of epidemiology of filariasis as a collaborative project. Serologically, rural as well as urban areas were screened and in rural areas, the incidence of 7.7 per cent infectivity rate was established. The department also carried on cholera carrier studies and screened about 3,000 human stool samples, 200 animal stool samples and 30 water samples in an endemic area in Parbhani.

The Institute has continued its interest in the field of cholera. Epidemiological studies were carried out in rural areas to assess the role of cholera carriers, both in the healthy and convalescents. Further extensive bacteriological studies on gastroenteritis are being undertaken. Studies on pathogenesis in the experimental cholera model have also been continued and the mechanism of action of berberin has been elucidated. Attempts were made to prepare a cholera vaccine by irradiation. This has given very promising results. For the first time, it has been possible to prepare an irradiated cholera vaccine which is more potent than the vaccine prepared by the conventional methods in experimental animals. It has also been possible to establish that the toxicity of the irradiated vaccine is very low. More extensive programme for the use of radiation in the preparation of cholera vaccine has been planned in collaboration with the Atomic Energy Commission and the Bhabha Atomic Research Centre. At the same time,

the Department of Bacteriology has undertaken live cholera vaccine production programme to see if this vaccine could be supplied in a lyophilised form, so as to eliminate the necessity of preservation of the vaccine under refrigerated condition in the field where it is required.

Studies on the growth requirements of the *Vibrio cholera*, both classical Ogawa and Inaba and 2 El Tor strains of Inaba and Ogwa, were continued in the Biochemistry Department. A new peanut protein hydrolysate medium was prepared to replace the usual casein hydrolysate medium. This medium was found to be as effective as the casein hydrolysate medium for the growth of cholera organisms.

In addition to research of a collaborative nature, project research and individual departmental research continued on the same pattern as before. The emphasis, as usual, has been on production-oriented research, with a view to improve the efficacy of the products manufactured by the Institute.

The Research Advisory Board met on 3rd October 1972 and the suggestions of the previous Research Advisory Board were implemented. The Director and senior members of the staff were greatly benefited by the advice and guidance rendered by the member of the Research Advisory Board. They put on record their grateful thanks to all the members of the Board for taking keen and active interest in the working of the Institute.

The Director and the members of the staff greatly appreciate the support they received from the administrative heads of Sachivalaya, which has resulted in bringing about accelerated changes in many areas.

Special thanks are due to the Commissioner, Food and Drugs Administration, who has not only exercised a vigil over the maintenance of the standard and the quality of the products manufactured at the Institute, but has given advice and guidance from time to time and assistance in procuring raw materials which were becoming extremely scarce.

Heads of departments and senior members of the staff significantly contributed to the different activities, both in the field of research, production and training. Without their active co-operation, enthusiasm and zeal in accomplishing the work as a joint responsibility, the Institute would not have been able to accomplish all that it has done during the year.

The success of the Institute depends upon the drive, integrity, conscientiousness and the skill of all the members of the staff, the scientists, technical staff, administrative staff and last but not the least, the workers in the different departments. 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 also takes this opportunity to thank all the fellow scientists, administrators and the workers for their unstinted support and loyalty, which enabled him to discharge his onerous duties, both administrative and scientific.

PART II
RESEARCH

- A. RESEARCH PROJECTS**
B. DEPARTMENTAL RESEARCH
-
-

A. RESEARCH PROJECT

CHOLERA PROJECT

Epidemiological studies of Cholera in non-endemic regions with special reference to the problem of carrier state during epidemic and non-epidemic period.

The role of carriers in the spread of cholera and its persistence in endemic areas has been well emphasized by studies conducted in various parts of the world. Recent Laboratory studies show that a large number of carriers persist during the inter-epidemic period. Hence, international sanitation regulations have not proved adequate in the prevention of cholera. We have observed that peculiar diarrhoeal cases occur in certain areas of the State of Maharashtra. It is also seen that these are areas, which are endemic as well as non-endemic to cholera in this State. The role of human carriers in the recurrence of epidemics every year in the affected areas is not fully understood. The role of animals as carriers in the maintenance of vibrios during inter-epidemic period is also not known.

It was also decided to find out the extent of persisting infection and non-vibrio bacterial infection responsible for these recurrent epidemics, particularly during summer and monsoon seasons.

There is no doubt that environmental factors, such as water supply and garbage and sewage disposal systems in these areas also play an important role, but the mode of persistence of vibrios during interepidemic seasons is not fully established.

A summary of findings with essential data

Present studies were conducted in Parthani District. Follow-up studies and household survey was carried out in Parbhani Town in general and in Khadradabad plot area in particular.

Examination of (i) human faecal samples (ii) faecal samples from extra human sources and (iii) water samples from different areas was carried out.

(i) Human faecal samples

So far, 3677 human stool samples have been processed. The pathogens isolated are as follows :—

<i>Vibrio cholerae</i>	...	55
<i>E. coli</i>	...	2240
<i>Proteus</i>	...	501
<i>A. aerogenes</i>	...	331
<i>Alk. fecalis</i>	...	281
<i>Pseudomonas</i>	...	75
<i>Staphylococci</i>	...	22

Details of the V. cholerae strains isolated

Total strains isolated	55
Isolates from urban cases	16 (inclusive of 7 from Khadrabad).
Isolates from rural cases	21
Isolates from convalescent carriers (Khadrabad).	5
Isolates from contact carriers (Khadrabad).	8
Isolates from repeat samples of carriers (Khadrabad).	5

All the isolates were found to be *V. cholerae* biotype *El tor* serotype *Ogawa*.

Antibiotic sensitivity test.—Results showed that only 50 per cent of the strains were sensitive to tetracycline (30/sug) whereas all (100%) were sensitive to chloramphenicol (30/ug.).

Pathogenicity test.—Fifteen strains studied so far were found to be pathogenic in experimental animals (method by Dutta and Habbu 1955).

Eleven strains of *V. parahacmolyticus* have been isolated from dog samples.

(iii) Examination of water samples.

Fifty-eight water samples, collected from tap water as well as from different areas of Parbhani town, were examined.

Four strains of vibrio-like (Non-agglutinable) organisms have been isolated, which are being studied further. Other pathogens isolated were *E. coli*, *Alk faecalis*, *Proteus* and *Pseudomonas*.

Biochemical studies of the action of Furazolidone on cholera organisms.

It was reported earlier that furazolidone inhibits the growth of cholera organisms by interfering with the protein metabolism. It is observed to uncouple the translation stage which results in the accumulation of RNA and inhibition of protein synthesis. Analysis of the base composition of the RNA indicates that the accumulated RNA is of the normal type.

Effect of furazolidone on oxygen uptake and on the oxidative and Transaminase enzymes of *V. cholerae* and *El tor* cholera.

To investigate whether the ATP or the GTP is the limiting factor for the protein synthesis, the oxygen uptake and the enzymes of the Krebs cycle were studied. The pyruvic, lactic and malic dehydrogenases are inhibited in the drug-treated organisms, indicating a general inhibition of the TGA cycle. The alpha keto glutamate dehydrogenase shows a higher specific

activity while the iso-citric dehydrogenase is unaffected. This observation, together with the high specific activities of GOT and citrate cleavage enzymes indicates that the drug accelerates the protein catabolism. These effects are less pronounced in the *El tor* strain. Both the strains show increased oxygen uptake which is typical of energy uncouplers. The glucose utilisation is more by the HMP. pathway, which is confirmed by the high specific activities of glucose-6-phosphate dehydrogenase in the drug-treated organisms.

Effect of furazolidone on the incorporation of glycine leucine and phenyl alanine.

Studies on the kinetics of synthesis of proteins, using labelled amino acids indicate that the drug inhibits the incorporation of glycine and leucine with no effect on phenylalanine. This can be due to some abnormal synthesis. Therefore, the soluble proteins of the normal and drug-treated organisms were isolated and fractionated by poly-acrylamide gel electrophoresis.

While fourteen protein bands are observed in the normal, the drug treated organisms show only twelve, with two of the lower molecular weight protein bands missing and displacement of some others. This gives a clue that some abnormal proteins are being synthesised by the drug-exposed organisms.

Biochemical studies on the effect of Chloramphenicol on cholera organisms.

(a) *Cellular composition*:—The organisms were grown in the synthetic medium for two hours under shaking conditions at 37°C, when the drug was added to the appropriate flasks to give a concentration of 0.5 µg/ml. Incubation was continued for six hours when the cells were centrifuged (12,000 rpm) washed with Tris-HCl buffer (pH 7.3) and dried in vacuum. Total nitrogen, RNA, DNA, proteins and lipids were done by the standard procedures used in the laboratory. It is seen that the drug inhibits the protein and phospholipid synthesis, while the synthesis of RNA is almost doubled.

(b) *Study of the protein spectra on acrylamide gel*:—The inhibition of protein synthesis was studied in detail using polyacrylamide gel techniques. Seven per cent. acrylamide and his acrylamide in Tris-glycine buffer (pH 8.3) at a voltage of 150 (5 maps/tube) for 3 hours was used. The protein bands were stained with commassive blue dye located on the densitometer. It is seen that 5-6 protein bands are missing in the protein fraction of the drug-treated organisms.

(c) *Characterisation of nucleic acids*:—The RNA and DNA were isolated by the phenol extraction method and the RNA fractions were separated on MAK column. The base composition of the total, transfer (t) and ribosomal (r) RNA was determined by high voltage electrophoresis. Significant changes were observed in the amount of nucleic acids. The quantity of r-RNA (16 and 23—S-RNA) was more. The base composition of the various RNA is not altered.

A distinct difference in the base composition of the light and heavy *Strands* of the DNA was observed.

(d) *Oxygen uptake and oxidative phosphorylation*:—The cells were harvested after 8 hours of growth, centrifuged, washed with tris buffer and diluted with buffer to give the required capacity. Aliquots were used for measuring the oxygen uptake on Warburg manometer and the oxidative phosphorylation studies were done spectrophotometrically, using succinate and glutamate as substrates. It is observed that the oxygen uptake is more in the drug-treated cells and the oxidative phosphorylation is inhibited.

(e) *Study of the enzymes of the TCA cycles*:—The lactic, pyruvic, malic, isocitric, alpha keto glutaric dehydrogenases and NADH oxides were studied spectrophotometrically by the standard procedures. The lactic, isocitric and alpha keto glutaric dehydrogenases are very much inhibited, while the malic dehydrogenase is unaffected. The pyruvic dehydrogenase is increased nearly four-fold. The NADH oxides are also inhibited.

(f) *Characterisation of the phospholipid*:—The total lipids were extracted and fractionated on silicic acid column. About five different types of phospholipids have been identified. Quantitation of the fractions is in progress.

Biochemical studies on cholera organisms grown in different media

(a) *Cellular composition*:—The nutritional requirements of *Vibrio cholerae* for amino acids and minerals were reported last year. The growth of the organism in the peanut protein hydrolysate was found to be better than that in the routinely used casein hydrolysate. A detailed study of the cellular composition of the organisms, grown in the four media—casein hydrolysate (c), peanut protein hydrolysate (p), the synthetic medium of Thind (T) and the formulated chemically defined medium (S) under stationary and shaking conditions were undertaken. The cells were grown, harvested, washed and dried in vacuum. Total nitrogen, proteins, reducing sugars, RNA, DNA and lipids were estimated by the standard procedures. The RNA content of the organism, grown in the synthetic media (T & S), are almost double that of the other two.

(b) *Oxygen uptake and protein synthesis V. cholerae (Ogawa)*:—The oxygen uptake and protein synthesis is 50 per cent more in the peanut protein hydrolysate. The synthetic media—T and S—seem to be rather poor in this respect.

(c) *Nucleotide composition of RNA of V. cholerae grown in different media Vibrio cholerae Ogawa*:—The Purine/Pyrimidine ratio from RNA from organisms grown in peanut protein hydrolysate is 1.25, while the others are higher.

Other studies

Lyophilization of bacterial cultures:—Lyophilization of *Vibrio cholerae* (Ogawa C₁—S₃) in small neutral glass tubes (110 mm. long with

6-7 mm. outer diameter), using gum acacia with nutrient both, gum acacia and glucose, different concentrations of sugars and combination of lactose with sodium glutamate, was reported last year. The percentage survival obtained was not more than 0.1 per cent.

During the year under review, lyophilization of this culture was done in ampoules (10.0 ml. capacity). Using 8.5% lactose and 8.5% sucrose as suspending media, one ml. of the culture was taken in each ampoule. The cultures were subjected to drying under vacuum for 20 and 44 hours respectively. After lyophilization each batch of the culture was examined for its viable count. The freeze-dried culture was reconstituted in 1.0 ml. of casein hydrolysate medium containing 270 mg. of nitrogen per 100.0 ml. For determining the viability, the dilutions of the reconstituted culture were also carried out in the casein hydrolysate medium. The viability obtained varied from 6 to 11% as shown in the table. In the experiments carried out so far, no significant difference was observed in the percentage viability when the cultures were dried for 20 hours and 44 hours respectively. Experiments would be carried out to study the effect of pH during lyophilization on the viability of an organism.

Radiation-killed cholera vaccine.—With a view to investigating whether the use of radiation for inactivating the cholera organisms would yield a better type of cholera vaccine, the studies were continued. It was reported last year that the cholera vaccine prepared by irradiating the organisms either for one hour or two hours were more potent than the usual formal in-killed and phenyl mercuric nitrate preserved cholera vaccine.

While assessing the efficacy of a vaccine, it is necessary to see that the vaccine is highly antigenically potent not only at the time of preparation but throughout the potency period. A good vaccine should not show any abnormal deterioration in its antigenicity during the shelf and potency period at the prescribed temperature of storage. With this object in view, the two cholera vaccine containing 6×10^9 organisms per ml. of the strains *Vibrio cholerae* 569 B Inaba and 6×10^9 organisms per ml. of the strain *Vibrio cholera* S/II/58 Ogawa inactivated by exposing to a 60 CO source for one hour and two hours respectively were tested periodically by active mouse protection test along with a standard cholera vaccine containing an equivalent number of organisms per ml. of the same two strains but killed by the addition of formalin and preserved by phenyl mercuric nitrate. The standard vaccine is preserved all the while in a lyophilized state. The results reveal that none of the two vaccines show any deterioration in the antigenicity even after a period of 18 months of storage at 4 to 10°C. The tests will be continued for some more time. vaccine show any determination.

Two more batches of cholera vaccine have been prepared by irradiating the cultures for one hour and 2 hours respectively. These will also be subjected to active mouse protection tests. It is also proposed to test these vaccine in young and adult-rabbits for their antigenicity.

Studies on cholera vaccine and allied investigations.—Monovalent, bivalent and quadrivalent cholera vaccines are studied for their protective value by passive immunisation technique. Hyperimmune serum is produced in adult rabbits against each vaccine under study. Individual serum is to be tested for antibody and vibriocidal titre and then used for passive immunisation of infant rabbits to be challenged with virulent strains Ogawa and Inaba serotypes of classical *V. cholerae* and *EL. tor* vibrio and toxins. Loop method in the immunised adult rabbits is being tried to judge the validity of the vaccine. The study will throw light on cross-protection of the vaccines. The data will be used to evolve a method for standardisation of cholera vaccine in comparison with other assay procedures.

The study is in progress.

EL tor water 6 culture filtrate.

Earlier, it has been reported that *EL tor* water 6, when used for the preparation of live cholera vaccine, gave complete protection to infant rabbits. Moreover, it was observed that *EL. tor* water 6 culture filtrate (Syncase toxin method of Finkelstein and Lankford, 1966) contains an antigenic component which gave a remarkable protection to the infant rabbits against bacterial challenge.

The filtrate was further studied in mice (active mouse protection test). The results obtained were as follows :—

Vaccine and dilutions used.	No. of Animals used	No. of Animals survived after challenge	
		24 hours	48 hours
<i>EL tor</i> water 6 Syncase toxin.			
5 mg. ...	16	16	16
1 mg. ...	16	15	14
0.2 mg. ...	16	16	15
<i>Vaccine :</i>			
Cholera <i>EL. tor</i> (Haffkine)			
AQ 40-5 Vaccine.			
I/400 dil ...	16	10	16
I/2000 dil ...	16	13	13
I/10,000 dil ...	16	15	15
<i>Control :</i>			
I/5000 dilution ...	10	0	0
I/50,000 ...	10	0	0
I/500,000 ...	10	2	2
I/5000,000 ...	10	2	2
I/50,000,000 ...	10	2	2

The results show that *El. tor* water 6 syncase toxin gave equivalent protection as afforded by our present quadrivalent cholera *El. tor* (Haffkine) vaccine, to experimental animals.

Study on NAG Vibrios.

Syncase toxins (method of Finkelstein and Lankford, 1966) were prepared from NAG starins-Sd. 280-13026; Sd. 281-13030; Sd. 282-13031 and Cz. 16/66-10125. Toxins, when studied in infant rabbits, did not produce cholera-like symptoms, suggesting that toxins do not have choleraogenic fractions (choleraenoid) in it, which is known to be responsible for choleraic symptoms in case of pathogenic vibrios. However, as reported earlier, same NAG cultures produced cholera-like disease when studied in infant rabbits.

An attempt was, therefore, made to produce toxin *in-viva* in infant rabbits Diarrhoeic stools-intestinal aspirates, when injected after sterilization by filtration into second lot of infant rabbits, produced cholera-like disease. The findings suggest choleraogenic (toxic) nature of the fluid. Furthermore, immuno-diffusion test results showed that two of the NAG strains have similar antigens, though it had only one common band (antigen) against classical *V. cholera* Ogawa antiserum. The findings further support, as reported earlier, that there is no cross-protection amongst classical and NAG vibrios, probably due to different antigenic patterns of the classical and NAG vibrios.

Further studies with regard to preparation of toxin by different methods in different media are under progress.

Purification and characterization of Cholera toxin

Choleraogenic toxin, as reported earlier, was partially purified and two fractions, F-1 and F-2, were obtained. Experiments were carried out to study nature and antigenicity of these fractions.

The fractions F-1 and F-2 were treated separately with different concentrations of lipase, trypsin B grade and *L-amylase*.

The enzyme-treated fractions were then studied in infant rabbits (method of Dutta Habbu, 1966) for their choleraogenic activity. The results showed that all the enzymes-tested, could inactivate the choleraogenic property of the Fraction 1. The findings suggest that the choleraogenic toxin (present in Fraction 1) is a protein-like-polysaccharide complex. Hydrolysis of any part of it by the enzymes reduce or destroys its choleraogenic activity.

The results of the studies with the different enzymes of the F-2 fraction indicated that the PF activity (skin permeability reaction) could be degraded by *L-amylase* and lipase, while the proteolytic enzymes had no effect on it. The observation suggests that F-2 (PF) might be low in protein or may be protein-free and could be a complex of lipid and polysaccharide.

Immune sera were prepared against both the fractions in adult rabbits. F1 produced 10^7 vibriocidal and 1 : 6400 agglutinin antibodies whereas F-2 gave a vibriocidal titre of 10^7 and agglutinin titre of 1:1600.

Passive protection test, using the above immune sera in infant rabbits, demonstrated that F-1 was antigenic; and statistically significant prolongation of the life of experimental animals was observed. On the other hand, F2 immune sera failed to give adequate protection. Furthermore, skin permeability test in adult rabbits demonstrated that the F2 is not suitable for producing immunity against PF. However, F1 completely resisted intracutaneous injection to Syncase toxin. The results indicate that PF (F2) is a poor antigen as compared to choleraogenic factor (F1).

Brief summary of work on berberine in toxin induced experiment cholera

(1) Berberine sulphate, when injected alone in dose below 10 mg. per loop (in adult rabbit ileum) does not cause any accumulation of fluid at the end of 18 hours.

(2) Berberine sulphate, given along with toxin, causes a reduction in fluid accumulation by three fold, was compared to those loops which are given toxin alone.

(3) Theophylline, when given prior to the administration of berberine and toxin, does not interfere with the action of berberine in reducing the fluid accumulation due to toxin. Loops which are given berberine and toxin, contain, on an average, the same volume of fluid as those given theophylline in addition.

DIPHTHERIA PROJECT

Diphtheria Toxin production

A synthetic medium was developed to study metabolism of *C. diphtheria* in relation to toxin production. Biotin or pimelic acid was found to nullify the inhibitory effect of iron on toxin production, suggesting alteration of permeability of cell membrane, iron making it less permeable and biotin counteracting the effect of iron. As the effect was considered on lipid composition of membrane, further study was carried out to find out unsaturation of fatty acids (responsible for fluidity) of cell envelope in medium containing iron and biotin both or none. Biotin appeared to increase the unsaturation three to four fold, but contradictory to our previous assumption, iron also stimulated unsaturation.

Attempts were also made to obtain maximum titres of diphtheria toxin production on a large scale. Routinely, only 50-60 LF toxin was obtained on an average. Hence, it was important to study this aspect further to get better titres. The factors studied were :—

(i) the toxigenicity of culture, (ii) the type of container and (iii) size of inoculum.

1. It was observed that toxigenicity of production culture was reduced and it was, therefore, necessary to revive it.

2. *Seven-day incubation*.—Roux bottle with 150 ml. medium yielded higher toxin production (100-110 Lf/ml) as against Haffkine flask with

900 ml. medium (55 Lf/ml.) Haffkine flasks with 300 ml. medium gave high toxin production (100-110 Lf/ml.) i.e. equivalent to Roux bottle with 150 ml. medium.

3. It was necessary to increase the inoculum for higher yield of production.

4. Even 900 ml. medium in Haffkine flask gave high toxin, but the incubation period had to be changed. This is still under study.

5. Toxin titre with the use of Analar quality sodium acetate was higher than with the use of sodium acetate (Apex) which was being used earlier.

PLAGUE PROJECT

Plague project studies were initiated in the State of Maharashtra to detect lingering foci of plague infection and to meet the threat of recrudescence by undertaking surveillance measures. These studies were undertaken in the year 1965 and are continued to-date.

The work carried out indicated favourable conditions for recrudescence of plague to occur, viz. heavy rodent infestation throughout the Maharashtra State and high flea index. *R. rattus* was the prevalent commensal rodent species and *X. cheopis* the prevalent rat flea. Commensal *R. rattus* showed high degree of resistance to *Y. pestis* in urban areas and high degree of susceptibility in hinterland areas where plague was not endemic. Serological studies indicated the likelihood of presence of low titre antibody to fraction I antigen in certain areas such as Mahad in Kolaba District and in Marathwada. However, no rodent plague or human cases have been reported from these areas during the last 15 years or more. Therefore, the presence of low titre antibody detected is difficult to interpret as far as latent infection is concerned.

Presently, these studies are continued in 'D' and 'E' ward areas of Bombay city. As reported earlier, *R. rattus*, *R. norvegicus* and *B. bengalensis* together constitute the predominant commensal rodent fauna. Of the three principal species, *B. bengalensis* is the most predominant species.

In all, 2,434 rats from 'D' ward area and 1,052 rats from 'E' ward area were examined. *X. cheopis* showed its predominance over the other species of fleas recovered, viz., *X. astia*, from 'D' ward 254 fleas and 179 fleas from 'E' ward were examined. Except these two species of fleas, no other species of fleas were recovered.

Susceptibility of fleas to insecticides

Fleas collected from 'D' and 'E' ward areas are being reared under laboratory conditions to assess their susceptibility to insecticides. Susceptibility status of dog flea *Ctenocephalides canis* to D.D.T., dieldrin and malathion was assessed. These fleas were recovered from stray dogs from Mahalaxumi dog kennel.

Susceptibility of rats to anticoagulant rodenticides

Assessment of susceptibility status of rats from 'D' and 'E' ward areas to anticoagulant rodenticide, viz., Warfarin, by using W. H. O. methods was continued. The work carried out so far indicated the presence of tolerance to Warfarin among *B. bengalensis* and *R. rattus*.

Serological studies in plague

Erythrocytes, sensitized with fraction I antigen of *Y. pestis*, were received from the Ministry of Health of the U.S.S.R., Middle Asian Research Institute on plague control, Zavodskaya, 14, Alma at a 74 for plague serological studies. About 50 rodent sera from 'D' and 'E' ward areas of Greater Bombay have so far been tested by HA test for the presence of antibodies against FI antigen. All the test sera showed negative results. Attempts are being made to examine rodent serum samples from other areas of Maharashtra State for the presence of antibodies against FI antigen.

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

Rats were collected from 'E' and 'D' wards and their susceptibility to plague infection was studied by the standard challenge method.

Materials

Approximately equal number of rats were taken and challenged with a fixed dose of about 5000 organisms of the virulent *Y. pestis* strain (strain 195/P) as before and their susceptibility was determined. Rats from 'E' ward belonged to the species *Bandicota bengalensis* and those from 'D' ward belonged to the species *Rattus norvegicus*. The passive haemagglutination test was also carried out for testing the sera of rats for the presence of antibody. The results revealed that rats belonging to the species *B. bengalensis* from 'E' ward were less resistant to plague than *R. norvegicus* from 'D' ward, the resistance rat in the latter species being 87 per cent.

The sera of rats collected from 'E' and 'D' wards were also tested by the passive haemagglutination test. None of the sera showed any agglutination even in a dilution of 1:5, thereby indicating the absence of any antibody to Fraction-I antigen. Fraction-I antigen was prepared from casein hydrolysate plague vaccine. This was done by fractional precipitation of the whole vaccine with ammonium sulphate. Fraction-I antigen was also prepared from the two newly isolated strains, viz., *Ronur A* and *Ronur B*. These strains were isolated from plague cases in Kolar district areas of the Mysore State. They were found to be resistant to plague phage and having low virulence in mice; hence, their identity was doubtful. They were, therefore, referred to this Institute for confirmation. Both these strains exhibited bipolarity on staining with methylene blue. They possessed Fraction-I antigen and also produced pesticin. These characteristics confirmed the identity of these two strains.

Detection of pesticin-I

Pesticin-I is a bacteriocin-like substance produced by majority of strains of *Y. pestis* group. It has the ability to inhibit the growth of certain strains of *Y. pseudotuberculosis*. Detection of this pesticin in dead rodents suspected of plague infection, would help in the rapid identification of plague infection. So, this method can be added to the other conventional methods for confirming the identity of the infecting organisms.

However, this method is not very specific, as it was found that the pesticin produced by some strains of *Y. pestis*, viz. 195/P, lyses its own cells.

The Bacteriology Department has got collection of a large number of strains of *Y. pestis* isolated during the last 75 years. These have not been studied in detail for some of their characteristics, such as presence of Fraction-I and pesticin production. Some of these strains (*Y. pestis* 195/P, *Y. pestis* 139/L., *Y. pestis* Ronur A and B, *Y. pestis*, Shillong and *Y. pestis* Poona) were, however, tested for their pesticin-producing activity and all of them exhibited this activity.

Preparation of fluorescent antibody for the rapid identification of *Y. pestis* in dried smears

With a view to investigating the possibility of rapid method for the detection of plague infection in rodents by the fluorescent antibody technique, antiserum was prepared by injecting whole cell antigen of the strain *Y. pestis* 195/P into rabbits. The globulin portion of the antiserum was conjugated with fluorescein isothiocyanate at 4°C. This globulin conjugate was used for staining smears prepared from blood and tissues of plague-infected as well as plague suspected rats. The work is still in progress.

B. DEPARTMENTAL RESEARCH

DEPARTMENT OF BACTERIOLOGY

The investigations on the development of submerged and continuous culture methods for the growth of micro-organisms were continued during the year under review. Studies on "Lyophilization of bacterial cultures", "Susceptibility of rodent population in Maharashtra to *P. pestis*" and "Radiation-killed cholera vaccine" were also continued. A new problem, viz. "Use of labelled glucose for sterility testing of biologicals", was also undertaken for study. In addition to the above, individual problems were being tackled by the post-graduate students on the staff of the Institute.

Development of submerged and continuous culture methods for the growth of micro-organisms

As reported earlier, batches of four different types of typhoid vaccines, viz. (i) formalin-killed and phenyl mercuric nitrate (P.M.N.) preserved (ii) heat-killed and phenol-preserved, (iii) alcohol-killed and alcohol-preserved and (iv) acetone-killed and dried (A.K.D.), were prepared from the cultures grown in a fermentor. These vaccines were found to be free from abnormal toxicity in guinea-pigs and mice.

The efficacy of these vaccines was tested by repeated active mouse protection tests in three-week old mice of the NIH strain along with a standard acetone-killed and dried typhoid vaccine prepared from *S. typhi* organisms of the Ty² strain, grown on routine solid medium. The results are summarised in Table 1.

It will be seen from the results that the first three types of vaccines, viz. (i) formalised, (ii) heat-killed and (iii) alcoholised vaccines, are much more potent than the standard A. K. D. vaccine, their relative potency being near about 3. However, A. K. D. vaccine, prepared from the washed cells of the fermentor culture, yielded poor protection in mice as compared to the standard A. K. D. vaccine.

In the preparation of A. K. D. vaccine from the fermentor cultures, it was necessary to use centrifuged and washed cells free from the supernatant, as some constituents of the whole culture interfered with the acetone treatment. To find out whether the poor antigenicity of this vaccine was due to the removal of some protective antigenic components along with the supernatant or to some other reason, three types of vaccines, viz. formalised, heat-killed and alcoholised, were prepared from the centrifuged and washed cells of the same fermentor culture which was employed for the preparation of fermentor A. K. D. vaccine. The supernatant separated from the fermentor-grown cells was sterilised by Millipore filtration and was preserved with 0.5 per cent phenol.

The vaccines prepared from the whole fermentor cultures (cells+supernatant) as well as those prepared from supernatant-free washed cells by the above three methods and phenol preserved cell-free supernatants were tested for their antigenicity in mice along with the standard A. K. D. vaccine. The

results are enumerated in Table 2. It will be seen from these results that the vaccines prepared from the supernatant-free washed cells yielded considerably poor protection (see Table 2) in mice as compared to that of either the standard A. K. D. vaccine or the whole culture vaccine of that type. The whole culture vaccines prepared from the fermentor cultures in three different ways, were about thrice more potent than the standard A. K. D. vaccine. But, the vaccine prepared from supernatant-free washed cells from the same fermentor cultures by the three different methods mentioned above, were only 1/6th to 1/9th as potent as the standard A. K. D. vaccine. Potency tests carried out on the cell-free supernatants showed that they were as potent or twice more than the standard A. K. D. vaccine. This showed that, while growing the typhoid culture in the fermentor, a large portion of the protective antigen was released into the culture medium.

In order to see whether the protective antigen is also liberated into the supernatant during the process of preparation of typhoid vaccine from the culture grown on a solid medium, four types of vaccines, viz. (i) formalin-killed and P. M. N. preserved (ii) heat-killed and phenol-preserved, (iii) alcoholised and (iv) A. K. D. were prepared from the whole cultures as well as from their supernatant-free washed cells. The cell-free supernatants were also preserved. Active mouse protection tests were carried out on the two types of vaccines viz. (i) heat-killed and phenol-preserved and (ii) alcoholised. Tests on the other types of vaccines and the supernatants will be undertaken shortly. The results are enumerated in Table 3. The results will show that the vaccines prepared from whole culture were almost as potent as the standard A. K. D. vaccine. However the vaccines prepared from the supernatant-free washed cells were only half or one-fifth as potent as the standard A. K. D. vaccine. This indicated that, in the preparation of typhoid vaccine from solid medium also, a portion of the protective antigen is liberated into the menstruum. This will be confirmed by carrying out further tests on the other two types of vaccines and the supernatants.

Lyophilization of bacterial cultures

The results on lyophilization of *Vibrio cholerae* (Ogawa C₁₄ S₂) in small neutral glass tubes (110 mm. long with 6.7 mm. outer diameter), using gum acacia with nutrient broth, gum acacia and glucose, different concentrations of sugars and combination of lactose with sodium glutamate were reported last year. The survival rate then obtained was not more than 0.1 per cent.

During the year under review, lyophilization of this culture was carried out in ampoules (10.0 ml. capacity). Using 8.5 per cent lactose and 8.5 per cent sucrose as suspending media, one ml. of the culture was taken in each ampoule. The cultures were subjected to drying under vacuum for 20 and 44 hours. After lyophilization, each batch of the culture was examined for its viable count. The freeze dried culture was reconstituted in 1.0 ml. of casein hydrolysate medium containing 270 mg. of nitrogen per 100.0 ml. For determining the viability, dilutions of the reconstituted culture were also prepared in the casein hydrolysate medium. The viability obtained varied from 6 to 11 per cent as shown in the Table 4. In the experiments carried

out so far, no significant difference was observed in the percentage viability when the cultures were dried for 20 hours and 44 hours respectively. Experiments will be carried out to study the effect of pH on the viability of an organism during lyophilization.

Radiation-killed cholera vaccine

It was reported last year that the cholera vaccine prepared by irradiating the organisms either for one or two hours was more potent than the usual formalin-killed and phenylmercuric nitrate preserved cholera vaccines. Studies were continued with a view to investigating whether the use of radiation for inactivating the cholera organisms would yield a still better type of cholera vaccine.

While assessing the efficacy of a vaccine, it is necessary to see that it is highly antigenically potent, not only at the time of preparation, but throughout its potency period. A good vaccine should not show any abnormal deterioration in its antigenicity during the shelf life and potency period at the prescribed storage temperature. With this object in view, the two cholera vaccines containing 6×10^9 organisms per ml. of the strains *Vibrio cholerae* 569 B Inaba and 6×10^9 organisms per ml. of the strain *Vibrio cholerae* S/II/58, Ogawa, inactivated by exposing to a ^{60}Co source for one hour and two hours respectively, were tested periodically by active mouse protection test along with a standard cholera vaccine containing an equivalent number of organisms per ml. of the same two strains, but killed by the addition of formalin and preserved by phenyl-mercuric nitrate. The standard vaccine was preserved all the while in a lyophilized state. The results are given in Table 5. To get a better idea, the results of some tests carried out during the previous year are also included in this table.

The results of these tests revealed that there is no deterioration in the antigenicity of the two vaccines even after a period of 18 months of storage at 4 to 10°C. The tests will be repeated for some more time for confirmation.

Two more batches of cholera vaccine were prepared by irradiating the cultures for one hour and two hours respectively. These batches will also be subjected to active mouse protection tests. It is also proposed to test them in young and adult rabbits for their antigenicity.

Preparation of allergenic extracts from the pollens of *Parthenium hysterophorus* L.

All the nine batches of allergenic extracts, reported last year, were prepared again and sent to the Pharmacology Department of this Institute for toxicity, antigenicity and other studies.

Effect of Calcium and Magnesium Ions on virulence of *Yersinia pestis*.

It has been reported by some workers that incorporation of an adequate amount of calcium and magnesium ions in 37 H medium, i.e. casein hydrolysate medium fortified with minerals and vitamins, is necessary for the

maintenance of virulence and prevention of lysis of *Y. pestis* strains. The present work was undertaken to study the effect of various concentrations of magnesium ions on the virulence of *Y. pestis* (195/P strain). In the past, optimum concentration of magnesium ions was worked out as 0.0025 M with 0.004 M calcium ions in the medium. The concentrations of magnesium, tried in the present study, were above as well as below the optimum level. These were 0.000625 M, 0.01 M and 0.04 M. Employing these concentrations, a growth of about 30 to 300 million organisms per ml. of the medium was obtained in a 12-hour old shaken culture. At the end of 72 hours, there was a considerable lysis and the number of organisms usually obtained was less than one million organisms per ml. of the medium.

Twelve and Twenty-four hours old cultures of *Y. pestis*, grown in a medium containing 0.04 M of magnesium, were found to be more virulent whereas, with 48 and 72 hours old cultures, no deaths were noticed in mice even when injected with a dose of about 300 organisms.

When 0.01 M and 0.000625 M of magnesium were incorporated in the medium the virulence of the organisms was less as compared to that of the organisms grown in a medium containing 0.04 M magnesium (see Table 6). Attempts were also made to assess the amount of calcium in plain casein hydrolysate medium by the precipitation method. However, the amount of calcium being very small, it could not be assessed. Hence, it was estimated by the flame photometry method and was found to be 70 ug per ml. of the medium.

Attempts were also made to grow the organisms in a casein hydrolysate medium devoid of calcium. In this case, all the calcium was removed by precipitation with ammonium oxalate. The growth obtained in this medium was much less as compared to that obtained in the casein hydrolysate medium without ammonium oxalate treatment.

About 80 per cent of the animals died when injected with only 5 organisms grown in the medium, wherefrom calcium was removed by ammonium oxalate precipitation. The work is being continued in order to confirm the findings further.

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

The work connected with plague surveillance in the State was continued. Rats from different areas, viz : 'E' and 'D' wards of Bombay City, were collected and their susceptibility to plague infection was studied by the standard challenge method.

Approximately equal number of rats were taken and challenged with a fixed dose of about 5000 organisms of the virulent *Y. pestis* strain (195/P) as before and their susceptibility was determined. Rats from 'E' ward belonged to the species *Bandicota bengalensis* and those from 'D' ward belonged to the species *Rattus norvegicus*. The passive haemagglutination

test was also carried out for testing the sera of rats for the presence of antibody. The results given in Table 7 revealed that rats belonging to the species *B. bengalensis* from 'E' ward were less resistant to plague than *R. norvegicus* from 'D' ward, the resistance rate in the later species being 87 per cent.

The sera of rats collected from 'E' and 'D' wards were also tested by the passive haemagglutination tests. None of the sera showed any agglutination even in a dilution 1:5 (see Table 8) thereby indicating the absence of any antibody to Fraction-I antigen. Fraction-I antigen was prepared from casein hydralysate plague vaccine. This was done by fractional precipitation of the whole vaccine with ammonium sulphate. Fraction -I antigen was also prepared from two newly isolated strains viz : *Ronur A* and *Ronur B*. These strains were isolated from plague cases in Kolar district areas of the Mysore State. They were found to be resistant to plague phage and having low virulence in mice; hence, their identity was doubtful. They were, therefore, referred to this Institute for confirmation. Both these strains exhibited bipolarity on staining with methylene blue, they possessed Fraction-I antigen and also produced pesticin. These characteristics confirmed the identity of these two strains.

Detection of Pesticin-I

Pesticin-I is a bacteriocin-like substance produced by majority of strains of *Y. pestis* group. It has the ability to inhibit the growth of certain strains of *Y. pseudotuberculosis*. Detection of this pesticin in dead rodents, suspected of plague infection, would help in the rapid identification of plague infection. So, this method can be added to the other conventional methods for confirming the identity of the infecting organisms. However, this method is not very specific, as it was found that the pesticin produced by some strains of *Y. pestis* viz. : 195/P, lysis its own cells.

The department has got collection of a large number of strains of *Y. pestis* isolated during the last 75 years. These have not been studied in details for some of their characteristics, such as presence of Fraction-I and pesticin production. Some of these strains (viz. : *Y. pestis* 195/P, *Y. pestis* 139/L, *Y. pestis*, *Ronur A* and *B*, *Y. pestis*, Shillong and *Y. pestis* Poona) were however, tested for their pesticin-producing activity and all of them showed pesticin-producing activity.

Preparation of fluorescent antibody for the rapid identification of *Y. pestis* in dried smears

With a view to investigating the possibility of rapid method for the detection of plague infection in rodents by the fluorescent antibody technique, antiserum was prepared by injecting whole cell antigen of the strain *Y. pestis* 195/P into rabbits. The globulin portion of the anti-serum was conjugated with fluorescein, isothiocyanate at 4°C. This globulin conjugate was used for staining smears prepared from bloods and tissues of plague-infected as well as plague-suspected rats. The work is still in progress.

TABLE 1

Potency tests of typhoid vaccines prepared from the cultures grown in the fermentor

Type of vaccine	Batch number	ED 50 of the vaccine	ED 50 of standard A. K. D. vaccine	Relative potency *
Formalin-killed and P. M. N. preserved ..	1	0.00147	0.00414	2.817
	5	0.002369	0.00788	3.328
Heat-killed and phenol-preserved ..	2	0.0007318	0.002304	3.148
Alcohol-killed and alcohol-preserved ..	3	0.0006248	0.001733	2.17
	4	>0.08	0.004141	<0.05
A. K. D. ..		0.06389	0.001733	0.0712
		0.1132	0.005	0.044
	8	0.06563	0.00788	0.12016

* Relative potency = $\frac{\text{ED 50 of the standard vaccine}}{\text{ED 50 of the test vaccine}}$

Relative potency of standard A. K. D. vaccine is taken as 1

TABLE 2

Potency tests of typhoid vaccines prepared from whole cultures, supernatant-free washed cells and cell-free supernatants of fermentor cultures

Type of vaccine	Prepared from	ED 50	ED 50 of standard A. K. D. vaccine	Relative potency
Formalin-killed P. M. N.-preserved	Whole culture ..	0.00147	0.004141	2.817
	Supernatant-free washed cells.	0.03667	0.004141	0.1129
Formalin-killed P. M. N.-preserved	Whole culture ..	0.002369	0.007883	3.328
	Supernatant-free washed cells.	0.08	0.007883	0.09854
Heat-killed and phenol-preserved	Whole culture ..	0.0007318	0.002304	3.148
	Supernatant-free washed cells.	0.01514	0.002304	0.1518
Alcoholised	Whole culture ..	0.0006248	0.001733	2.77
	Supernatant-free washed cells	0.03361	0.005	0.1508
Phenol-preserved Supernatants	Cell-free Supernatants	0.004208	0.004141	0.9842
		0.003853	0.007883	2.055

TABLE 3

Potency tests of typhoid vaccines prepared from whole cultures and supernatant-free washed cells grown on solid fortified medium

Type of vaccine			Prepared from	ED50	ED50 of standard A.K.D. vaccine	Relative potency
Heat-killed and phenol-preserved	and	phenol-	Whole culture	.. 0·002125	0·002304	1·08
			Washed cells	.. 0·01094	0·002304	0·2107
Alcoholised	..	..	Whole culture	.. 0·003451	0·005	1·15
			Washed cells	.. 0·01009	0·005	0·4958

TABLE 4

Viability of Vibrio cholerae (Ogawa C₁₄S₆) during lyophilization

Experiment number	Suspending fluid		Number of viable organisms/ml before processing (X × 10 ²)	Number of viable organisms/ml after lyophilization (X × 10 ⁶)	
				Drying period 20 hrs.	44 hrs.
1	8·5 % Lactose		16	1·62 (10·12)	1·4 (8·75)
2	Do.		14·32	1·6 (11·18)	1·2 (8·84)
3	Do		15·8	1·87 (11·83)	1·085 (6·87)
4	8·5 % Sucrose		12·45	1·12 (9)	1·35 (10·84)
5	Do.		35·15	3·25	2·37
6	Do.		14·05	1·33 (9·47)	1·23 (8·76)

Figures in parenthesis indicate percentage survival

TABLE 5

Periodical potency tests on irradiated cholera vaccine

Period of test	Challenge strain	Standard vaccine		Vaccine irradiated for one hour		Vaccine irradiated for two hours	
		ED 50	Relative potency *	ED 50	Relative potency *	ED 50	Relative potency *
August 1971	.. 569 B Inaba	.. 0·00006	1	0·00003	2·0		
„	.. 5321 Ogawa	.. 0·000071	1	0·00002387	2·97	0·00004395	1·6
February 1972	.. 569 B Inaba	.. 0·0000894	1	0·00003085	2·97	0·0000412	2·17
„	.. 5321 Ogawa	.. 0·000067	1	0·00005	1·34	0·000017	3·9
June 1972	.. 569 B Inaba	.. 0·0001137	1	0·00003625	3·14	0·0000393	2·9
„	.. 5321 Ogawa	.. 0·0001117	1	0·0000393	2·8	0·00004463	2·5
October 1972	.. 569 B Inaba	.. 0·000112	1	0·00005	2·24	0·00005	2·24
„	.. 5321 Ogawa	.. 0·000123	1	0·00005	2·46	0·0000559	2·2
February 1973	.. 569 B Inaba	.. 0·0001233	1	0·00004055	3·04	0·0004154	2·7
„	.. 5321 Ogawa	.. 0·000125	1	0·00004463	2·8	0·00005	2·5

$$* \text{ Relative potency} = \frac{\text{ED of the standard vaccine}}{\text{ED of the test vaccine}}$$

TABLE 6

Growth and virulence of Y. pestis with Ca⁺⁺ and Mg⁺⁺ ions at different incubation periods

Expt. No.	Concentration of added		Incubation -12 hours		Incubation-24 hours		Incubation-48 hours		Incubation-72 hours.	
	Ca ⁺⁺	Mg ⁺⁺	Growth Organisms/ml (in millions)	Virulence LD 50	Growth Organisms/ml (in millions)	Virulence LD 50	Growth Organisms/ml (in millions)	Virulence LD 50	Growth Organisms/ml (in millions)	Virulence LD 50
Control	0.004 M*	0.0025 M	< 341	3 orgs.	554	< 1.8 orgs.	557	4 orgs.	1.10	> 500 orgs
1	**	0.01 M	440	170 orgs.	300	76.19"	400	6.59 orgs.	21	> 10500 orgs"
2	**	0.04 M	350	4.7 "	950	2.67 "	900	139 "	Less than 1	No deaths.
3		0.000625M	..	..	160	80 "	365	182.5 "	602.5 "	> 301000 orgs
4	*	0.01 M	10.75	5.37 "	60	20 "	20	76.72	10.15	5.07 orgs.
4-A	0.004 M*	0.0025 M	14.5	72	12.5	6.2	10	5 "	0.7	92.9

* Calcium initially present in the casein hydrolysate medium was removed by precipitation with ammonium oxalate.

** Calcium in the casein hydrolysate medium was not removed.

TABLE 7

Susceptibility of rats to Y. Pestis (strain 195/P)

Place of collection	Species used	No. of rats in-oculated			No. of deaths in rats within 15 days			No. of rats found positive of plague			Percentage Susceptibility	Percentage Resistance	
		Male	Female	Total	Male	Female	Total	Male	Female	Total			
D-Ward	<i>R. nervegious</i>	..	22	28	50	5	3	8	4	2	6	12.5	87.5
E-Ward	<i>B. bengalensis</i>	..	22	28	50	18	27	45	17	24	41	89.1	10.9

TABLE 8

Haemagglutination titres of rat sera

Place	Species used	Serum Pools used	Pools showing titre			
			* L1/5	1/5- 1/10	1/10- 1/640	
D-Ward	.. <i>R. rattus</i>	4	-	-	-	
E-Ward	.. <i>R. rattus</i>	5	*	*	*	
Standard			+	+	+	

* L-Less than

DEPARTMENT OF BIOCHEMISTRY

Biochemical studies of the action of Furazolidone on cholera organisms

Furazolidone is a member of the nitrofuran group of synthetic drugs and is being used extensively as an anti-fungal and anti-bacterial agent since the last two decades. It has a wide spectrum of anti-bacterial activity and is specially effective against most members of the enterobacteriaceae group including *Vibrio cholerae*. Reports on the use of furazolidone as an anti-vibrio drug in laboratory and clinical trials are encouraging and indicate that this drug can be an effective alternative to tetracycline and chloramphenicol in the treatment of cholera infections. The drug is reported to be less toxic to the host and does not induce much resistance in the organisms. Work was undertaken to study in detail the effect of the drug on the two strains of cholera organisms, viz. *Vibrio cholerae* and its biotype *El. tor cholerae*.

It was reported earlier that furazolidone inhibits the growth of cholera organisms by interfering with the protein metabolism. It is observed to uncouple the translation stage which results in the accumulation of RNA and inhibition of protein synthesis. Analysis of the base composition of the RNA indicates that the accumulated RNA is of the normal type.

(a) *Effect of furazolidone on oxygen uptake and on the oxidative and a transaminase enzymes of V. cholerae and El. tor cholerae* :—The investigator whether the ATP or the GTP is the limiting factor for the protein synthesis, the oxygen uptake and the enzymes of the Krebs cycle were studied. As shown in Table 1, the pyruvic, lactic and malic dehydrogenases are inhibited in the drug-treated organisms, indicating a general inhibition of the TCA cycle. The alpha keto glutarate dehydrogenase shows a higher specific activity while the isocitric dehydrogenase is unaffected. This observation, together with the high specific activities of GOT and citrate cleavage enzymes, indicates that the drug accelerates the protein catabolism. These effects are less pronounced in the *El tor* strain. Both the strains show increased oxygen uptake which is typical of energy uncouplers. The glucose utilisation is more by the HMP pathway, which is confirmed by the high specific activities of glucose-6-phosphate dehydrogenase in the drug-treated organisms.

(b) *Effect of furazolidone on the incorporation of glycine, leucine and phenylalanine*.—Protein synthesis is dependent upon the uptake and incorporation of amino acids into the proper sequence. Studies on the kinetics of synthesis of proteins, using labelled amino acids indicate that the drug inhibits the incorporation of glycine and leucine with no effect on phenylalanine (Table 2). This can be due to some abnormal synthesis. Therefore, the soluble proteins of the normal and drug-treated organisms were isolated and fractionated by polyacrylamide gel electrophoresis. While fourteen protein bands are observed in the normal, the drug-treated organisms show only twelve, with two of the lower molecular weight protein bands missing and displacement of some others. This gives a clue that some abnormal proteins are being synthesised by the drug-exposed organisms.

Biochemical studies on the effect of Chloramphenicol on cholera organisms.

Since chloramphenicol is widely used in cholera infections, a detailed biochemical study of the effect of this drug on vibrio and its biotype *El tor* was taken up.

(a) *Cellular composition* :—The organisms were grown in the synthetic medium for two hours under shaking conditions at 37°C, when the drug was added to the appropriate flasks to give a concentration of 0.5 ug/ml. Incubation was continued for six hours when the cells were centrifuged (12,000 rpm) washed with Tris-HCl buffer (PH 7.3) and dried in vacuum. Total nitrogen, RNA, DNA, proteins and lipids were done by the standard procedures used in the laboratory. The results are given in Table 3. It is seen that the drug inhibits the protein and phospholipid synthesis while the synthesis of RNA is almost doubled.

(b) *Study of the protein spectra on acrylamide gel* :—The inhibition of protein synthesis was studied in detail using polyacrylamide gel techniques. 7.0 per cent acrylamide and bis acrylamide in Tris-glycine buffer (PH 8.3) at a voltage of 150 (5 m amps/tube) for 3 hours was used. The protein bands were stained with comassive blue dye and located on the densitometer. It is seen that 5-6 protein bands are missing in the protein fraction of the drug-treated organisms.

(c) *Characterisation of nucleic acids* :—The RNA and DNA were isolated by the phenol extraction method and the RNA fractions were separated on MAK column. The base composition of the total, terminal (t) and ribosomal (r) RNA was determined by high voltage electrophoresis. Significant changes are observed in the gross rate of formation of nucleic acids. There is an acceleration of r-RNA (16 and 23-S RNA) synthesis. The base composition of the various RNA are not altered.

The two DNA strands were separated on MAK column and the base composition was determined after processing. The results are given in Table 4. As seen from the figures, there is a distinct difference in the base composition of the light and heavy strands of the DNA.

(d) *Oxygen uptake and oxidative phosphorylation* :—The cells were harvested after 8 hours of growth, centrifuged, washed with tris buffer and diluted with buffer to give the required opacity. Aliquots were used for measuring the oxygen uptake on Warburg manometer and the oxidative phosphorylation studies were done spectro-photometrically, using succinate and glutamate as substrates. It is observed that the oxygen uptake is more in the drug treated cells and the oxidative phosphorylation is inhibited.

(e) *Study of the enzymes of the TCA cycle* :—The lactic, pyruvic, malic, isocitric, alpha keto glutaric dehydrogenases and NADH oxidase were studied spectrophotometrically by the standard procedures.

The results are given in Table 5. The lactic, isocitric and alpha-keto glutaric dehydrogenases are very much inhibited while the malic dehydrogenase is unaffected. The pyruvic dehydrogenase is increased nearly four-fold. The NAD H oxidase is also inhibited.

(f) *Characterisation of the phospholipid*.—The total lipids were extracted and fractionated on silicic acid column. About five different types of phospholipids have been identified. Quantitation of the fractions is in progress.

Biochemical studies on cholera organisms grown in different media.

(a) *Cellular composition*.—The nutritional requirements of *Vibrio cholerae* for amino acids and minerals were reported last year. The growth of the organisms in the peanut protein hydrolysate was found to be better than that in the routinely used casein hydrolysate. A detailed study of the cellular composition of the organisms, grown in the four media casein hydrolysate (c), peanut protein hydrolysate (p), the synthetic medium of Thind (T) and the formulated chemically defined medium (S) under stationary and shaking conditions were undertaken. The cells were grown, harvested, washed and dried in vacuum. Total nitrogen, protein, reducing sugars, RNA, DNA and lipids were estimated by the standard procedures. As seen from Table 6, the RNA content of the organism grown in the synthetic media T and S are almost double that of the other two.

(b) *Oxygen uptake and protein synthesis*.—*V. cholerae* (Ogawa) was grown on nutrient agar for 18 hours at 37°C. The cells were scraped and suspended in distilled water to give a suitable opacity. Aliquots were transferred to Warburg flasks containing the particular medium and allowed to attain the log phase for 1-1½ hours before measuring the oxygen uptake. Readings were taken for 1 hour at 13 minutes intervals and the initial and final protein concentrations were estimated. As seen from Table 7, the oxygen uptake and protein synthesis is 50 per cent more in the peanut protein hydrolysate. The synthetic media-T and S seem to be rather poor in this respect.

(c) *Nucleotide composition of RNA of V. cholerae grown in different media*.—*Vibrio cholerae* (Ogawa) was grown in Roux bottles containing agar (1.85 per cent) impregnated medium for 24 hours at 37°C. The cells were harvested by centrifugation (12,000), washed with saline and the RNA was isolated by the standard procedure used in the laboratory. The base composition (Table 8) was determined by high voltage electrophoresis. The Purine/Pyrimidine ratio from RNA from organisms grown in peanut protein hydrolysate is 1.25, while the others are higher.

Use of Peanut protein hydrolysate for cholera vaccine preparation.

The growth of the *Vibrio cholerae* in peanut protein hydrolysate medium had indicated that it could be useful for vaccine preparation. Using the standard procedure adopted in the Bacteriology Department, cholera vaccine, containing *V. cholerae* (Inaba 569 B and Ogawa S/II/58) and El tor Inaba and Ogawa was prepared and referred to the Bacteriology Department for potency test in mice.

Dietary fat and lipid metabolism in the albino rat.

(a) *Effect of carbohydrate*.—It, was reported earlier that the hypo cholesterolemic effect of an unsaturated fat like safflower oil could be nullified by

the carbohydrate-sucrose in the diet of the albino rat. This effect was studied in detail by interchanging the carbohydrate in the diet starch/sucrose during the experimental period. After four hours of initial feeding, the carbohydrate portion was interchanged twice at intervals of two weeks. Six animals in each group were sacrificed at appropriate stages for the estimation of serum and liver cholesterol. After the first reversal, the starch group showed a rise in the cholesterol level while the opposite effect was seen in the sucrose group. The second reversal, however, did not give clearcut results as expected, indicating that the two weeks interval is rather too short for quick adaptation.

(b) *Effect of dietary carbohydrates on lipogenic enzymes.*—Lipid synthesis is dependent on the availability of NADPH and acetyl CoA. This experiment was planned to study the enzymes which are known to generate these metabolites. Groups of male albino rats were fed starch/sucrose diet, with safflower oil as the dietary fat, for 8 weeks. At the end of this period, the animals were sacrificed and the liver processed for the study of the enzymes.

Increased levels of Malic enzyme, citrate cleavage and glucose-6-phosphate dehydrogenase are observed in the livers of sucrose-fed animals with no change in the transaminase levels.

(c) *Effect of hormones.*—It was reported earlier that sucrose in the diet elevates the cholesterol and triglyceride levels in the serum and liver of albino rats. This work was further extended to study the effect of lipid regulating hormones—adrenaline, thyroxine and insulin. Male albino rats were kept on starch/sucrose diets with safflower oil as the source of fat for 10 weeks. After this period, the hormones were administered daily for one week when the animals were sacrificed to study the serum and liver cholesterol levels. The results indicate that the three hormones lower the serum cholesterol level irrespective of the type of carbohydrate in the diet. Adrenaline has no effect on the liver cholesterol, while thyroxine lowers the level. Insulin, on the other hand, lowers the hepatic cholesterol in the starch group with no effect on the sucrose-fed animals (Table 9).

Lipid profile in health and disease.

(a) *Long-term effects of oral hypoglycaemic agents.*—Oral hypoglycaemic drugs have become therapeutically important but their long-term effects still remain to be investigated. There are controversial reports about the effects of their continued use on degenerative changes in the body. Since these effects are mainly on the lipid metabolism, a study of the lipid profile of diabetic subjects who are under treatment was undertaken. About 112 samples have been screened so far for triglycerides, cholesterol, lipoproteins and phospholipids. These subjects are controlled either by diet or by oral hypoglycaemic drugs—sulphonylurea/biguanide or both. Normal controls and fresh cases without treatment are to be included for comparison.

(b) *Effect of insulin and tolbutamide on the development of atherosclerosis in the rabbit.*—Mahler (1966), and Stout (1968) have reported that intravenous injection of labelled glucose/acetate along with insulin to albino rats leads to a higher incorporation into the aortic lipids as compared to the

controls without insulin. This suggests that insulin may have some inhibition on the lipase activity in the arterial tissue, causing accumulation of lipids and leading to the formation of atherosclerosis. It is also known that the amount of circulating insulin is abnormally high in some diabetic patients and the condition *Diabetes mellitus* is known to be strongly associated with ischemic vascular disease. This work was planned to study the long-term effect of insulin and tolbutamide in rabbits with reference to the development of atherosclerosis. Normal and cholesterol fed rabbits were given doses of insulin/tolbutamide daily and the serum lipids were screened once in 15 days. Aortic, hepatic and serum lipids will be estimated at the end of 12 weeks.

TABLE 1

The effect of furazolidone on the specific activity of enzymes of cholera organisms.

Name of the Enzyme System		V. Cholerae(Inaba)	569B	El tor (Inaba)	
		Control	Test	Control	Test
Lactic Dehydrogenase	..	32	22	36	28
Pyruvic Dehydrogenase	..	22.6	13.7	26.1	19.5
Isocitric Dehydrogenase	..	18.4	17.9	21.0	22.6
Alpha-keto glutarate Dehydrogenase	..	45.3	59.5	42.4	51.7
Malic Dehydrogenase	..	15.9	4.5	18.3	9.1
Glucose-6-Phosphate Dehydrogenase	..	10.0	25.0	14.8	27.5
6-Phospho gluconate Dehydrogenase	..	8.1	17.7	11.6	21.1
Citrate cleavage enzyme	..	36.2	59.3	43.1	55.4
Glutamate oxaloacetate transaminase	..	115	142	121	137
Glutamate Pyruvate Transaminase	..	24	36	26	32

TABLE 2

Effect of furazolidone on the specific incorporation of amino acids by Vibrio cholerae

			Time in Hours					
			0	1	2	3	4	5
Glycine	..	C	8833	10036	15884	19000	20168	20937
		T ¹	6666	8423	10981	12142	14122	15680
		T ²	5323	10981	9826	11000	12926	13437
Leucine	..	C	8733	11818	13166	14246	17770	22260
		T ¹	7416	8800	9518	9839	10826	12087
		T ²	7002	8476	9125	9318	10934	11243
Phenyl alanine	..	C	7000	8609	10729	11382	12637	13108
		T ¹	8066	8236	9727	11163	12432	12936
		T ²	6924	8122	8988	9500	10812	11943

C=Control; T¹=0.5 Mg/ml; T²=1.0 Mg/ml.

TABLE 3

Effect of chloramphenicol on the cellular composition of cholerae organisms : mg per cent

Strain		Proteins	RNA	DNA	Sugar	Phospho- lipids
569 B	.. C	54	10.5	4.1	18.0	0.9
	T	46	19.0	4.0	17.6	0.65
El tor Inaba	.. C	56	9.5	4.3	16.0	0.88
	T	40	16.3	4.4	16.2	0.63

TABLE 4

Effect of chloramphenicol on the base composition of DNA

DNA strands			A	G	C	T	Pu/Py
Light (L)	..	..	24.6	26.4	23.1	25.9	1.0
Heavy (H)	..	..	22.1	24.6	26.9	26.5	0.9
L + H	..	..	24.0	26.5	25.5	24.0	1.0
Native	..	..	24.5	26.0	25.0	24.5	1.0

TABLE 5

Effect of chloramphenicol on the specific activity of the enzymes of the TCA cycle in Vibrio cholerae

Enzymes				Control	Test
Lactic dehydrogenase	..	..	..	98.4	38.8
Pyruvic dehydrogenase	..	..	..	80.0	311.0
Isocitric dehydrogenase	..	..	..	21.2	4.5
Alpha-keto glutaric dehydrogenase	..	..	..	18.9	5.4
Malic dehydrogenase	..	..	..	60.2	60.8
NADH Oxidase	..	..	..	27.4	15.5

TABLE 6

Effect of media on the cellular composition of V. cholerae Ogawa S/II/58

Media	*mg/100 mg of dry cells							
	Total nitrogen	Protein	Ash	Lipids	Sugars	RNA	DNA	RNA/DNA
Stationary condition in liquid medium, 48 hours, 37° C								
C (135 mg N%)	11.2	61.8	5.2	12.2	7.6	7.6	3.8	2.0
P "	11.6	62.8	5.4	10.5	7.9	7.5	3.8	2.0
T "	11.3	57.1	6.3	11.4	13.0	10.7	3.8	2.8
S	11.8	57.2	5.9	10.4	14.0	11.5	4.1	2.8
Shaking condition (100 r, p.m.) in liquid medium, 24 hours, 37° C								
C (135 mg N%)	12.5	62.2	5.2	13.4	6.4	7.7	3.8	2.0
P "	12.2	64.0	4.8	13.0	5.4	7.5	3.8	2.0
T "	11.8	58.6	6.8	11.6	12.0	10.7	3.8	2.8
S	11.8	59.3	6.0	12.2	11.6	11.5	4.1	2.8
Surface growth in 1.85% agar medium in Roux bottles, 24 hours/37°C								
C (200 mg N%)	12.1	62.1	5.2	11.6	7.4	6.8	3.8	1.8
P "	11.8	63.2	5.0	11.9	6.3	6.7	3.5	1.9
T "	11.2	57.9	5.8	10.4	13.8	12.7	5.0	2.5
S	11.5	58.4	5.8	10.4	14.0	12.7	4.7	2.7

* Values are the average of two experiments.

C — Casein hydrolysate ;

P — Peanut protein hydrolysate ;

T — Synthetic medium of Thind (1961) ;

S — Formulated chemically defined medium.

TABLE 7

Effect of media on oxygen uptake and protein synthesis of vibrio cholerae, ogawa S/II/58

Media	Oxygen uptake and protein synthesis					
	QO ₂	IPr	FPr	Pr	Pr/QO ₂	FPr/IPr
Casein hydrolysate 135 mgN/100 ml	93	98	233	135	1.44	2.38
Peanut protein hydrolysate 135 mg N/100 ml	125	98	270	172	1.38	2.75
Synthetic medium of "Thind"	37	98	144	46	1.24	1.47
Formulated chemically defined medium	48	98	160	62	1.29	1.63

QO₂=Oxygen uptake (μ /hr); IPr=Initial protein (μ g/ml);
 FPr=Final protein (μ g/ml); Pr=Protein synthesized (μ g/ml/hour)
 The values are the mean of two experiments in duplicate

TABLE 8

Effect of media on nucleotide composition of Ribose Nucleic Acid (RNA) of Vibrio cholerae, Ogawa S/II/58

Media (1.85 per cent agar in Roux bottles)	Nucleotides of RNA (Percentage)							
	A	G	C	U	A+G	C+U	$\frac{P_{\mu}}{Py} = \frac{A+G}{C+U}$	
Casein-hydrolysate 200 mg N/100 ml	31.1	27.0	26.8	15.1	58.1	41.9	1.41	
Peanut protein hydrolysate 200 mg N/100 ml	21.8	34.2	23.2	20.8	56.0	44.0	1.27	
Formulated chemically defined medium	24.5	35.5	22.2	17.8	60.0	40.0	1.50	

A=Adenylic acid G=Guanylic acid; C=Cytidylic acid;
 U=Uridylic acid; Pu=Purines; Py=Pyrimidines
 The figures are the mean of the two experiments

TABLE 9

Effect of hormones on serum and liver cholesterol in starch/sucrose fed albino rats

Hormone	Serum Cholesterol mg %		Liver cholesterol mg %	
	Starch	Sucrose	Starch	Sucrose
Adrenaline	103 $\pm$ 11	100 $\pm$ 8.7	600 $\pm$ 150	563 $\pm$ 27.2
Thyroxine	94 $\pm$ 13.8	75 $\pm$ 4.0	427 $\pm$ 16	424 $\pm$ 56
Insulin	113 $\pm$ 11.6	94 $\pm$ 5.6	614 $\pm$ 58	557 $\pm$ 15
Control	123 $\pm$ 8.7	152 $\pm$ 5.7	504 $\pm$ 40	610 $\pm$ 45

DEPARTMENT OF CHEMOTHERAPY

Chemotherapy of tuberculosis and leprosy: 5-Mercapto-1, 3, 4-oxadiazoles

2-4'-amino-2'-hydroxy) phenyl-5-hydroxy-1, 3, 4-oxadiazole, a cyclic derivative of PAS, has been reported to possess the same order of activity both *in vitro* and *in vivo* as PAS and it has also clinically been found to be equally effective at a much lower dosage and also better tolerated than PAS. Accordingly, a series of analogues, viz. 2-4'-amino-2'-hydroxy) phenyl-5-mercapto 1, 3, 4-oxadiazole and related compounds were synthesized. Some of them have shown *in vitro* antitubercular activity and are being studied further in experimental animals for serum tuberculostatic activity.

N-N'-bis (ketone hydrazones) of terephthalic acid

Terephthalanilides and terephthalamides have been described as possessing interesting activities, such as antitubercular and antitumor. The newer terephthalamides of p-aminophenyl-2, 5-disubstituted thiazole synthesised earlier could not be tested *in vitro* since they were practically insoluble in usual solvents used for screening purposes. As further work in this series, N-N'-bis hydrazones of terephthalic acid with acetophenone, propiophenone and butyrophenone were prepared and will be submitted for screening.

4[p-(p-alkoxyanilino) acetamido (or ethylamino) phenyl]-3, 5-dimethylthiazoles.

A series of thiazolyl thiocarbanilides were reported earlier to possess higher order of *in vitro* antitubercular activity and one of them a dimethyl thiazole derivative showed activity at 0.04 mg/ml. In order to see if the ethylenediamine analogues of this series and other related compounds would offer better compounds, probably possessing favourable physico-chemical characteristics, a new series of compounds were synthesized, wherein the CS. of the thiocarbanilide was replaced by CH_2 , CH_2 or CO-CH_2 . They have been screened and many of them have shown a good order of antitubercular activity.

Synthesis and study of compounds for possible antiparasitic activity

2-Benzthiazoles and pyridothiazoles.—Many benzthiazole derivatives have been reported to possess a high order of antiparasitic and antibacterial activity. Furthermore, the incorporation of basic moieties in these molecules potentiates the activity. It was, therefore, proposed to synthesize newer derivatives of benzthiazoles and pyridothiazoles having piperazines as the additional basic moieties.

While pyrido (3, 2-d) thiazole-2-piperazines were prepared from 2-chloro-isothiocyanato pyridines with N'-substituted piperazines, 2-piperazino-5-nitrobenzthiazoles were prepared by reaction of 6-chloro-3-nitrophenylisothiocyanate with N'-substituted piperazines. In another related series, piperazines have been replaced by different substituted anilines. So far, 35 compounds have been synthesised and submitted for evaluation of their antiparasitic activity.

N-(5-nitrofurylamidomethyl)-N⁴-phenyl piperazines

Nitrofurfural derivatives have established themselves as highly potent antibactericidal agents. With a view to combining piperazine moiety with nitrofurfural, a series of Mannich bases were prepared by reacting 5-nitrofurfuryl-2-carboxamide and N-substituted piperazines. These compounds have been submitted for antibacterial, antiprotozoal and anthelmintic activities.

Tetrahydropyrimidines

A wide spectrum of activity, viz antibacterial, antiviral and antitumor, has been reported in respect of tetrahydropyrimidine derivatives. In the present study, a series of 2-oxy or 2-thio tetrahydropyrimidines, to which are attached active moieties in 4, 5 or 6 positions, has been prepared. Nearly 60 compounds have been prepared. So far they have been screened for antimicrobial activity only, wherein some compounds have been found to show a low to moderate order of activity. These compounds are being studied further for their antitumor as well as antiprotozoal activities.

β -N-piperazinylethylpyrimidines

Earlier, a series of 2, 4-disubstituted-5- β -hydroxyethyl (or propyl) pyrimidine compounds have been synthesised for the purpose of broad screening. These compounds have now been converted into β -N-piperazinylethylpyrimidines with a view to studying them also for possible antiprotozoal activity.

Synthesis and study of compounds for antitumor activities: Schiff-bases from benzaldehydebimethane sulphonates and 4-(p-aminophenyl) thiogoles.

Earlier, some of the Schiff-bases from benzaldehyde nitrogen mustard were reported as active antileukemic agents against Dunning Leukemia L-1210 lymphoid leukemia, P 388 lymphocytic leukemia and Walker carcinosarcoma 256 (intramuscular) in rats.

With a view to reducing the toxicity and obtaining compounds with better tolerance; the preparation of bimethane sulphonates analogous to a series of active nitrogen mustards reported earlier, was continued and a large number of bimethane sulphonates of the active Schiff-bases from thiazole amines have now been synthesised and screened. Forty-six compounds of this series are being screened by C. C. N. S. C. Bethesda, U.S.A.

Schiff bases from benzaldehyde bimethane, sulphonate and aromatic amines

Earlier Schiff bases from 4-[N, N-bis (2-chloroethyl) amino] benzaldehydes and m-haloanilines, 2-6-dihalo-4-aminophenols have shown a high order of activity against L-1210 lymphoid leukemia and P 388 lymphocytic leukemia. With a view to increasing the tolerance, the chloro groups in nitrogen mustard arms were replaced in this series by bimethane sulphonates. Nineteen representative compounds from the series have been screened by the CCNSC, U.S.A.

DEPARTMENT OF CLINICAL PATHOLOGY

Studies on Resistance factors (Drug Resistance)

Fifty-nine strains of multiple drug resistant enterobacteriaceae, isolated mainly from urinary-tract infections, were screened for the presence of resistance factors. Twenty-eight of these strains could transfer their resistance to a recipient *E. coli* K12F mutant strain. Thirteen of these twenty-eight strains carried resistance markers for Sulphadimidine, Streptomycin, Ampicillin, Chloromycetin and Tetracycline. These resistance markers could be transferred en block to a recipient strain.

Two hundred and ten strains of *Salmonella*, isolated from blood samples of enteric fever cases, were tested for drug sensitivity against Sulphadimidine, Streptomycin, Ampicillin, Chloromycetin and Tetracycline. Eighteen cultures of *Salmonella typhi* and ten cultures of *Salmonella para A* were found to be resistant to two or more drugs. Three of these cultures were resistant to Chloromycetin. However, this drug resistance in *Salmonella* is of a transient nature. The drug resistance particularly of chloromycetin, was lost on sub-culture. Only five, out of 18 drug resistant strains of *Salmonella*, could transfer their resistance to recipient *E. coli* K12F strain.

Laboratory investigations in gonorrhoea

Preliminary work on the isolation of *N. gonorrhoeae* from 192 samples collected from acute cases of gonorrhoea yielded growth only in 35 per cent of cases on chocolate-agar medium. The medium was then improved by incorporating Trimethoprim and Mycostatin to inhibit the growth of contaminants.

Thereafter, 181 samples of infective discharges were collected in Stuart's Transport medium and processed. One hundred and twenty-one out of 181 samples (i.e. 86 per cent), yielded positive results by using the selective medium.

Attempts are being made for preservation and large-scale cultivation of the isolated strains of *N. gonorrhoeae*.

Studies on Brucellosis

A sero-epidemiological investigation was undertaken to assess the Prevalence of Brucellosis in man and animals. Five hundred and twenty-two human sera and 345 animal sera were studied. The human sera were collected from professionally pre-disposed persons and their family contacts, clinically suspected cases of brucellosis and cases of pyrexia of unknown origin. The animal study group included samples of sera from cattle, goat, pig and sheep.

A pilot survey showed incidence of Brucellosis to the extent of 12 per cent and 9.5 per cent among the occupational group of dairy and piggery workers respectively. The incidence among different animals varied from 6.6 per cent to 70 per cent.

Different laboratory media for the isolation of *Brucella* organisms were standardised. The work is in progress.

Cholera carrier studies

Isolation and characterisation of *V. cholerae* from material collected from Parbhani District.

Laboratory study began in June 1972.

A. Faecal samples from human patients and their contacts :—

3,677 faecal samples were studied.

Fifty-five strains of *V. cholerae* were isolated.

The other most frequently isolated pathogens were :—

Escherichia coli, *Proteus*, *Alkaligenes Faecalis*, *Aerobacter aerogenes* and *Pseudomonas*.

The isolated strains of *V. cholerae* were further characterised by :—

Sugar fermentation test.

Haemagglutination test.

Haemolysis test.

Polymyxin B (50 units) sensitivity.

Phage typing.

All the isolates belonged to *V. cholerae* Ogawa biotype *El tor*.

Antibiotic sensitivity test was also carried out. Only 50 per cent of the strains were sensitive to Tetracycline (30 ug.) and all were sensitive to chloromycetin (30 ug.).

Pathogenicity test (Method of Dutta and Habbu, 1955) confirmed the toxicity of the strains.

B. Faecal samples from extra-human sources :—

Buffaloes	...	51	Samples
Cows	...	51	Samples
Sheep	...	59	Samples
Horses	...	45	Samples
Dogs	...	142	Samples
Asses	...	42	Samples
Fowls	...	62	Samples
Fish	...	28	Samples

Vibrio like organisms were isolated from one faecal sample of fowl and five faecal samples of dog No *V. parahaemolyticus* has been isolated so far.

Two strains isolated from dog were found to be toxic in infant rabbits Method of Dutta and Habbu, 1955).

C. Water samples :—

A total of 58 water samples were examined. Four *Vibrio*-like organisms were isolated. Two of the strains were found to be toxic by infant rabbit method (Dutta and Habbu, 1955).

D. Serological investigations will be undertaken soon.

Labelling of S. typhi antigen with Chlorella protein hydrolysate C-14 isotope.

Since March 1972, 3 batches of *S. typhi* antigen were labelled with *Chlorella* protein-hydrolysate C-14 and radio-immune assay carried out with 6 *S. typhi* anti-sera. The results in 2 batches with 2nd and 3rd batches of antigen were satisfactory. The work is in progress.

DEPARTMENT OF IMMUNO-HAEMATOLOGY

Studies in Australia antigen

The studies on Australia antigen, commenced earlier were continued in its various aspects. The earlier work was published in 'Vox Sanguinis' during the year.

(i) *Screening for HBA and HBAb.*—The screening of the sera of patients suffering from various clinical conditions was continued further. The agar-gel double diffusion technique (AGD) using agarose and counter-immune electrophoresis (CEP) techniques were employed for this screening procedure. Table 1 gives the incidence of HBAG in various clinical conditions.

(ii) *Status of HBAG and liver function tests in clinical condition.*—This study, which was commenced last year, was pursued further. The sera collected for screening for HBAG were also tested for S. G. O. T. S. G. P. T., serum bilirubin and alkaline phosphates. The results are given in the Tables 2, 3 and 4.

It is too early to draw any definite conclusions. However, it is interesting to note that in leukaemia, the liver function tests do not reveal any great variation between HBAG positive and HBAG negative cases unlike the infective hepatitis cases.

(iii) *Production of HBAb in laboratory animals.*—Rabbits and guinea pigs were immunised with HBAG positive serum alongwith Freund's adjuvant. The response was not very satisfactory, though the antibody with low titre was produced. The immunisation is being continued further.

Recently, sub-groups of HBAG have been described in literature, though the actual significance of these sub-typing is not known. N. I. H. has kindly supplied some of these standard and sub-typing sera and the work of sub-typing or serum samples, positive for HBAG, is being undertaken.

Studies on alpha-fetoprotein (AFP)

In 1944, Pederson demonstrated a protein present in fetal calf serum which was absent in adult bovine. On purification, the fetal protein was found to have a molecular weight of 50,000. Borgstrand and Czar (1958) first showed the occurrence of alpha-fetoprotein (known as AFP) in an adult. Appearance of an embryo-specific globulin (now known as AFP) in an adult mammalian was first shown by Abeloo (1964), particularly in adult mice bearing primary hepatoma. Tatarinev (1966) showed the presence of AFP in humans 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. Linsoll, Chief of Nairobi Regional Centre W.H.O. investigating malignancy of liver in Uganda (Africa), has suggested possible relationship

between HBAg and AFP in malignancy of liver and 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, 164 suspected cases of hepatoma were investigated at this Institute for the AFP. Results are shown in Table 5.

Immunoglobulins

The importance of starch-gel electrophoresis in the diagnosis of multiple myeloma is well established. The location of paraprotein 'M' band is more prominent by starch-gel electrophoresis than by conventional paper electrophoresis. During the past years, we have investigated 110 cases of multiple myeloma and found monoclonal type of Ig G 51.8 per cent, IgA 14.5 per cent and IgM 10.9 per cent. Twenty-five cases (i.e. 22.7 per cent) showed generalised gammopathy with an increase in all, immunoglobulin levels and thus termed as polyclonal type. During the year, 30 cases (20 males and 10 females) were referred to this Institute and, amongst these 8 cases were tested before they were given any treatment, while the remaining 22 had already received chemotherapeutic treatment. One case, which was followed up for a period of four months, showed a change from initial polyclonal pattern to the formation of monoclonal immunoglobulin IgG.

Immuno-biochemical aspects of rheumatoid syndrome

Rheumatoid disorders are collagen disorders supposed to be of auto-immune in origin, leading to various clinical features like polyarthrit's fibrositis. The exact nature of the rheumatoid factor is not definitely known, but is presumed to be immunoglobulin 'M' with sedimentation of 1922 S. 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.

A preliminary study of screening of suspected cases of rheumatoid arthritis and related conditions was undertaken. Besides latex fixation test, the sera were investigated for other biochemical parameters, such as haptoglobins, immunoglobulins.

Sixty suspected cases of rheumatoid arthritis were investigated for RA factor test, haptoglobins, immunoglobins, blood group. The samples were received from K. E. M. Hospital's Rheumatic Clinic. Results are given in Table 6.

Haptoglobins

Studies on haptoglobins, which are alpha-2 heamoglobin-binding proteins, have been carried out by the department since 1965-66. In earlier studies, these genetic markers were mainly used in population studies. However,

in recent years, haptoglobins have also been studied in some of the diseased conditions like haemolytic anaemias and necrotic liver disorders, where it has some significance. Various studies carried out by the department are summarised below :—

(a) *Haptoglobins in haemolytic anaemia*.—Since haptoglobins are haemoglobin-binding proteins, the haemoglobin released due to intravascular haemolysis in haemolytic anaemia is bound to the haptoglobin proteins and eliminated from circulation. Thus, in haemolytic anaemia the quantity of haptoglobins is markedly decreased and often reaches the level where they are not demonstrated by starch-gel electrophoresis giving the pattern of Hp 0-0 type. Various studies in the literature have shown increased incidence of Hp 0-0 in different haematological syndromes. The department has been investigating various haemolytic anaemia cases as a routine and has also studied haptoglobin pattern in such cases. Table 7 gives analysis of the cases investigated so far during the last few years.

(b) *Haptoglobins in neo-natal jaundice*.—There is a presumptive evidence that haptoglobins are synthesized by the liver. Various reports have shown very low incidence of new-born infants with demonstrable haptoglobin types. Also, since the haemoglobin-binding capacity of 3 common haptoglobin phenotypes is different, it is likely that the lower levels of haptoglobins at birth may show a variable incidence as compared to that observed in adults. The incomplete development of hepatic cells at neo-natal stage, if true, may also grossly affect the haptoglobin patterns in new born infants developing jaundice in early neo-natal life. With this end in view, a study of the status of haptoglobins in normal and neo-natal jaundiced infants was undertaken. Two hundred and forty cases of neo-natal jaundice and 168 normal infants, together with their mothers, were tested for haptoglobin types. The findings showed that there were no difference in haptoglobin types in mothers of normal and jaundiced infants. While 66 per cent of the normal new-born infants showed Hp 0-0 type, the incidence in jaundiced infants was 56 per cent. It may be pointed out that few earlier reports in the literature have shown Hp 0-0 in new-born infants, ranging from 76-91 per cent. Lower incidence in our population may be either racial or due to the fact that 48 hour old infants were studied in the present series (since one of the reports suggested that nearly 60 per cent of undeveloped haptoglobins are developed within the first 8 days). Secondly, the higher incidence if Hp 0-0 at birth could be due to poor expression of haptoglobin gene at birth. This was ruled out, since the jaundiced infants of the same age group showed significantly low incidence of Hp 0-0. On the other hand, the circumstantial evidence in the literature suggests the synthesis of haptoglobins in liver and, therefore, incomplete development of hepatic cells at birth may lead to higher incidence of Hp 0-0. The lower incidence of Hp 0-0 in jaundiced infants could, therefore, possibly suggest the hyper activity of the generating hepatic cells in icteric infants. The data also showed that the severely affected infants had much higher incidence of Hp 0-0 than the mildly affected jaundiced infants. This could possibly be explained by assuming that, at a break-down in this compensatory

mechanism to produce the haptoglobin proteins. The data, when analysed on the basis of haemolytic and non-haemolytic jaundice, fully confirmed the findings of others that Hp 0-0 incidence is higher in haemolytic than in non-haemolytic jaundice.

Analysis of mother-child combination also showed an interesting feature that the icteric infants of Hp 2-1 type born to Hp 2-1 mothers were far in excess than the expected values. Similar excess of 2-1 type infants has been reported by Kirk from Australia. Thus, in view of the findings of Kirk and those of ours, it may be worthwhile investigating the possibility of the protection, if any, to Hp 2-1 infants against haemolytic anaemia, neo-natal jaundice and their susceptibility to the development of Kernicterus. Table 8 gives the incidence of various haptoglobin types in neo-natal jaundice.

Specific immunoglobulins I G)

(a) *Tetanus immunoglobulins*.—Hyperimmunisation of donors with injections of Tetanus oxid had been started at the Yerawada and Nasik Prisons. So far, 58 volunteers have received 2 doses and 21 volunteers have received 1 dose. The programme is being extended to other prisons also to get more donors.

(b) *Anti-Rh immunoglobulins*.—The batches of Anti-D prepared showed low titre and so have been further purified by DEAE Sephadex chromatography and will now be re-evaluated.

Red cell enzymes

Enzymes of glycolytic and pentose phosphate pathways are important in maintaining the integrity and viability of red cells *in vivo*. However, under certain physiological stress, the sequence of enzyme reaction gets altered, resulting in changes which lead to the disintegration of the cell on slightest provocation. Many of these red cell enzyme deficiencies are hereditary and the incidence of such enzyme deficiencies in population pose an important public health problem. Some of these enzymopathies like G-6-PD deficiency were, therefore, studied in greater detail than others. Pyruvate kinase and glutathione reductase are important enzymes, dependent on ATP—a chemical reservoir of energy. Estimate of total glutathione and its stability thus provides an important clue to the biochemical abnormalities present in various chronic and acute forms of haemolytic anaemia. The department has been surveying the population as well as the clinical patients for G-6-PD deficiency for almost a decade. Many cases of haemolytic anaemia of unknown etiology are being referred for the purpose. Since the last 2 years, the department has also standardized the methods for quantitative assays of the 3 enzymes viz. G-6-PD, PK and GSH and studied the enzyme profile in the cases of haemolytic anaemia. Some of the preliminary findings on these quantitative aspects were reported last year. The more comprehensive data collected during the year are presented here.

Various anti-coagulants have been used by different workers for the collection of blood for enzyme studies. The use of various suitable anti-coagulants and the period for which enzyme studies could be considered

reliable, have great practical importance. A study was, therefore, undertaken to evaluate 4 different anti-coagulants by carrying out quantitative assays of G-6-PD, PK and GSH up to a period of 13 days. The summary of these findings is also given below :—

(a) *Screening of G-6-PD.*—During the year, 98 cases were referred for G-6-PD deficiency test from various city hospitals. Eight out of these were found to be G-6-PD deficient.

(b) *Quantitative assays of G-6-PD, GSH and PK in various haemolytic anaemias.*—During the year, 56 cases of different types of haemolytic anaemia were investigated for this assay. Also, cases of normal adults (40 males and 20 females) and mothers of G-6-PD deficient males were investigated. The results are presented in Table 9.

The findings suggest that the levels of G-6-PD were nearer zero in deficient males, while heterozygote females had nearly 50 per cent values of this enzyme in comparison to normal females. It is interesting to note that GSH levels were also lower in G-6-PD deficient males.

This data, when further divided into those having crisis of haemolytic episode and those with no crisis showed that G-6-PD deficient cases without crisis had GSH levels equal to the heterozygote females. GSH levels were very low in those having haemolytic episode. However, levels of PK were not greatly altered in these individuals. Amongst other categories of haemolytic anaemia, G-6-PD as well as GSH levels were not affected, except in Thalassaemia major, where GSH levels were slightly elevated. No gross difference in PK was noticed.

Anti-coagulants in enzyme studies

In order to assess the suitability of 4 different anti-coagulants, 6 samples of blood were collected each in 4 different anti-coagulants, as follows :—

1. ACD (NIH) Formula A—1 ml./5ml. blood.
2. Heparin (without preservative) 20 USP Units—0.3 ml./5 ml. blood.
3. Ammonium oxalate—7 mg./5 ml. blood.
4. Di-sodium EDTA 10 mg./5 ml. blood.

Enzyme profiles were studied on the day of collection, i.e. on the 7th day and 13th day. In each case, percentage GSH stability was also calculated. Findings are given in Table 10 and the results are expressed as mean of 6 blood samples studied.

The findings suggested that ACD was not a suitable anti-coagulant, particularly for GSH studies, because of the presence of extra glucose in ACD.

Heparin and Oxalate both showed fall in GSH levels nearly 50 per cent values in 2 weeks. The stability was also equally affected. There was no proportionate fall of PK and G-6-PD in all the 3 anti-coagulants (ACD, Heparin and Oxalate) on the 7th day, while both the enzymes showed reduced levels to nearly 50 per cent on the 13th day. EDTA anti-coagulant appeared somewhat better than the others, since the fall in GSH values was less and stability was correspondingly better. Only slight fall in PK activity was noticed, while the G-6-PD activity remained constant for a period of 2 weeks.

Biochemical and immunological studies of fibrinogen and cryoprecipitate

Fibrinogen was prepared from fresh plasma with different methods. Clottable protein was estimated on a weight basis converting fibrinogen into fibrin. Purified fibrinogen was prepared by the methods described by Blomback.

Mono-specified anti-sera against fibrinogen were prepared. The fibrinogen prepared by the above methods was tested immunologically against anti-fibrin and anti-human serum for purity.

The cryoprecipitate was prepared from fresh human plasma by (1) slow freezing method at 70°C in a deep freeze (Pool and Shanon 1965), (2) rapid freezing method with dry CO and alcohol and (3) precipitation with alcohol 3 per cent at 3°C (Moyar 1972). Estimation of AHF activity of the above cryoprecipitate was carried out, and was found to be 200-260 per cent. Anti sera against AHF was prepared by injecting cryoprecipitate prepared by Hoyer's method. These anti-sera give a single line against plasma and AHF and not against fibrinogen. Purification of fibrinogen and cryoprecipitate is now being carried out with column chromatography using Sephadex G 200.

Genetics and biochemical studies of R. B. C. in normal and diabetic

Diabetes, a disease of carbohydrate metabolism, has been the subject for extensive clinical, biochemical and biological studies. Tissue metabolism in diabetes has been well-documented, while only scattered literature is available on the red cell metabolism. Two major pathways, namely Embden-Mayerof and glycolytic pathway (anaerobic) and Penthose phosphate pathway (aerobic), are operative in a mature red cell. An integrated approach to study the red cell metabolism, including biochemical and genetic parameters in diabetes, has been made. The study involves a number of enzymes and intermediates as well as the genetic markers on the red cell.

Blood samples from 100 diabetic patients were studied. Results are enumerated in Tables 11 and 12.

TABLE 1
Incidence of HBAg and HBAb in various clinical conditions

Serial No.	Types of cases	No. of samples tested for HBAb and HBAb	HBAb +ve samples	%	HBAb +ve sample	%
1	Multi-transfused ..	190	10	5.2	11	7
2	Viral hepatitis ..	708	73	10.1		
3	Chronic active hepatitis ..	8	2	25		
4	Jaundice other than hepatitis ..	10				
5	Cirrhosis ..	249	9	3.6		
6	Hapatic amoebiasis ..	8				
7	Leprosy ..	130	12	9.2		
8	Leucoemia ..	50	2	4	1	2
9	Staff of renal dialysis unit ..	62	2	3		

TABLE 2

Liver function tests in viral hepatitis cases, positive and negative for Australia antioen

L.F.T.				Normal mg. %	Au + ve	Au - ve
S.G.O.T. (Karmen Units)	..	..	..	25.7	115.6	99.4
S.G.P.T. (Karmen Units)	..	..	..	21.25	159.9	127.00
Alkaline phosphatase	..	..	..	3.22	4.18	4.98
Bilirubin mg. %	..	..	..	2.5	6.54	7.8

TABLE 3

Liver function tests in leukemia cases, positive and negative for Australia antigen

L.F.T.					Au + ve mg. %	Au - ve mg. %
S.G.O.T.	..	..	..	..	33.2	28.45
S.G.P.T.	..	..	..	..	31.4	23.6
Alkaline phosphatase	..	..	..	..	2.8	2.92
Bilirubin	..	..	..	..	2.5	2.5

TABLE 4

Liver function tests in leprosy cases, positive and negative for Australia antigen

L.F.T.					Au + ve mg. %	Au - ve mg. %
S.G.O.T.	..	..	..	..	58.7	34.4
S.G.P.T.	..	..	..	..	43.5	19.66
Alkaline phosphatase	..	..	..	..	2.9	2.55
Bilirubin	..	..	..	..	2.5	2.5

TABLE 5

Alpha-fetoprotein (AFP)

	No.	AFP Positive	HBAG
Suspected cases of hepatoma and other liver diseases	.. 164	*14	2

* All cases were confirmed as hepatoma later on. Two were found positive for both HBAG and AFP.

TABLE 6

Immunological findings in suspected rheumatoid cases

RA Test		Haptoglobin type					Immunoglobulins				
Positive	Negative	2	2	2	1	0—0	Normal	IgG	IgG	MgI	IgM
33	27	44		10		3	38	3	2	1	1
Only 45 samples were tested											

Only 45 samples were tested

Blood group study did not reveal anything uncommon.

TABLE 7

*Distribution of Hp phenotypes in various haemolytic disorders **

Group	No.	Hp Phenotypes				Hp 0-0
		1-1	2-1	2-2	0-0	
Normal	220	6	67	142	5	2.5
Thalassemia						
— minor	51	1	4	14	26	52
— major	15	0	2	6	13	86
Chronic non-spherocytic haemolytic anaemia	16	0	3	6	7	45
Drug-induced haemolytic anaemia	69	1	9	27	22	30
Leukemia						
Myeloid	36	0	7	18	4	11
Lymphoid	8	0	3	4	1	12
Auto-immune haemolytic anaemia	9	0	1	4	4	36
Multiple myeloma						
— monoclonal	65	2	8	32	23	36
— polyclonal	14	0	1	6	7	50
Cardiac prosthesis	26	0	4	10	12	48
Transfusion reactions	23	0	3	9	11	49
Iron deficiency anaemia	6	0	1	5	0	0
Unexplained haemolytic anaemia	17	0	1	6	10	59
Miscellaneous including nutritional anaemias	60	0	18	31	9	15

* Findings indicate higher incidence of Hp 0-0 in practically all types of haemolytic anaemias. The higher incidence of Hp 0-0 in myeloma may be due to the increase in total gamma globulin levels, thus reducing the levels of α_2 globulin. Efforts will also be made to correlate Hp 0-0 with Hb levels.

TABLE 8

Incidence of various heptoglobin types in a neo Natal jaundice

Hp types	Normal		Icteric.		X ²
	No	Percent	No.	Percent	
2-2	35	20.8	58	27.2	14.9 *
2-1	19	11.3	39	15.6	3.6
1-1	3	1.8	3	1.2	0.02
0-0	111	66.1	140	56.0	3.8 *

* Significant at 0.05 level.

TABLE 9

Quantitative assay of enzymes in normal adults, heterozygote females and cases of haemolytic anaemia

			No. of cases	G-5-PD activity		GSH levels		PK activity	
				Range	Mean	Range	Mean	Range	Mean
<i>Normal controls</i>									
Male	..	..	40	2.95—553	3.84	41—88.6	63.9	26.3—50	41.8
Females	..	..	20	2.4—5.53	3.6	43.4—76.6	64	26.3—45.5	36.6
<i>G-6-PD heterozygote</i>									
Females	..	..	6	0.736—1.843	1.545	9—43.1	30.8 *		
Nee-natal Jaundice	..	..	10	3.5—4.98	4.55	46.4—85.6	65	28.8—48.1	37.7
<i>Thalassemia</i>									
Major	.	.	12	2.93—5	4.45	65.5—100.5	85.4	35.4—50.6	42.8
Minor	..	..	8	2.9—5.35	4.86	55.3—90.2	70.8	30.5—49.8	40.8
Unexplained haemolytic anaemia			20	3—5.53	3.8	45—88.6	65.5	26.3—52.2	39.2
<i>G-6PD deficient—</i>									
Haemolytic anaemia	..	..	6	0	0	5—17.2	11.3	30.5—55.4	42.9*
Non-haemolytic anaemia	..	..	4	0	0	25.08—32.5	28.59	..	..

*GSH levels of only 3 samples

*PK activity done in 2 cases only

G-6-PD activity expressed in units/ml. RBC/min.

PK activity expressed in units/ml. RBC/Min.

GSH Levels expressed in mg. % RBC.

TABLE 10

Evaluation of four different anti-coagulants for the quantitative levels of G-6-PD, GSH and PK (Mean values of six samples.)

First day			Seventh day			Thirteenth day			
			GSH in mg %			*	*		
Anti-coagulants used			PCV %	B. I. with APH		A.I. with APH		%Stability	
PK	activity	G-6PD	activity	PCV. .percent.					
ACD	...	..	43	56.8	55.4	98 %	34.6	4.61	43
Heparin	..	..	45	65.4	54.4	83 %	34.0	4.32	45
Oxalate	..	..	47	63.5	50.0	79 %	33.0	4.03	47
Edta	..	..	47	64.8	61.5	95 %	35.2	4.54	47

B. I. * before incubation with APH.

A. I. + after incubation with APH.

First day		Seventh day				Thirteenth day			
		GSH in mg %				GSH in mg %			
B.I.	A.I.	%Stability	PK activity		PCV %	B.I.	A.I.	%Stability	
PK	activity	G-6-PD	activity						
54.2	53	98%	33.4	4.62	42	50	45.3	91%	30.2
55.0	55.5	65%	34.0	4.20	42	57.3	15.8	42%	25.4
55.0	25.6	47%	32.3	4.0	38	35.0	20.4	58%	20.2
57.5	35.4	62%	34.5	4.45	46	45.4	35.0	78%	29.4

× GSH levels expressed in mg. of GSH/100 ml. RBC.

PK activity expressed in units ml. RBC/min.

G-6-PD activity expressed in units/ml. RBC/min.

TABLE 11

Changes in red cell G-6-PD, PK and GSH in diabetic patients

	No.	G-6-PD	PK	GSH
Diabetic	100	0	61 deficient.	39 deficient.

TABLE 12

Serum haemoglobin binding proteins in diabetic patients

Total	Haptoglobin				Transferrin C-C
	0-0	1-1	1-2	2-2	
100	3	4	39	54	100

DEPARTMENT OF IMMUNOLOGY

Diphtheria toxin production

A synthetic medium was developed to study metabolism of *C. diphtheriae* in relation to toxin production. Biotin or pimelic acid was found to nullify the inhibitory effect of iron on toxin production, suggesting alteration of permeability of cell membrane-Iron making it less permeable and biotin counteracting the effect of iron. As the effect on lipid composition of membrane was considered, further study was carried out to find out unsaturation of fatty acids (responsible for fluidity) of cell envelope in medium containing iron and biotin, both or one. Biotin appeared to increase the unsaturation three-four fold, but contradictory to our previous assumption, iron also stimulated unsaturation.

Attempts were also made to obtain maximum titres of diphtheria toxin production on a large scale. Routinely, only 50-60 LF toxin was obtained on an average. Hence, it was important to study this aspect further to get better titres. The factors studied were :—

(i) The toxigenicity of culture, (ii) the type of container and (iii) size of inoculum.

(1) It was observed that toxigenicity of production culture was reduced and it was, therefore, necessary to review it.

(2) Seven-day incubation : Roux bottle with 150 ml. medium yielded higher toxin production (100-110 Lf/ml) as against Haffkine flask with 900 ml. medium (55 Lf/ml.). Haffkine flasks with 300 ml. medium gave high toxin production (100-110 Lf/ml.) i.e. equivalent to Roux bottle with 150 ml. medium.

(3) It was necessary to increase the inoculum for higher yield of production.

(4) Even 900 ml. medium in Haffkine flask gave high toxin, but the incubation period had to be changed. This is still under study.

(5) Toxin titre, with the use of Analar quality sodium acetate, was higher than with the use of sodium acetate (Apex) which was being used earlier.

Tetanus toxin production

Haffkine Institute uses Oxoid Tryptone and Bengal Immunity Tryptone for Tetanus Toxin production according to Massachusetts method. It was interesting to study the effect of skimmed milk instead of tryptone, which is cheaper substitute and is also locally available medium. Skimmed milk was digested with trypsin at optimum PH and temperature. Digested product was directly taken for toxin production. However, digestion followed by deferration and then charcoal treatment showed good toxin production.

Charcoal treatment was carried out at different PH levels. PH-7 was found to be the best for toxin yields.

Composition of the medium with this as the base, using optimum amounts of glucose and salts, was prepared, varying the amounts of amino-acids and vitamins. It was observed that Tryptophane, tyrosine and 'B' group vitamin-Thiamine were inessential items.

An I. C. M. R. Scheme on tetanus toxin is being proposed for studying toxicological and pharmacological properties of Tetanus Toxin, in collaboration with the Departments of Bacteriology, Biochemistry and Pharmacology.

Studies on immune response

Shri P. M. Naik attached to this Department, is studying the immune response of steroid hormones. He compared effects of DNA, Oilgonucleotides, Poly A-U, Poly I-C and Vitamin A acetate as adjuvants on antibody production with those of Freund's complete and incomplete adjuvants and Adjuvant 65. The ability of phyto-haemagglutinin, both M and P, to enhance or related antibody production was also determined.

Ovarectomy and subsequent treatment with Ethinyl Oestradiol and progesterone suppressed anti-body production significantly Progesterone, Diethyl Stilboesterol and Anovlar were also tried in the study of effect of response and suppression of response.

Studies on immunogenicity of diphtheria toxin

Whether cellular products of bacterial antigen help increase in antigenicity resulting in response in horses was studied.

Diphtheria toxin was prepared under different conditions of incubation and its Lf and MLD were tested.

It was observed that the MLD content of toxin increased with the increase in the period of incubation, thereby indicating that the toxin fraction increases with increased incubation period.

The pooled toxin was higher in MLD as compared to routine toxin. It is expected that this carries some DNA material which would have adjuvant effect during immunization. The intracellular toxin also may be available by prolonged incubation.

This was used for immunization of horses. The general trend was marginally higher response when compared with the results of the previous 10 years. Experiments have been based to study individual reactions of the different toxins from the same source and the results are awaited.

Studies on immunogenicity of tetanus toxin

Tetanus Toxin was prepared by incubating organisms at different temperatures. The starting lot was divided into two groups and incubated

for different periods. The toxin is being used in two groups of horses for immunization. The results of the analysis will indicate the difference in immunogenicity, if any. However, it was observed that there was slight fall in Lf, but no perceptible fall in MLD of toxins prepared by the two methods. No increase in toxicity was observed in toxins after prolonged incubation.

Within a period of observation for 4 months, no marked difference was noted in the response of horses. The experiment was discontinued as the condition of horses in both the control and report groups seemed normal. Horses used were old and accelerated stimulus was used in the immunization process.

Snake Venoms.

Monovalent immune anti-sera against Russell's Viper venom was prepared for use in immunoelectrophoretic studies. Krait venom was studied analytically by electro-phoresis on starch-gel, starch block agar-gel and polyacrylamide gel. Fractions were tested for enzyme activity and toxicity. Nuclease and Lamino oxidase were found to be present in cathodic fractions, proteases in anodic fraction and cholinestrases on the site of application. Four toxic components were separated, out of which three were highly toxic and were not associated with any of the tested enzymes. The less toxic component showed some proteolytic activity.

Crude Krait venom was fractionated on the column of Sephadex G-50 and G-100. G-50 fraction, on separation, gave two components, both of which were toxic. The non-toxic components contained cholinestrase and C-amino Oxidase. G-100 gave 3 components, 2 of which were toxic. Fractionation on G-75 is still in progress.

Fractionation of Krait and Cobra venoms was carried out on Pevikon Block. The fractions are being characterised. After characterisation, the fractions will be put on Sephadex G-50 to isolate toxic factors.

DEPARTMENT OF PHARMACOLOGY

Studies in antileprosy drugs

The Indian Council of Medical Research continued to support the scheme for studies in antileprosy drugs under Dr. M. B. Bhide, Shepard's method of cultivation of leprosy bacilli in mouse foot pad was standardised. Out of the 12 batches inoculated, 7 have showed good growth curves. The growth was detectable after 6-8 months as against 2-4 months as reported by Shepard *et al.* The difference in the two results may be due to higher temperatures in animal colony at the Institute. The rest of the batches will be examined after completion of 8 months from inoculation. Growth curves in thymectomised and ALS treated mice were higher than those in the control ones.

A very sensitive spectrophotofluometric method has been standardised for the estimation of DDS and its acetylated products DADDS and MADDS.

Experimental amoebiasis

The department continued to test synthetic organic compounds received from the Department of Chemotherapy. The screening was carried out on hepatic infection of hamsters and caecal infection of rats. No compound screened showed promising activity.

Studies in testing of drugs for their antifilarial activity

At present, Diethyl-carbamazine is the only available drug for the treatment of filariasis. There is need for a more effective drug. With this end in view, the testing of drugs against filariasis has been undertaken. Efforts are being made to standardise the techniques for *in vitro* maintenance of *L. carini* in Tyrode and *Wuchereria bancrofti* in tissue culture V E R O cell-line. Cotton rats infected with *L. carini* are maintained by the Department of Zoonosis. Efforts are being made to obtain infected microfilaria from *Culex* mosquito maintained and infected in the laboratory.

Studies on experimental Giardiasis

Giardia lamblia, the causative organism of human giardiasis was cultivated *in vitro*, using Pavlova's medium and modified Karpetyan's medium for the first time. Different cultural parameters of the organism were studied. The effect of pH, the redox potential on the culture of giardia and the growth behaviour of the parasite were studied. Also, various aminoacids were incorporated in the medium to study whether there is any enhancement of the growth.

Drug screening programme

For an effective collaboration between the Chemotherapy Department and the Pharmacology Department, a new drug screening programme was evolved. The following techniques were standardised :—

- (a) Screening of anti-convulsant drug by chemically and electrically convulsions

(b) Screening of anti-inflammatory drugs in (x) caragenaninduced inflammation in rat paw :—

- (i) Cotton pellet method in rats ; and
- (ii) Granuloma pouch in rats.

Evaluation of new drugs—pharmacological and toxicological studies

Toxicological evaluation of R-585 was carried out in the department. The results are being analysed to assay the toxicity.

Assay of Pantothenic Acid

The work undertaken last year was concluded this year. Suitability of the use of 'Schizosaccharomyces pombe' yeast organism was established for the assay of Pantothenic Acid and its salts. The proposed method fulfils the usual criteria for the validity of the microbiological method. The standard deviation was 7.0. The results obtained by this method were compared and were found to be in conformity with those obtained by using the method described in U.S.P. XVIII. However, the proposed method has a distinct advantage over the U.S.P. method wherein the reading is obtained at the end of 40 hours as against 80 hours in the previous method.

Studies on haemolytic drugs

Normal and glucose-6-phosphate dehydrogenase deficient red blood cells were used as the test material. Haemolytic drugs, such as acetyl phenyl hydrazine, primaquin, ascorbic acid nitrofurantoin and menandione were incubated with these cells at 37°C for 3 hours. The drugs inhibit glucose-6-phosphate dehydrogenase activity and catalase activity and cause increase in methemoglobin levels and lowering of reduced glutathione levels.

DEPARTMENT OF QUALITY CONTROL

Stability of vitamins in light

Work on this problems was continued.

Using sunlight and artificial light as the source of destructive agent, stability of different water-soluble vitamins, viz. vit. B₁, B₂, B₆, folic acid, ascorbic acid, niacin and calcium pantothenate, was studied. Role of (OH) moiety to stabilize the photo-sensitive vitamins was investigated; different combinations of vitamins were examined in order to study the influence exerted on each other in the presence of light.

Vit B₂, B₆, and folic acid are photo-labile, while others are stable, B₁ is stabilized by benzylalcohol to a limited degree; folic acid does not get oxidized under experimental conditions in the presence of ascorbic acid, while in BHA, destruction rate is lowered. Folic acid is destroyed at faster rate in the presence of riboflavin. This mixture, in the presence of ascorbic acid, is more stable. Niacinamide is found to be ineffective as far as folic acid stability towards light is concerned.

Riboflavin is a photo-sensitizer and the reaction once started can proceed in the dark also.

Pharmaceutical preparations containing vitamins are liable to deteriorate in potency on exposure to ordinary tungsten lamp. Riboflavin acts as a photo-sensitizer. The stability can be increased by using proper wrapping or also by adding certain chemicals which can quench riboflavin in its excited state.

A comparative biochemical and immunochemical study of antibodies against various antigens.

The following factors were studied :—

(1) To find out proper dilution and period at which particular antigen with particular animal species gives maximum titre of antibody; (2) to prepare serum of maximum antibody titre on a large scale (3) to study the above sera for the isolation and characterization of Ig-G and its fragments with the biochemical and immuno-chemical techniques, such as gel-chromatography, immuno-diffusion and immuno-electrophoresis.

Different dilutions of tetanus toxoid were injected into rabbits and guinea-pigs subcutaneously. Dose was given according to the body weight of the animal. Antitoxin content was determined by bio-assay. A titre of 8 O. I. U./ml. was observed. More experiments will be carried out to get a still higher titre. Work is in progress on the same basis as diphtheria toxoid.

Isolation and characterisation of Ig-G and its fragments from ready-made horse anti-serum against tetanus or diphtheria toxoids will be carried out.

Comparison of neutralisation test and haemagglutination Inhibition test in the study of rabies infection.

This investigation covered HA (haemagglutination) and HAI (haemagglutination inhibition) by different strains of rabies virus and comparison of the filter paper disc method with serum dilution method.

The comparative study of neutralization test and haemagglutination inhibition test threw light on whether HAI test could be substituted for the neutralization test.

Studies on the bacterial flora in fish in the coast of Bombay, with particular reference to *V. parahaemolyticus*

V. parahaemolyticus is found responsible for causing food poisoning or gastro-enteritis and this has been well established in Japan and a few other countries. Attempts were made to isolate *V. parahaemolyticus* from varieties of fish and stool samples of gastro-enteritis cases.

Twelve and six strains of *V. parahaemolyticus* were isolated from shell fish and oysters respectively.

Two hundred and sixty-five and one hundred and ninety-one stool samples of gastro-enteritis cases from Parbhani and Kasturba Hospital (Bombay) respectively were studied culturally and found to be negative for *V. parahaemolyticus* sixty-four stool samples of gastro-enteritis cases and 191 pooled samples of animal faeces from Parbhani were collected. Suspected colonies are under study.

Studies on Cholera vaccine and allied investigation

Monovalent, bivalent and quadrivalent cholera vaccines were studied for their protective value by passive immunisation technique. Hyperimmune serum was produced in adult rabbits against each vaccine under study. Individual serum is to be tested for antibody and vibriocidal titre and then used for passive immunisation of infant rabbits to be challenged with virulent strains-Ogawa and Inaba sero-types of classical *V. cholerae* and El Tor vibrio and Toxins. Loop method in the immunised adult rabbits is being tried to judge the validity of the vaccine. The study will throw light on cross protection of the vaccines. The data will be used to evolve a method for standardisation of cholera vaccine in comparison with other assay procedures.

The study is in progress.

Studies of Immunoglobulins with special reference to anti-body patterns in pooled globulin and purified immunoglobulins.

The investigation concerns the efficacy of purified mono-specific immunoglobulins as a preventive against diseases based on anti-body determination.

Standardisation of diphtheria and tetanus toxin anti-toxin systems by counter and immuno-electrophoresis has been carried out.

As a first step for isolation of specific immunoglobulins, Sephadex G-200 column was set-up and fractionation is being carried out.

The work is in progress.

DEPARTMENT OF VIRAL VACCINES

Poliomyelitis surveillance

In Greater Bombay during January, to December 1972, the Unit recorded 1338 cases of Paralytic Poliomyelitis (Table 1). This number is the highest recorded so far in any single year in Greater Bombay. The corresponding figures for the previous two years were 545 for 1970 and 810 for 1971. The unusually high incidence of paralytic poliomyelitis in the population of Greater Bombay has been observed after a lapse of 3 years. Figures of incidence of paralytic poliomyelitis in Greater Bombay indicate progressive rise in the endemicity. The data also indicate significant rise in the incidence in successive epidemic years. Incidence of paralytic poliomyelitis in Greater Bombay during the last five epidemic years is given in table 2.

The incidence of paralytic poliomyelitis, on the basis of recorded cases, for the susceptible population (between 0-3 years) of Greater Bombay is shown in table 3. The figures in table 3 clearly indicate the progressive rise in the incidence of paralytic poliomyelitis.

Seasonal distribution of paralytic polio

Even though the incidence of paralytic poliomyelitis has shown significant increase during the last 10 years in Greater Bombay, the seasonal pattern has remained the same. During 1972, approximately 60 per cent of the total cases were recorded during the period June to September. It is observed that the seasonal pattern of the disease remains constant, irrespective of epidemic or non-epidemic years.

The incidence of paralytic polio during different parts of the year during the last five years is shown in table 4.

Age distribution of paralytic polio

Like the constancy of seasonal distribution, the age pattern of paralytic polio in Greater Bombay has also been steady. Irrespective of high or low incidence during a year, the maximum number of cases have occurred during the early childhood.

Approximately, 70 per cent of the total cases were recorded in the age group of 7 months to 2 years and 90 per cent or more of the total cases occurred in children below the age of 3 years (Table 5).

The constancy of the age pattern is seen in the data of the last three years (1972 was the epidemic year and 1970 and 1971 were non-epidemic years).

The figures in table 6 clearly indicate that the most vulnerable population is that below the age of 3 years. The implication of the above findings in the immunisation programme is obvious.

Status of residual paralysis

The Enterovirus Unit attempts to follow up cases of paralytic poliomyelitis to determine the status of residual paralysis. The aim of this study is to evolve a more accurate count of true paralytic poliomyelitis and exclusion of cases of transient paralysis due to other etiological factors. The data thus collected also give the true account of morbidity caused on account of paralytic poliomyelitis. The detailed analysis for the period January to December 1972 is shown in table 9.

It will be seen from table 7 that in 12.6 per cent of the cases (168 cases), there was no residual paralysis, while in 45.7 per cent of the cases, there was either severe or partial residual paralysis.

Virological studies in cases of paralytic poliomyelitis

To keep a watch on the pattern of virus etiology of paralytic poliomyelitis, the Unit carries out virus isolation studies on approximately 10 per cent of the total number of cases. For this purpose, cases to be studied are selected on weekly basis. During the period under report (January-December 1972), out of the 1338 reported cases 177 cases were studied for virus isolation. Using tissue cultures as well as infant mice for isolation of viruses, it was possible to isolate one or more viruses from 138 cases (77 per cent). Details of the virus studies are presented in Table 8. Approximately 65 per cent of the cases studied showed excretion of polio virus. Of the 89 cases showing excretion of polio virus, 70 per cent were found to be excreting polio virus type I.

Seasonal distribution of Enteroviruses

During the virus isolation studies on cases of paralytic poliomyelitis in 1972, all the three types of polio viruses were encountered. Polio virus type 1 was the predominant virus. Besides polio virus, 21 serotypes of non-poliovirus were also encountered. These were represented as Echo viruses; 9 sero types; Coxsackie A; 7 serotypes and Coxsackie B; 5 serotypes. The seasonal appearance of these viruses is shown in table 9. It would be seen from this table that, except for the month of November 1972, polio virus type 1 was detected in all the remaining 11 months. In contrast, polio virus type 3 was first isolated in the month of May. The pattern of non-polio viruses presents some interesting features. During the first three months of the year (January—March 1972), apart from polio viruses, only 4 serotypes of non-polio viruses were encountered. In contrast, during the summer months (April to August), as many as 17 serotypes of non-polio viruses were encountered. Again, during September to December period, only 6 serotypes of non-polio viruses were encountered. Coxsackie B virus types were not isolated during the first five months of the year (January to May).

Paralytic illness amongst vaccinated individuals

Out of the 1338 cases of paralytic poliomyelitis recorded during January—December 1972 in Greater Bombay, 194 cases had a history of receiving oral polio vaccine in the past. Of these, 55 had received only one dose of

the vaccine, 41 had received two doses while 98 cases had received three doses of the vaccine. Analysis of age distribution and seasonal distribution of these 194 cases does not show any differences when compared with those in the un-vaccinated cases. Details of these are presented in tables 10 and 11.

It was possible to collect materials for virological studies from 184 out of the 194 cases. Of the 184 cases studied, 123 cases were found to be excreting virus. The details of the virus studies are presented in table 12. Polio virus was isolated in 80 cases. Forty-three cases were found to excrete only a non-polio virus, while no virus could be isolated in 81 cases. Polio virus type 1 was isolated in 58 cases, type 2 in 16 cases and type 3 in 8 cases, respectively.

The seasonal distribution of the viruses encountered in this study is shown in table 13. In addition to all the three types of polio viruses, 13 serotypes of non-polio viruses were encountered from these cases. It is interesting to note that 12 non-polio serotypes encountered during the same period from non-vaccinated cases, were not isolated from cases of paralytic illness amongst vaccinated individuals (Echo. types 6, 8, 9, 12, Cox A types 2, 6, 10 and 15, Cox B types 1, 2, 4 and 6). In contrast, 4 serotypes of non-polio viruses (Echo types 10, Cox A type 3 and Cox B type 5), encountered in the vaccinated group, were not isolated from non-vaccinated cases.

Whereas the seasonal distribution of polio virus types 1 and 2 isolated was identical in both the vaccinated and non-vaccinated cases, amongst polio viruses, the isolation of polio virus type 3 in January and February 1972 was restricted only to the vaccinated cases.

Of the 194 cases of paralytic illness amongst vaccinated individuals, blood samples for antibody studies would be collected only from 80 cases. All these samples were collected during the acute stage of the illness. It is presumed that the anti-body patterns observed in the acute sera of these cases would reflect either the natural exposure to polio virus in the past or that these anti-bodies represent the response to oral polio vaccine. Details of the results on the immunity status of these 80 cases at the onset of disease are presented in table 14.

Antibodies to polio virus type 1 were detected in 72.5 per cent of the cases. The corresponding figures for polio virus types 2 and 3 were 62.5 per cent and 31.25 per cent respectively. In 8 cases (10 per cent), there were no antibodies to any of the three types of polio viruses. Table 16 also shows the results of virus isolations in these 80 cases. It is interesting to note that polio virus could be isolated only from 33 cases (41 per cent) out of 80 cases studied., the corresponding percentage of cases with no history of oral polio vaccine being 65 per cent.

Genetic Marker study on polio virus isolates

During the period under report, 114 strains of polio virus isolated from cases of paralytic illness with history of oral polio vaccine in the past, were studied for genetic marker tests. The marker test used was "reproductive

capacity at a temperature of 40°C". Of the 114 strains in this study, 77 belonged to polio virus type 1, 24 to polio virus type 2 and 15 were polio virus type 3. The details of the results are presented in table 15.

Study on response to oral Polio Vaccine

The aims, objectives and the methodology used in this study have been described in the annual report for the year 1971-72. Infants in the age group of 3 to 6 months were selected for this study. Pre-tested trivalent oral polio vaccine was administered on a three dose schedule at an interval of six weeks. Pre-vaccination stool/rectal swabs and blood samples were collected on the day of the administration of the first dose of vaccine. Stool samples for virological studies were collected after 7 days of administration of each dose of vaccine. The post-immunisation blood sample was collected two weeks after the administration of the third dose of the vaccine.

So far, 119 infants have been recruited for this study and 387 stool samples, 118 rectal swabs and 204 blood samples have been collected for virological and serological studies. Virus studies on samples collected from the first 85 infants have been completed. Paired sera from the first 30 infants have been tested.

Results, of virological studies carried out on pre and post-immunisation samples from 85 vaccinees are prescribed in table 17. Since these studies are in progress it is not possible at present to draw any firm conclusions. However, a few observations could be made on the completed study.

It will be seen from table 16 that, as many as 36 vaccinees out of 84 were found to be excreting a virus in the stool at the time of the administration of the first dose of the vaccine. All the three types of polio viruses were encountered in the faecal samples collected from pre-vaccinated children. After the first dose of trivalent vaccine, the number of children excreting a virus rose significantly. As many as 58 vaccinees, out of a total of 79, were found to be excreting a virus. The pattern of polio virus excretion after the first dose shows certain interesting features. The excretion of polio virus types 2 and 3 rose very significantly. Excretion of polio virus type 1 was found to be of a very low order. After the second dose of vaccine, the excretion of the three types of polio viruses was found to be equally distributed. After the third dose, excretion of polio viruses 1 and 2 was found to be totally absent while excretion of polio virus type 3 continued (these observations are based on the available data in relation to the identified strains). The feature of interest is the excretion of multiple types of polio viruses in single samples. After the first dose of vaccine, 4 vaccinees were found to excrete all the three types of polio viruses, while 6 vaccinees excreted types 2 and 3 only. Excretion of the mixtures of polio viruses was not observed after 2nd and 3rd doses of oral polio vaccine. Results of virus excretion, after each dose of polio vaccine, are shown in table 16.

The results of studies on a group of 30 vaccinees, where serological studies on pre and post-immunisation blood samples have been completed, are presented in table 17.

It will be observed from results presented table 17 that 12 infants did not have antibodies against type 1 polio virus before vaccination. Eleven and 25 infants did not have anti-bodies against polio virus types 2 and 3 respectively. After administration of three doses of trivalent oral polio vaccine, the number of vaccinees lacking anti-bodies against poliovirus 1, 2 and 3 were 6, 2 and 5 respectively. One vaccinee (Sr. No. 14) remained triple negative after administration of 3 doses of vaccine.

In table 18 are presented the results of serological studies on these 30 vaccinees typewise and arranged according to the pre-vaccination titres. It will be observed from this table that out of the 30 vaccinees, 16 showed antibody rise against type 1, 27 against type 2 and 23 against 3. Thus, the sero-conversion rates after three doses of polio vaccine in this group work out as 53.3 per cent for type 1, 90 per cent for type 2 and 76 per cent for type 3 respectively.

These studies are in progress and further results will be reported in the annual report for the year 1973-74.

Risk of paralytic poliomyelitis in the un-vaccinated and vaccinated

In the following section, an attempt has been made to calculate the risk of paralytic poliomyelitis in the un-vaccinated susceptible population and the vaccinated population in Greater Bombay. The figures are best available approximates.

Extent of immunisation

Approximately 1,00,000 children, out of 1,60,000 born every year, receive oral polio vaccine in Greater Bombay. Thus, it is estimated that approximately 3,00,000 children below 3 years would have received oral polio vaccine in a total population of 4,25,000.

Incidence of paralytic polio in the child population in Greater Bombay

On an average, approximately 500 cases of paralytic poliomyelitis are recorded in Greater Bombay during a non-epidemic year. Nearly 40 per cent of these occur in children below one year, another 40 per cent occur in children between 1 to 2 years of age and about 10 per cent of the total cases are recorded in children between 2 to 3 years of age.

The figures in table 19 show that, while one child in every 250 between the age group of 0 to 2 years gets paralysed if un-vaccinated, the ratio for vaccinated population in the same age group works out at 1 in 4,000. For a child between 2 to 3 years of age, the ratio for un-vaccinated and vaccinated work out at 1,600 and 1,11,000 respectively.

The ratios for the vaccinated groups shown above do not take into consideration the number of doses of vaccine administered. When this factor is taken into consideration, the ratio works out as follows :—

	Unvaccinated	Vaccinated with 3 doses of O. P. V.
1 to 2 years	1:250	1:8,000
2 to 3 years	1:600	1:22,000

Study of Simian intestinal viruses

Monkeys are routinely used for the preparation of primary renal cell cultures and employed for various studies at the Enterovirus Unit. Since these animals are known to harbour intestinal viruses, a study for characterisation of Simian Viruses has been undertaken.

Intestinal contents from 100 *Macacca mulatta* (Rhesus) and 50 *Macacca radiata* (Bonnett) monkeys were studied for the presence of viral agents. Of the 150 monkeys studied, 92 (61 per cent) were found to harbour viral agents in their intestinal tract.

On the basis of cyto-pathogenic effect (CPE), in primary monkey kidney tissue cultures, these virus isolates could be broadly divided into 2 groups : (a) those showing adenovirus type CPE and (b) those showing enterovirus type of CPE.

These Simian viruses have been further characterised by :—

- (1) Ether sensitivity test.
- (2) Thermal stability in the presence of 1M Mg Cl₂.
- (3) Haemagglutination activity.
- (4) Capacity to produce plaque under agar overlay.
- (5) Animal pathogenicity and
- (6) Antigenicity.

To summarise, the results of the above studies are as follows :—

- (i) All the strains were ether-resistant, i.e. lacking in lipoid envelope.
- (ii) All were found to be stable at 37°C for 30 minutes, but unstable at 56°C.
- (iii) Most of the adenovirus type strains show HA activity with rat erythrocytes.
- (iv) Amongst enterovirus type isolates with the exception of one strain, no HA activity could be demonstrated with rat, human, sheep and monkey erythrocytes.
- (v) Under agar overlay, 38 out of 42 adenovirus type strains produced plaques resembling those produced by measles virus.
- (vi) Thirty seven out of 50 enterovirus type strains produced plaques resembling those produced by Echovirus.
- (vii) All the Simian isolates proved to be non-pathogenic to infant as well as adult mice.

(viii) Adenovirus type isolated were found to have better antigenicity. Enterovirus type isolates were antigenically poor.

Preparation of antigens and antisera

During the period under report, Coxsackie A, types 15, 16, 17, 18, 19, 20 and 23 antigens were prepared in infant mice. The virus strains used for this work were obtained from the National Institutes of Health U. S. A. All these antigens were titrated and vialled. The details of the strains used and the titres of antigens prepared are shown in table 20.

Hyperimmune sera for identification of unknown isolates were prepared against Coxsackie A viruses. All those anti-sera have been prepared in rabbits. The titres of anti-sera prepared are shown Table 21.

Maintenance of continuous cell lines

While bulk of the routine virological and serological studies are carried out using primary monkey kidney tissue cultures, the unit also maintains and use the following continuous cell lines:—

- (1) Hela.
- (2) HEp-2.
- (3) Vero

All the above cell lines were brought from the Virus Research Centre, Poona, and are being maintained since last one year. The passages undergone by the above cell lines in the Unit are as follows:—

Hela cells	78 passages
HEp-2 cells	5 passages
Vero cells	14 passages

TABLE 1
Paralytic Poliomyelitis in Greater Bombay
(1972)
Monthly Distribution

Month	Total Number of cases	Percentage	Cumulative percentage
January	71	5.3	5.4
February	48	3.6	8.9
March	44	3.3	12.2
April	93	6.9	19.1
May	123	9.2	28.3
June	151	11.3	39.6
July	221	16.6	56.2
August	223	16.7	72.9
September	201	15.0	87.9
October	91	6.7	94.6
November	38	2.8	97.4
December	34	2.5	99.9
Total	1,338		

TABLE 2

Incidence of paralytic poliomyelitis in Greater Bombay

Epidemic Year	Cases of paralytic polio
1960	514
1964	723
1966	820
1968	1,120
1972	1,338

TABLE 3

Incidence of paralytic poliomyelitis in susceptible (0-3 years) age group

Year	Case incidence per 10,000 population
1961	9.9
1962	12.1
1963	11.4
1964	20.2
1965	11.3
1966	21.1
1967	9.8
1968	26.6
1969	12.2
1970	12.4
1971	18.0
1972	29.1

TABLE 4

Incidence of paralytic polio during the last 5 years

Year	Total No. of cases	February- May	June- September	October- January
		*		
		Per cent	Per cent	Per cent
1968	1,110	18.4	67.0	14.6
1969	525	10.8	62.3	26.9
1970	545	21.4	51.7	27.9
1971	810	14.9	49.4	35.7
1972	1,338	23.0	59.6	17.4

* % of total cases

TABLE 5

Paralytic poliomyelitis in Greater Bombay (1972)
Age distribution

Age group in years				Total No. of cases	Percentage	Cumulative percentage
0- $\frac{1}{2}$	..	..	..	91	6.8	6.8
$\frac{1}{2}$ -1	..	..	..	460	34.3	41.2
1-2	..	..	..	572	38.2	79.4
2-3	..	..	..	173	12.9	92.3
3-4	..	..	..	51	3.8	96.1
4-5	..	..	..	18	1.3	97.4
5-6	..	..	..	10	0.7	98.1
6-14	..	..	..	22	1.6	99.7
14-20	..	..	..	1	0.07	99.77
Total				1,338		100.0

TABLE 6

Distribution pattern of paralytic poliomyelitis 0-3 years age group

Year	Age group			Total No. of cases
	0-6 months	$\frac{1}{2}$ -2 years	0-3 years	
	Per cent	Per cent	Per cent	
1970	6.0	71.4	90.4	545
1971	7.1	70.7	91.7	810
1972	6.8	72.77	92.4	1,338

* % of total cases

TABLE 7

Status of residual paralysis after 60 days from the onset

	No.	Per-centage
Paralytic poliomyelitis cases with residual paralysis at 60 days--		
(a) Severe residual paralysis	99	(7.4%)
(b) Partial residual paralysis	512	(38.3%)
Paralytic poliomyelitis cases without report on residual paralysis ..	454	(33.9%)
Fatal cases	105	(7.8%)
Paralytic poliomyelitis case count	1170	(87.4%)
Paralytic poliomyelitis cases without residual paralysis (complete recovery).	168	(12.6%)

TABLE 8
Paralytic poliomyelitis in Greater Bombay (1972)
Virus studies

Month	Total cases			Polio viruses				Polio + Non-polio	Non-polio or un-identified
	Recorded	Studied	Positive	Ty 1	Ty 2	Ty 3	Mixed		
January	71	4	4	0	0	0	0	3	1
February	48	5	4	2	0	0	0	1	1
March	44	6	5	3	0	0	0	1	1
April	93	16	12	5	0	0	0	3	4
May	123	65	53	12	6	1	1	13	20
June	151	19	18	4	0	0	0	4	10
July	221	16	14	4	0	0	1	4	5
August	223	19	11	2	0	1	1	3	4
September	201	10	7	2	1	0	1	1	2
October	91	9	6	3	1	0	0	0	2
November	38	3	1	0	0	1	0	0	0
December	34	5	3	2	0	1	0	0	0
Total	1,338	177	138	39	8	4	4	33	50

TABLE 9
Paralytic poliomyelitis in Greater Bombay (1972)
Seasonal distribution of Enteroviruses

Month	Polio viruses					ECHO viruses								Coxsackie A					Coxsackie B					
	Ty 1	Ty 2	Ty 3	1	3	6	7	8	9	11	12	19	2	4	5	6	9	10	15	1	2	3	4	6
January	*													*										
February	*								*															
March	*	*					*											*						
April	*	*			*		*							*		*		*						
May	*	*	*	*	*		*			*		*	*	*		*	*							
June	*	*	*				*							*		*		*		*				
July	*	*	*				*	*			*	*				*		*		*		*		
August	*	*	*		*		*									*		*			*			
September	*	*	*		*	*												*			*			
October	*	*						*										*			*		*	
November	*		*																					
December	*		*																					

* Indicates the serotype encountered

TABLE 10
Paralytic illness amongst vaccinees
 (1972)

Age distribution

Age in Years			No. of cases	Percentage	Commulative percentage
0 - 1	..	..	5	2.6	2.6
1 - 2	..	..	43	22.1	24.7
2 - 3	..	..	79	40.7	65.4
3 - 4	..	..	40	20.6	86.0
4 - 5	..	..	12	6.2	92.2
5 - 6	..	..	7	3.6	95.8
Above 6	..	..	3	1.5	97.3
			5	2.6	99.9
Total			194		

TABLE 11
Paralytic illness amongst vaccinees
 (1972)

Monthly distribution

Month			No. of cases	Percentage
January	..	..	11	5.7
February	..	..	9	4.6
March	..	..	4	2.1
April	..	..	11	5.7
May	..	..	19	9.2
June	..	..	21	10.2
July	..	..	40	20.6
August	..	..	24	12.4
September	..	..	27	13.9
October	..	..	13	6.7
November	..	..	9	4.6
December	..	..	6	3.1
Total			194	

TABLE 12
Paralytic illness amongst vaccinees (1972)
Virus studies

Month		Total No. of cases reported	No. of cases having vaccination history			Total No. of cases studied	Virus isolation							
			1 dose	2 dose	3 dose		Polio virus			Mixed	Polio and non- polio mixed	Non-polio or unidentified	Negative	
							Ty 1	Ty 2	Ty 3					
January	..	..	11	2	1	8	11	2	..	2	1	4	1	1
February	..	..	9	2	2	5	9	4	..	..	..	2	1	2
March	..	..	4	2	1	1	4	2	1	..	..	..	..	1
April	..	..	11	1	3	7	11	2	1	..	..	3	3	2
May	..	..	19	9	2	8	17	6	1	..	..	4	2	4
June	..	..	21	6	5	10	21	8	..	..	..	3	4	6
July	..	..	40	14	8	18	35	7	3	1	..	..	9	15
August	..	..	24	6	3	15	22	2	..	..	..	2	10	8
September	..	..	27	6	8	13	26	6	1	..	1	..	9	8
October	..	..	13	3	4	6	13	3	1	..	..	3	1	5
November	..	..	9	3	2	4	9	2	..	..	..	..	2	5
December	..	..	6	1	2	3	6	1	..	..	..	..	1	4
Total	..	..	194	55	41	98	184	45	8	3	2	22	43	61

TABLE 13

*Paralytic illness amongst vaccinees (1972)**Seasonal distribution of enteroviruses*

Month	Polio virus			Echo virus						Coxsackie A					Coxsackie B	
	Ty 1	Ty 2	Ty 3	1	3	7	11	15	19	3	4	5	9	10	3	5
January	..	* @	*								*	*				
February	..	*	*								*	*				
March	..	*	*													
April	..	*	*		*				*		*					
May	..	*	*		*						*					
June	..	*				*										*
July	..	*	*	*	*	*									*	
August	..	*	*			*	*	*		*			*		*	
September	..	*	*	*	*	*	*							*	*	
October	..	*	*													
November	..	*														
December	..	*														

@ Indicates the serotypes encountered

TABLE 14

*Paralytic illness amongst vaccinees
Greater Bombay (1972)*

Virological and serological studies of 80 cases

Virus isolated *	Anti-body		pattern against polio virus					Total
	+00	0+0	++0	0++	+0+	+++	000	
Polio virus type 1	5	1	6	2	2	5	4	25
Polio virus type 2	3	2	..	..	..	2	1	8
Echo 1	..	2	..	..	..	..	..	2
Echo 3	..	..	..	..	..	1	..	1
Echo 7	..	2	1	..	..	1	2	6
Cox B5	..	..	..	..	..	1	..	1
Echo 15	..	1	..	..	..	..	..	1
Un-identified	..	4	1	..	..	1	..	6
Negative	..	6	5	8	1	9	1	30
Total	20	11	16	3	2	20	8	80

* +00 = Anti-body titre of 1—16 or more for type 1, less than 1—16 for type 2 and 3 respectively.

Cox = Coxsackie Virus

TABLE 15

Paralytic illness amongst vaccinees

Genetic marker studies

Marker character				Poliovirus types			Total	
				1	2	3		
rect	+	(Wild)	..	..	66	13	5	84
40								
rect	+	(Intermediate)	..	..	10	4	3	17
40								
rect	—	(Vaccine like)	..	..	1	7	5	13
40								
Total			..	..	77	24	13	114

rect = rodentic capacity at temperature 40° C.

40

TABLE 16
Response to oral polio vaccine
virus excretion (1972)

Sample	Total studied	Total +ve	Total -ve	Polio Virus			Unidenti- fied	Nonpolio	To be identified
				Ty1	Ty2	Ty3			
S (pre-vaccination)	84	36	48	5	1	3	1	12	14
S1 (7 days after 1st dose)	79	58	11	5	20	28	0	6	13
S2 (7 days after 2nd dose)	73	50	23	6	5	7	2	9	21
S3 (7 days after 3rd dose)	69	55	14	0	0	9	1	16	29

TABLE 17

Response to oral polio vaccine
Serological studies of 30 paired sera (1972)

Serial No.	Pre-vaccination			Post-vaccination		
	Ty1	Ty2	Ty3	Ty1	Ty2	Ty3
	*		a			
5	128	128	16	128	128	0
6	32	16	0	64	128	16
7	8	8	0	128	128	128
8	16	0	0	16	128	64
9	8	0	8	0	128	128
10	8	8	0	128	128	0
14	0	0	0	0	0	0
15	C	8	0	0	0	128
16	16	0	0	32	128	128
17	128	16	0	128	128	128
18	0	0	0	64	128	128
19	16	8	0	8	128	128
20	8	8	0	128	128	128
22	32	16	16	128	128	64
23	0	32	0	128	128	128
24	0	8	0	128	128	128
25	8	0	0	C	128	128
29	32	32	8	128	128	128
30	128	8	0	128	16	32
31	0	C	0	16	128	128
32	0	8	0	0	128	128
34	0	0	0	32	128	64
35	0	16	0	128	128	32
36	64	16	8	8	128	128
37	128	8	0	128	128	128
38	0	0	0	64	128	128
39	0	0	0	128	128	128
41	0	0	0	128	128	32
42	0	0	0	0	128	0
43	32	8	0	0	128	0

a Reciprocal of serum dilution neutralizing approximately 10⁵ TCID₅₀ of virus

* Indicates equal to or more than 128

TABLE 18

Response to oral polio vaccine [Serological studies I (1972)]

O 5053-76

Serial No.	Polio Pre	Type-1 Post	Serial No.	Polio Pre	Type-2 Post	Serial No.	Polio Pre	Type-3 Post
	*				^a			
14	0	0	8	0	128	6	0	16
18	0	64	9	0	128	7	0	128
23	0	128	14	0	0	8	0	64
24	0	128	16	0	128	10	0	0
31	0	16	18	0	128	14	0	0
32	0	0	25	0	128	15	0	128
34	0	32	34	0	128	16	0	128
35	0	128	38	0	128	17	0	128
38	0	64	39	0	128	18	0	128
39	0	128	41	0	128	19	0	128
42	0	0	42	0	128	20	0	128
						23	0	0
						24	0	128
						25	0	128
7	8	128	7	8	128	30	0	32
9	8	0	10	8	128	31	0	128
10	8	128	15	8	0	32	0	128
20	8	128	19	8	128	34	0	64
25	8	C	20	8	128	35	0	32
41	8	128	24	8	128	36	0	128
			30	8	16	38	0	128
			32	8	128	39	0	32
			34	8	128	41	0	128
8	16	16	43	8	128	42	0	0
16	16	32				43	0	0
19	16	8						
29	32	128	6	16	128			
			17	16	128			
			22	16	128	9	8	128
			35	16	128	29	8	128

TABLE 18—(contd.)

Serial No.	Polio Pre	Type-1 Post	Serial No.	Polio Pre	Type-2 Post	Serial No.	Polio Pre	Type-3 Post
6	32	64	36	16	128	36	8	128
22	32	128	29	32	128			
43	32	0						
			23	32	128	5	16	0
36	64	8	5	128	128	22	16	64
5	128	128						
17	128	128						
30	128	128						
37	128	128						

* Reciprocal of serum dilution neutralizing approximately 100 TCID₅₀ of virus.

a Indicates equal to or more than 128.

TABLE 19

Incidence of paralytic poliomyelitis among vaccinated and non-vaccinated population of Greater Bombay.

Age group	Vaccinated		Non-vaccinated		Total	
	No. of cases	No. Vaccinated	No. of cases	No. unvaccinated	No. of Cases	Total population
0—1	26/100,000 (1 : 4000)		185/47,000 (1 : 250)		211/147,000	
1—2	26/100,000 (1 : 4000)		186/41,000 (1 : 250)		212/141,000	
2—3	9/100,000; (1 : 11,000)		62/37,000 (1 : 600)		71/173,000	

Foot Notes : 1 Total population 5.5 millions
2 Total population below 3 years : 424,000
(excluding deaths in first 3 years)

TABLE 20

Preparation of Cocksackie A reference virus stocks in infant mice

Type					Strain	Titre/0.03 ml.
Cox A 15	..	..	..	..	G 9	.. 106.9
Cox A 16	..	..	..	..	G 10	.. 105.7
Cox A 17	..	..	..	..	G 12	.. 105.0
Cox A 18	..	..	..	..	G 13	.. 104.5
Cox A 19	..	..	..	..	Dohi	.. 105.0
Cox A 20	..	..	..	..	1A 35	.. 105.5
Cox A 22	..	..	..	..	Chulman	.. 106.5
Cox A 23	..	..	..	..	Vispo	.. 105.0

TABLE 21

Preparation of Cocksackie A virus antisera in rabbits

Virus type					Titre of antisera	ID 50 used in the test
Cox A 14	..	..	..	..	1,400	316
Cox A 15	..	..	..	..	15.8	316
Cox A 16	..	..	..	..	2,314	200
Cox A 17	..	..	..	..	314.2	316
Cox A 18	..	..	..	..	682.3	100
Cox A 19	..	..	..	..	682.3	100
Cox A 20	..	..	..	..	794.3	100
Cox A 22	..	..	..	..	955.0	87

DEPARTMENT OF VIROLOGY

Epidemiology of rabies

(a) *Carrier state in dogs.*—On 1st August 1972, one more normal dog was reported as having caused death from rabies in a human patient. The dog was brought to the Institute and kept under observation. Daily samples of saliva, collected for virus isolation, were found to be negative. The dog remained alive and in good health.

(b) *Human patients.*—Three samples of saliva, 1 tongue scraping, 1 sputum sample, 1 urine sample, 3 C.S.F. samples and 4 blood samples were collected from human patients suffering from rabies and admitted to various hospitals in the city. The saliva, tongue scraping, sputum, CSF. and urine samples were inoculated into infant mice for virus isolation. All were negative. Blood samples will be tested for the presence of antibodies.

(c) *Trial of an indigenous drug.*—On request from a private party from Satara, an indigenous herbal product, called "Tukaram Aushadh", which was claimed to be effective against hydrophobia, was tested for its efficacy against a standard fixed virus challenge in white mice. The product was toxic to mice and as, such, no conclusion could be reached as to its efficacy, because the controls as well as the test animals died in the course of the experiment.

(d) *Rats and bandicoots.*—The study of these animals for their role as vectors of human rabies was interrupted for some time due to some organizational problems with the Municipality and later due to an episode of infection in the mouse colony, resulting in shortage of mice. The work has been recommenced.

Influenza surveillance activities

Surveillance activities were continued and reports of influenza outbreaks were investigated.

Isolation.—Twenty throat washings were collected from suspected cases of influenza as shown in Table 1. No virus was isolated from any of these cases.

Serology (i).—Tests were completed for assessing HI antibodies against influenza virus strains prevalent at different periods in various age groups of the population. From tables drawn up for each prototype strain and for age-wise prevalence of such antibodies, it was possible to draw general conclusions regarding the history of influenza and the successive infective strains of this virus, prevalent amongst the population of Bombay (see Tables 2, 3 and 4). The overall findings and the historical sequence resemble the findings reported from other parts of the world.

(ii) *Incidence of HI antibodies in 45 pairs of mother and cord samples collected in 1967.*—Transfer of antibodies to the extent of 68-88 per cent to the child from the mother was seen in the different prototype tests,

indicating thereby that children up to 6 months of age maintain the passive immunity obtained from the mother (see Table 2).

(iii) *Distribution of anti-bodies in commercial samples of gamma globulins.*—Twenty-nine samples of gamma globulin (15 pre-pandemic and 14 postpandemic), prepared at this institute, were tested. Results obtained (see Table 5) suggest the usefulness of regular sampling of gamma globulin preparations as representative of the young adult population to assess the epidemic situation.

(iv) *Further variation of the A2/Hong Kong/68 strain.*—The World Influenza Centre reported a further variant of the Hong Kong strain A2/England/42/72, which resembled the bulk of the isolates obtained in Southern India in the latter part of 1971 (Weekly Epi. Rec., 1972).

The likelihood of the circulation of such strains in Bombay prompted the Department to take up a quick survey of a few sera from the May 1972 collection against the A2/Coonoor/99/71 variant and the A2/Hong-kong/68 variant.

Antibody titres were either identical or showed two-fold difference against both the viruses in 22 sera, while 9 sera showed a four-fold difference. In all cases, the titres were lower in respect of the Coonoor strain.

From the results, it would appear that the Coonoor strain has not circulated extensively in Bombay. However, considerable cross-immunity is conferred against the current strain by the previous variant. Attempts at isolation and serological surveillance for newer variants are continuing. Sero-epidemiology of measles.

Sero epidemiology of measles.

The studies to assess correlation between history of measles and presence of antibodies in the serum were completed. In such interrogative studies, the importance of the confidence of the subjects in the investigator and the care the investigator should take to ensure that his questions are properly understood, cannot be over-estimated. Studies in which these pre-conditions were known to be adequately satisfied are classified as A, whereas other are classified as B. All sera were tested with the ignorance of the history, a comparison having been made in each study after the tests were completed. The two tables (Tables 6 and 7) show that a close correlation was obtained in all studies classified as A. It is not possible to express any opinion on the B studies, since the conditions were not known. However, even when A and B studies are grouped together, this discrepancy is very small.

Subacute Sclerosing Panencephalitis (SSPE)

A total of 18 sera and 27 C. S. F. samples from 29 cases of this condition were referred, during the year, for measles anti-bodies. The results of 5 sera and 4 C. S. F. samples tested are given in Table 8. Other sera are yet to be tested.

Since it has been suggested that the development of SSPE is due to a deficient antibody forming mechanism, a study of differential immunoglobulins in 34 samples was kindly carried out, on our behalf, by Dr. Girish Vyas at the Department of Clinical Pathology and Laboratory Medicine, University of California, San Francisco.

The results in comparison with the anti-body titres obtained are still under study.

Smallpox vaccination

Collection of sera from children vaccinated against small-pox was continued. Total sera obtained were as follows :—

Mother's blood at delivery	...	518
Cord blood (pre-vaccination)	...	518
One month after vaccination	...	132
Six months after vaccination	...	44
One year after vaccination	...	4

Rats as vectors of human disease

In previous years, isolation of encephalomyocarditis (EMC) virus and presence of antibodies in rats collected from villages of Kalundre and Palaspe have been reported. The investigation was, therefore, extended to human population from the same villages for the presence of antibodies against the same (EMC) virus. The number of sera obtained was, however, small. The results are given in Table 9.

The incidence of anti-bodies to EMC virus in human beings was found to be 9 per cent as compared to 37.8 per cent in the rats from the same area.

Rubella

During the year, a total of 13 cases of infants and children were referred to the Department for detection of rubella virus and serological studies.

Blood samples from all the cases and 18 contacts were studied serologically. Eight, out of 13 cases and 16 out of 18 contacts showed presence of rubella specific antibody in serum samples.

Prospective studies in relation to serological status of pregnant women during each trimester have been initiated.

Biochemical studies

Though mouse inoculation test for rabies is reliable, it is expensive and time-consuming. Hence, attempts were made to see whether it can be replaced by a simpler method like immuno-disc electrophoresis. Fixed rabies virus was the antigen used, against which specific antiserum prepared in horse, was allowed to react in polyacrylamide gel containing a central

core for serum agarose mixture. The pore size of the polyacrylamide gel was found to be too small for the virus to enter. At present, electrophoretic runs, using antigen in the central core of the gel, are in progress.

Polyacrylamide gel electrophoresis of normal and infected brain tissue proteins was carried out, but no significant difference in the protein pattern was noticed. Similarly, there was no drastic change in the total protein concentration of normal and infected brains.

TABLE 1

Throat gargles collected for influenza surveillance

Date of collection	Place	No. of cases
30th October 1972	 Ship MV. APJ Ambika	7
17th January 1973	 Aurangabad	4
20th February 1973	 Aurangabad	9
Total		20

TABLE 2

Incidence of HI antibodies in children

Influenza strains	Period of collection					
	Pre-pandemic Pre-October 1968 (a)				Intra-pandemic October 1968-69 (b)	
	0-6 months		6 months-11 years		6 months-6 years	
	Per cent positive	A. mean titre	Per cent positive	A. mean titre	Per cent positive	A. mean titre
Swine 15/30	.. 4.0*	1:1	2.0	1:1	..	..
A/PR/8/34	.. 18.0	1:6	2.0	1:1	..	..
A1/FM/1/47	.. 50.0	1:39	2.0	1:2	2.0*	1:1
A2/Singapore/1/57	.. 29.0	1:12	2.0	1:1	12.0	1:30
A2/England/12/64	.. 96.0	1:198	42.0	1:51	45.0	1:27
A2/Tokyo/3/67	.. 75.0	1:52	60.0	1:35	60.0	1:22
A2/Hong Kong/8/68	.. 11.0	1:6	15.0	1:7	23.0	1:24
B/Lee/40	.. 85.0	1:127	14.0	1:10	2.0	1:1
A/Equi-2/Miami/63		..	..	..	..	..
Total No. of samples	.. 28	..	65	..	55	..
						

* Sera having titres $\geq 1:20$ were considered positive.

* Indicate a titre < 10

A. Mean titre = Arithmetic mean titre

TABLE 3
Incidence of HI antibodies in adults

Influenza strain	Period of collection									
	Pre-pandemic			Intra-pandemic			Post-pandemic			
	1964-65			Pre-October 1968			October 1968-69			
	Per cent positive	A. mean titre	Per cent positive	Per cent positive	A. mean titre	A. mean titre	Per cent positive	A. mean titre	Per cent Positive	A. mean titre
Swine/15/30	..	13.3*	10.4	1:1	1:17	1:17	51.4	1:32	10.4	1:8
A/PR/8/34	..	6.7	12.5	1:10	24.3	1:17	39.2	1:34	66.0	1:27
A1/FM/1/47	..	64.0	64.5	1:32	83.7	1:74	93.8	1:158	76.0	1:40
A2/Singapore/1/57	..	18.6	20.8	1:5	49.6	1:31	70.3a	1:102	74.4	1:24
A2/England/12/64	..	62.6	85.4	1:108	91.4	1:296	4.6	1:24	192	
A2/Tokyo/3/67	..	49.0	60.4	1:62	79.8	1:38				
A2/Hong Kong/8/68	..	28.0	22.9	1:11	33.3	1:52				
B/Les/40	..	52.0**	85.4	1:150	56.5	1:36				
A/Eq-2/Miami/63	..	..	..	..	..	..				
Total No. of samples	..	75	48		129					

A. mean titre=Arithmetic mean titre.

* Sera having titre $\geq 1:20$ were considered positive.

a Sera having titres $\geq 1:40$ were considered positive.

** Indicates titres ≥ 10 .

TABLE 4

Age distribution of HI antibodies to A/Swine/15/30 and equine strain A/Equi-2/Miami/63 in the oldest group of sera collected during the 1970-72 period

Age group (years)	No. of samples	Strain			
		A/Swine/15/30		A/Equi-2/Miami/63	
		Per cent positive*	A. mean titre	Per cent positive	A. mean titre
61-65	4	100	1 : 40	25	1 : 10
66-75	5	100	1 : 128	40	1 : 8
76-80	5	80	1 : 22	40	1 : 8
81-85	3	100	1 : 33	100	1 : 46
86-90	3	66	1 : 30	66	1 : 5
99	1	100	1 : 20	100	1 : 40

*Titres $\geq 1 : 20$ were considered positive.

*Titres are reciprocals of dilutions used.

A. mean titre = Arithmetic mean titre.

The second equine strain A/Equi-1/Prague/56 was also tested, but no antibodies were seen.

TABLE 5

Incidence of HI antibodies in commercial preparations of gamma globulins

Strains	Period of collection			
	1964-September 1968		1971	
	Per cent positive*	A. mean titre	Per cent positive	A. mean titre
A/Swine/15/30	93.0	1 : 45	57.14	1 : 15
Ao /PR/8/34	53.2	1 : 17	0	1 : 10
A1 /FM/1/47	100.0	1 : 149	92.8	1 : 55
A2 /Singapore/1/57	93.5	1 : 23	100.0	1 : 93
A2 /England/12/64	100.0	1 : 336	100.0	1 : 320
A2 /Tokyo/3/67	87.2	1 : 70	100.0	1 : 70
A2 /Hong Kong/8/68	13.7	1 : 11	92.8	1 : 220
B/Lee/40	98.0	1 : 95	100.0	1 : 49
A/Equi-2/Miami/63				
Total No. of samples	15		14	

*Titres $\geq 1 : 20$ were considered positive.

A. mean titre = Arithmetic mean titre.

TABLE 6

Location, number and age distribution of children serologically assessed for measles

Serial No. of study	Place	Year	Category (See text)	Number of children	Age (years)			
					½ to 1	1 to 3	3 to 5	above 5
1	Bombay	.. 1967	A	200*	50	50	50	50
2	Bombay	.. 1969	B	56**	17	27	12	..
3	Aurangabad	.. 1969	A	79**	31	42	6	..
4	Aurangabad	.. 1970	A	61†	11	32	18	..
5	Indore	.. 1969	A	348**	140	176	20	12
6	Indore	.. 1971	B	106	15	66	25	..
7	Poona	.. 1970	B	40		1	11	28
Total ..				890	264	394	142	90

Reproduced by permission

*Form Mehta *et al* (1972) Indian Journal of Medical Research 60 : 661 1669.

**Pre-vaccination serum samples collected during measles vaccine trial conducted by Indian Council of Medical Research.

†From Shah *et al* (1972) Bull : Wld. Heth. Org. 46 : 130-138.

TABLE 7

Correlation of history with presence of measles antibodies

Serial No. of study		Number of children serologically assessed	History corresponded with serum antibody results		History did not correspond with serum antibody results	
			Number	Per-centage	Number	Per-centage
1	A	200	197	98.5	3	1.5
2	B	56	52	92.9	4	7.1
3	A	79	79	100.0	..	..
4	A	61	59	96.7	2	3.3
5	A	348	348	100.0	..	..
6	B	106	89	84.0	17	16.0
7	B	40	40	100.0	..	..
Total (A only) ..		688	684	99.4	4	0.6
Total ..		890	865	97.2	25	2.8

TABLE 8

Measles antibody titre in sera and C.S.F. from SSPE patients

Case No.	Sex	Age (years)	History of measles (age)	Interval between onset of measles and onset of neurological symptoms	Duration of neurological symptoms	Anti body titre*		
						HAI	CF	N
58 1	M	11	—	—	1 ½ years	40	< 4	
58 a 2			—	—		< 5	< 4	
59 a	M	6	—	—	2 weeks	< 5	< 4	
60	F	10	No history	—	7 months	640	4,096	
60 a						5	64	
61	M	9	No history	—	15 days	160	1,024	256
62	M	10	4 years	6 years	1 month	1,280	4,096	2,048
62 a						20	16	32
62 a 3						640	4,096	1,024

1 = Serum sample tested.

2 = C.S.F. sample tested

3 = Second sample of serum tested.

4 = Not known.

* Titres expressed as reciprocals of dilutions used.

TABLE 9

EMC antibodies in human population

Serial No.	Age groups years				No. of sera tested	No. of sera + ve
1	Up to 10	..	..	..	11	2
2	11- 20	..	..	..	14	2
3	21- 30	..	..	..	12	0
4	31- 40	..	..	..	11	1
5	41- 50	..	..	..	4	0
6	51- 60	..	..	..	3	0
Total					55	5 (9%)

DEPARTMENT OF ZOONOSIS

Plague epidemiological studies

The studies were continued in 'D' and 'E' ward areas of Greater Bombay. As in other areas of Greater Bombay in these wards *R. rattus*, *R. norvegicus* and *B. bengalensis* together constituted the predominant commensal rodent fauna.

In all 2,434 rats from 'D' ward area and 1,052 rats from 'E' ward area were examined. *X. cheopis* showed its predominance over the other species of fleas recovered, viz *Xastia*. From 'D' ward, 254 fleas and from 'E' ward 179 fleas were examined. Except these two species of fleas, no other species of fleas were recovered.

Laboratory evaluation of anti-coagulant rodenticides

Laboratory evaluation of Drat—an anti-coagulant-based rodenticide was undertaken. The compound was evaluated against *R. rattus*, *R. norvegicus* and *B. bengalensis*. The compound showed very good efficacy as a rodenticide against all the three species of rats.

Serological studies

Erythrocytes, sensitized with Fraction I antigen of *Yersinia pestis*, were received from the Ministry of Health of the U. S. S. R., Middle Asian Research Institute on Plague Control, Zavodakaya, 14, Alma-ata-74, for plague serology. About 50 rodent sera from 'D' and 'E' ward areas of Greater Bombay have so far been tested by haemagglutination test for presence of anti-bodies against F-1 antigen. All the test sera showed negative results.

Susceptibility of rats to anti-coagulant rodenticides

Assessment of susceptibility status of rats from 'D' and 'E' ward areas to anti-coagulant rodenticide, viz. Warfarin, by using W. H. O. method was continued. The work carried out so far indicated the presence of tolerance to Warfarin among *B. bengalensis* and *R. rattus*.

Susceptibility of fleas to insecticides

Fleas collected from 'D' and 'E' ward areas were reared under laboratory conditions to assess their susceptibility to insecticides. Susceptibility status of dog flea *Ctenocephalides canis* to D. D. T. dieldrin and malathion was assessed. These fleas were found to be highly susceptible to all the three insecticides. The fleas were recovered from stray dogs from Mahalaxmi dog kennel.

Studies in insecticide resistance and vector control

Evaluation of susceptibility status of fleas, as reported earlier, viz. mosquitoes, *Culex fatigans*, house flies, *Musca d. nebulosa* to various insecticides was undertaken by using W. H. O. techniques. Susceptibility status of house

fly. *Musca domestica nebulosa* from different localities of Greater Bombay was assessed to DDT, Dieldrin, Malathion, OMS. 33 and OMS 43. The results obtained so far indicated high degree of susceptibility among flies to all the above insecticides tested.

Susceptibility status of *Culex p. fatigans* from 'R' ward area, where filariasis incidence is high was assessed in relation to D. D. T., dieldrin and Malathion. The studies carried out so far showed high degree of resistance to D. D. T. and Malathion. The mosquitoes were found to be susceptible to Dieldrin.

Studies were initiated to investigate the pathogenic organism harboured by mosquitoes, namely *Culex p. fatigans*. The role of this particular mosquito species in transmission of these pathogens to man and pathogenicity of these organisms to the mosquito and possibility of exploring the pathogenicity of these organisms for mosquito control will be studied after isolation of these organisms. So far, 100 mosquitoes have been screened. From these 26 strains were isolated, viz. Proteus species *Staphylococcus albus*, *Alkaligenes faecalis*, *Staphylococcus* and *Serratia marcescens*. The others were found to be contaminated with spores and yeast etc.

The studies are in preliminary stages.

Studies in filariasis

Studies in filariasis were undertaken with following objects in view :—

(1) Epidemiology (2) Serology, (3) Laboratory studies relating to transmission studies, (4) Studies in Animal filariasis.

(a) *Epidemiology*.—Epidemiological studies were carried out in Bhayandar area of Thana District. These studies were carried out in four villages, viz. Murdhe Rai, Morva and Dongri, from 27th March to 31st March 1973. These four villages together constitute a population of 5,000. One thousand four hundred and forty persons were screened for the presence of micro-filaria by night blood smear examination. The examination of smears collected is underway. About ninety-six micro-filaria carriers were detected. *Culex P. fatigans* was found to be the prevalent vector species. Mosquito density per man-hour was observed to be 13.24, 6.10, 7.5 and 18.8 at Murdhe, Rai, Morva and Dongri respectively. Infectivity rate ranged from 0.0 at Morva to 7.7 per cent at Rai, where mosquito density per man-hour was also found to be the highest.

(b) *Serology*.—From Thana area, skin tests were undertaken with the FST antigen supplied by the W. H. O. The findings are summarised below :

Thirty-nine disease positive cases were examined, out of which 35 showed positive reaction. Out of 208 micro-filaria positive cases studied, only 139 gave positive reaction. Three-hundred and thirty-nine contacts were examined, out of which 141 showed positive reaction.

An attempt was made to use haemagglutination test in the diagnosis of filarial infection. Haemagglutination test was positive (titre 1.32) in 79 cases out of 117 m.f. positive cases and it was positive in 11 out of 12 disease positive cases. Examination of serum samples from non-endemic area, viz. Parbhani, showed 27.9 per cent samples to be positive.

(c) *Laboratory studies*.—A laboratory colony of *L. carinii* was established to conduct filaria transmission studies, serological studies and drug evaluation.

(d) *Animal filariasis*.—Studies in animal filariasis were undertaken to determine incidence of different filarial infections in different animals. So far, 500 dogs, 119 horses, 6 jungle crows, 6 house crows, 41 sheep, 6 monkeys, 6 fowls, 43 cats, and 80 rats have been examined. Filarial infection was observed only in dogs, horses and crows. The species recorded were *Dirofilaria repens*, *Setaria sp* and *Chandlerella sp*.

Cholera carrier studies.—A cholera carrier study unit was established in the Institute in June 1972 to conduct "Cholera carrier studies" in Parbhani town of Marathwada region. This study was conducted in Khadrabad Plot area of Parbhani town, because every epidemic of cholera in Parbhani town, in the past, had its beginning from the Khadrabad plot area. The main object of the study is to trace and mark carriers (convalescent as well as healthy) in the population during epidemic and inter-epidemic periods, thereby pin-pointing the possible sources of the persistence and spread of the disease. The studies also include testing of the water and evaluation of animal sources in the vicinity of the study area to find out the factors responsible for the maintenance and spread of cholera infection.

So far 3000 human stool samples 200 animal pools and 30 water samples have been examined.

Fifty-three strains of *V. cholerae* bio-type *El-tor* Ogawa have been isolated from human samples. Five convalescent and 8 contact carries have been detected. The cases and carriers are being followed up to study the pattern of status.

Studies will be continued to detect more and persistent human carriers. Role of water and animals in the spread of the disease will be evaluated. Serological status of the population will be assessed by studying different anti-bodies against *V. cholerae* in the serum samples.

Studies in Leptospirosis

Leptospiral strains were grown in this laboratory in synthetic medium. The medium consisted of mineral salt and vitamin solutions. One non-pathogenic strain and three pathogenic strains were able to grow in this medium. Further work is being done in this field. A slide antigen, as described by Galton et al (1968), was prepared in the laboratory. This is very useful in quick screening of the sera. Four-hundred and fifty-four

sera were tested by this new antigen and the test was compared with our standard microscopic agglutination test. The antigen is found to be quite sensitive.

A type specific TM antigen is being extracted from *Leptospiral* cells. This extract will help explore the complexity of the antigenicity of leptospiral organism.

Studies in amoebiasis

Swiss strain of *E. histolytica*, obtained from Ciba, was maintained in the diphasic medium. Antigen was prepared both for the haemagglutination test and the complement fixation test. Trials of both the tests were carried out. Minimum diagnostic titre was taken as 1,32 for haemagglutination test. About 140 sera were tested with the above antigen. Anti-human gamma globulin conjugate was prepared. Preparations for the axenic culture of *E. histolytica* are being made.

Studies in Dracunculosis

Epidemiological studies in Dracunculosis were initiated in Nagothne area of Kolaba district on 21st March 1973. The studies are presently in progress at the village Tisse of Roha taluka of the Kolaba District.

PART III
ROUTINE DEPARTMENTAL ACTIVITIES

DEPARTMENT OF BACTERIOLOGY

During the year under review, the department continued its usual activities of production and supply of Cholera-El-Tor, T. A. B. and Plague Vaccine. Due to short rain fall and the resulting a drought and scarcity conditions prevailing in many parts of the country in general and in the Maharashtra State in particular, heavy demands for Cholera Vaccine were anticipated during the ensuing year. The production of Cholera Vaccine was, therefore, maintained at a high level during the year under review, itself, to meet the emergency.

Heavy demands for cholera vaccine usually start pouring in the month of June. However, they started much earlier, i.e. in the month of February 1973 and the department started supplying about 2 million doses of Cholera-El-Tor vaccine per month from February 1973, to the scarcity areas in Maharashtra.

The bacteriological examination of samples of water for potability, which was discontinued since long, was restarted in response to many enquiries from the city of Bombay and other places. Eight samples of water, one sample of wheat flour and two samples of soil manure were examined for viable counts during the year.

The following quantities of vaccines were produced and supplied during the year :

Product	Quantity produced (litres)	Quantity supplied (litres)
Cholera-El-Tor vaccine	11127-176	10978-833
T. A. B. vaccine, I. P.	284-697	242-481
Plague vaccine, I. P.	159-320	67-720

Shri R. S. Karbhari took over as Assistant Director of the department in June 1972 vice Shri M. E. Kulkarni transferred to the Department of Immunology as Assistant Director.

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

Dr. A. F. B. Standfast, the W. H. O. Expert on D. T. P. Vaccine, was specially invited to visit this department for three weeks from 15th December 1972 to 4th January 1973, to supervise and guide the production and testing of Pertussis Vaccine. His valuable suggestions have gone a long way in developing the production of Pertussis Vaccine, which was started on a regular basis since January 1973.

The sections of the Department of Immunology, concerned with the production of diphtheria and tetanus toxins and toxoids, were brought under the administrative control of the Department of Bacteriology with effect from 15th February, 1973.

MEDIA SECTION

The Media Section continued the manufactures 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.

During the year under review, two new products, family planning programme and peritoneal dialysis fluid for viz 20 per cent sodium Chloride required for the treatment in cases of kidney dysfunction were taken up for manufacture.

In addition to the above, the Section provided general facilities for sterilization, cleaning of glassware etc.

The Ampoule Cutting Section supplied out and sterilized ampoules to the Departments of Bacteriology, Immunology, Antirabic Section and other production departments. The production figures of the different items are presented below : —

Culture Media

Product	Quantity (Litres)
Casein hydrolysate medium (Cholera) ...	41,575-800
Meat-digest Agar (Typhoid) ...	247-000
Aerobic medium ...	7,634-880
Anaerobic medium ...	7,902-770
Sabouraud medium ...	5,172-480

Parenteral Solutions

Product	Quantity (Litres)
Dextrose injection, 5 per cent w/v, I. P.	52,954-000
Dextrose and sodium chloride injections, U. S. P.	47,526-000
Sodium chloride injection, I. P.	40,987-000
Water for injection, I. P.	9,528-000
Dextrose injection, 25 per cent w/v, I. P.	94-000
Sodium lactate, B. P.	10,300-000
A. C. D. solution 'A', U. S. P.	362-000
Sodium chloride injection, 20 per cent.	342-000
Peritoneal dialysis solution 'A'	60-000
Peritoneal dialysis solution 'B'	5-000

The other various media and stains and solutions, which were prepared during the year, are given below :—

Product	Quantity (Litres)
Acid-digest of meat agar	641.400
Nutrient agar	1038.500
Maconkey agar	302.000
Sabouraud agar	63.500
D. E. C. Agar	21.000
Löffler's medium	
2235 test tubes X	10 ml.
Lowenstein Jensen's medium	
920 test tubes	10 ml.
Nutrient broth	168.000
Glucose broth	43.000
Peptone water	324.300
Glucose phosphate medium	13.500
Koser's citrate medium	2.500
Lab-Lemco broth	16.500
Thioglycolate medium	36.000
Ox-bile medium	26.800
Heartley's broth	7.200
Christensen's medium	2.000
Bordet Gengou medium	6.000
Macconkey broth	21.000
Sabouraud broth	45.000
Alkaline peptone water	61.000
Selenite broth	10.700
Muller and Hinton medium	6.000
Hoyle's base	3.500
Buffer saline	24,327.000
Normal saline	11,190.600
Litmus milk	2.250
2 per cent Citrate saline	14.000
T. C. B. S. agar.	6.000
Standardstaphylococcal broth	8.000
Gelatinised buffer saline	56.000
Glucose	30.700
Lactose	30.450
Mannitol	28.480
Sucrose	30.050
Dulcitol	10.000
Xylose	1.400
Maltose	3.100
Mannose	1.950
Arabinose	3.250

Diagnostic Reagents

Product	Quantity (Litres)	
Ziehl Neelsen carbol fuchsin	...	158·000
Albert stain No. 1	...	15·000
Albert No. 2	...	8·000
Benedict's solution	...	2,029·000
Safranine	...	24·000
Methylene blue	...	126·000
J. S. B. stain No. 1	...	30·000
J. S. B. stain No. 2	...	18·000
Gentain violet	...	28·000
Preservative fluid for W. B. C.	...	22·000
Preservative fluid for R. B. C.	...	27·000
Gram stain No. 1	...	16·000
Gram stain No. 2	...	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 Employees' State Insurance Scheme and to Podar Ayurvedic Hospitals. Increasing number of the staff members of the Institute availed of this facility. There were more demands for special tests like Serum Protein bound Iodine, Serum Transaminases and Urinary 17-keto steroids. A total of 4,879 tests were carried out during the year as shown in Table 1 appended herewith.

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.) Shanta M. Modak continued to be on study leave.

Shri J. K. Kamath was appointed as Junior Scientific Officer. Kum. T. R. Kamala and Kum. K. S. Koregaokar were taken up as fresh Senior Technical Assistants in the department.

Academic.—Smt. Lakshmi Jairam was awarded M.Sc. degree of the Bombay University on the basis of the thesis "The Role of Haptoglobins in Liver Function under clinical and experimental conditions", Shri V. D. Rane submitted his thesis for the M. Sc. degree of the Bombay University.

Kum. Ranjana Buit submitted her synopsis "Biochemical Studies on the action of Furazolidone on *Vibrio cholerae*" for the Ph.D. degree of the Bombay University.

Shri Syed Raziuddin, Junior Research Fellow, was registered for the Ph.D. degree under Dr. S. D. Ambegaokar.

Shri P. M. Nayak and Shri Chhatwal from the Immunology Department were registered under Dr. A. Venkataraman after the retirement of Dr. S. S. Rao as research guide.

TABLE 1

Showing the tests carried out during the year 1972-1973.

Name of the test	No. of tests
<i>Blood-Serum—</i>	
Aldehyde Test	1
Antimony Test	1
Icteric Index	72
Vanden Bergh	87
Amylase	3
Bilirubin	365
Calcium	5
Chlorides	23
Creatinine	2

Name of the test				No. of tests
Cholesterol	...	...	...	101
Phosphorus	...	...	...	3
Non-protein Nitrogen	...	...	...	8
Acid phosphatase	...	...	...	2
Alkaline phosphatase	...	...	...	200
Proteins, Total	...	...	...	309
Proteins, Albumin	...	...	...	277
Proteins, Globulins	...	...	...	277
Blood Sugar	...	...	...	1152
Thymol Turbidity	...	...	...	264
Urea Nitrogen	...	...	...	107
Uric Acid	...	...	...	20
Fibrinogen	...	...	...	1
Blood Ketone (acetone)	...	...	...	1
Total				3281

Urine and Stool—

Bile Salts and Pigments	...	...	...	142
Acetone bodies	...	...	...	1
Calcium	...	...	...	5
Chlorides	...	...	...	4
Phosphorus	...	...	...	9
Porphyrins	...	...	...	1
Sugar	...	...	...	120
Occult blood	...	...	...	3
Urobilinogen	...	...	...	1
Albumin	...	...	...	8
Total				294

C. S. F. and other Body Fluids—

C. S. F. Chlorides	...	...	...	3
C. S. F. Proteins	...	...	...	10
C. S. F. Sugar	...	...	...	3
S. M. Test	...	...	...	8
Total				24

Name of the test	No. of tests
<i>Special Tests—</i>	
Amino-acids (urine)	3
Gastric analysis	2
Glucose Tolerance Tests	48
Iron (Serum)	8
Lange's Test (C. S. F.)	28
Potassium (Serum)	32
Protein-bound Iodine (Serum)	38
Proteins by paper electrophoresis (Serum)	18
Sodium (Serum)	38
17-Ketosteroids (Urine)	28
S. G. O. T.	470
S. G. P. T.	474
Urinary stone	8
Vinyl mandelic Acid (Urine)	1
Total ...	1196
<i>Special food analysis :</i>	
Sukhadi (Horse-gram, Groundnut cakes and HNC Nutro-proteins) for—	
Calories	13
Carbohydrates	13
Fat	13
Minerals	13
Moisture	13
Proteins	13
Nutro-proteins (HNC)	6
Total ...	84
Blood	3281
Urine and Stool	294
C. S. F. and other Body Fluids	24
Special Clinical Tests	1196
Special food analysis	84
Grand Total ...	4879

DEPARTMENT OF CHEMOTHERAPY

The synthesis and study of newer compounds for their possible use against tuberculosis, leprosy, protozoal infections like amoebiasis, filariasis, helminthic infestation and neoplastic diseases were continued during the year under review. Over 300 new compounds were synthesized, characterized and representative compounds from each of these series were subjected to preliminary screening, except for the antileprosy, antifilarial and anthelmintic tests, which are being standardized.

Two compounds reported earlier, viz. R-2870 and R-2617 (ketonic analogues of tibrone), which had exhibited in *vitro* antitubercular action of much higher order than tibione, were studied for serum tuberculostatic activity after oral feeding and compared with tibione, because facilities for in *vivo* testing for antitubercular action by infection of the animals are not yet developed. Although, both these compounds were much better, tolerated in mice and guinea-pigs than tibione, they did not reach the adequate tuberculostatic levels in the sera of experimental animals.

In view of the non-availability of Diacetyl diaminodiphenylsulphone-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 this product. The method was worked out and sufficient quantity was prepared and got converted into the depot injectible. This new product has passed all the tests and will be taken up for controlled clinical trials soon. Also, another powerful antihelmintic drug, Tetramisole, was taken up for working out the details of manufacture by a comparative study of the different possible routes.

Two compounds, R-1230 (a piperazine derivative showing potent hypotensive activity) and R-585 (a triazole derivative having long-acting potent hypoglycaemic activity, both in normal and alloxan diabetic animals), are still undergoing detailed toxicological studies in the Pharmacology Department of this Institute.

During the year, 78 new compounds were sent to Cancer Chemotherapy National Service Centre (CCNSC), Bethesda, U. S. A., for screening anti-tumor activity. One hundred and twenty reports were received from CCNSC on the screening of compounds. These included the reports on compounds sent in the previous year also. A few of the compounds have shown high order of activity with low toxicity.

During the year under review, Sarvashri D. T. Chaudhari, M.Sc., K. Y. Mahajan, M.Sc., the Government of India Scholars and Shri B. G. Khadse, M.Sc., University Grants Commission Research Fellow, continued to work on their research problems for Ph.D. degree. Shri S. V. Kekatpurey and Shri P. P. Dalvi, joined the department as Junior Research Fellow. In all, nine students worked for their post-graduate degrees in the department. During the year, Shri D. R. Shekawat was awarded the Ph.D. degree and Kum. B. K. Kapadia obtained her M.Sc. degree.

The Micro analytical Section of the department carried out elemental microanalyses of all the new compounds synthesised.

The Antimicrobial screening unit continued to screen the compounds synthesised by the department, against various pathogenic organisms.

Three research papers were published during the year.

A new Pilot Plant Committee was appointed to look into the possibility of utilising 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, Nicotinic acid, and Nicotinamide were completed and the manufacture of Nicotinamide was taken up on pilot plant during the period under review.

During the year the department continued to manufacture and supply the major requirements of Government hospitals, Employees' State Insurance Scheme and public health bodies. The list of items and their output are given in the following table :—

Name of the Product			No. of tablets manufactured
Vitamin A. D. (P)	...	...	79,21,800
Vitamin A. D.	...	...	13,90,900
Vitamin B. C.	...	...	1,84,91,500
Vitamin B1	...	...	11,60,900
Vitamin B2	...	...	1,94,000
Vitamin B2 (mild)	...	...	1,92,822
Vitamin C	...	...	1,00,15,000
Vitamin C (strong)	...	...	23,63,392
Nicotinic acid	...	...	6,86,860
Vitamin BC3	...	...	9,43,000
Poly Vitamin	...	...	1,04,58,600
Vitamin B-Complex	...	...	96,08,300
Vitamin K	...	...	82,300
Iodochlorohydroxy quinoline	...	...	17,05,100
Di-Iodo-hydroxyquinoline	...	...	23,39,600
Calcium with Vitamin D	...	...	24,60,700
INH	...	...	71,57,000
Thiacetazone, INH	...	...	23,87,900
Sulphaguanidine	...	...	9,97,000
Sulphadimidine	...	...	70,78,000
Phthalyl sulphathiazole	...	...	30,75,200
Aspirin	...	...	1,81,18,750
Na-P. A. S. (Sodium para amino salicylate)	...	...	55,89,300
Tolbutamide	...	...	13,15,500
Chlorpropamide (100 mg.)	...	...	85,600
Chlorpropamide (200 mg.)	...	...	1,03,700
Berberine Hydrochloride	...	...	5,87,100
Total			11,65,09,224
Pemon	...	...	35,951 litres
Tolbutamide	...	...	81 kg.
Nicotinamide	...	...	70 kgs.

DEPARTMENT OF CLINICAL PATHOLOGY

Dr. N. B. Nadkarni, Medical Officer, Class III, Typhoid Unit, was transferred as Medical Officer M. M. S. Class, II, to the Blood Bank Department on 30th June 1972.

Dr. (Smt.) V. P. Purohit, was appointed as Medical Officer, M. M. S. Class III, Typhoid Unit, on 20th November 1972.

Shri K. N. Joishy, the Government of India Research Scholar, left the department on 28th February 1972.

Shri Taranath L. Shetty joined the department as a Junior Research Fellow under the Diamond Jubilee Scheme on December 9, 1972.

Two Senior Technical Assistants and three Laboratory Attendants were transferred to the Department of Zoonosis in April 1972 and the Epidemiology Unit was completely amalgamated with the Zoonosis Unit.

Dr. (Smt.) J. K. Dave participated in a symposium on "Mycobacterium leprae" at the acworth Leprosy Hospital in February 1973. She spoke on "The Genus Mycobacterium".

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. Histopathological study of the tissues of all experimental animals, received from post-graduates of various departments of the Institute, was also undertaken. The tissues referred by the Pharmacology Department of this Institute were studied for drug toxicity reactions.

Twelve students were admitted for the D. M. L. T. Course and out of these, eleven continued the course. Eighteen short-term trainees completed the training course in Clinical Pathology.

With the help of an I. C. M. R. Scheme, laboratory investigations were undertaken in this department on the material collected from Parbhani district, which has been selected as an endemic focus of Cholera in the State of Maharashtra. Smt. S. Kumar was appointed from June 1972 as Assistant Research Officer in this scheme.

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

	Total
A. <i>Histo-Pathology</i>	
Histo-pathological examinations	6022
B. <i>Haematology</i>	
Haemoglobin estimation	755
Total R. B. C. Count	724
Total W. B. C. Count	792
Differential W. B. C. Count	793
P. C. V. Estimation	3
E. S. R. Estimation	141

Prothrombin Time	...	...	1
Platelet count	...	...	1
Bleeding Time	...	...	2
Clotting Time	...	...	2
<i>C. Clinical Microscopy</i>			
Urine for routine examination	...	...	488
Stool for routine examination	...	...	238
Semen examinations	...	...	15
C. S. F. for Cytological examination	...	...	14
Other puncture fluids for cytological examination.			3
Pregnancy Tests (Qualitative)	...	...	1,775
Pregnancy Tests (Quantitative)	...	...	242
<i>A. Bacteriology</i>			
Blood for cultural examination	...	...	31
Blood cultures for enteric organisms	...	...	604
Urine for cultural examination	...	...	189
Urine for enteric organisms	...	...	5
Stools for cultural examination	...	...	3
Stool cultures for enteric organisms	...	...	104
Throat swabs for cultural examination	...	...	12
Antibiotic Sensitivity Tests	...	...	2,828
Autovaccines	...	...	64
Cultures of specimens for Tuberculosis	...	...	71
Specimens for guinea-pig inoculation for T. B.	...	...	36
Smears for A. F. B.	...	...	91
Cultures for Diphtheria	...	...	210
Material for Tetanus. cultural examinations.	...	...	187
Stools for cholera culture examinations.	...	...	54
Smear examinations for Gonorrhoea	...	...	375
Cultures for genorrhoea	...	...	375
Miscellaneous cultural examinations	...	...	163
<i>B. Serology</i>			
Widal Tests	...	...	595
Weil-Felix Tests	...	...	595
Tests for Brucellosis	...	...	595
Vi Agglutination Tests	...	...	1
Paul Bunnell Tests	...	...	13
V. D. R. L. Tests	...	...	394
Kahn Tests	...	...	96
Wassermann Tests	...	...	19
Casoni's (Skin) Tests	...	...	6

Total investigations ... 19,727

Supply of diagnostic reagents during the year was as follows :—

			Supply (Ml.)
Dead emulsions	...	...	4,11,305
High-titre sera	...	...	7,174
Kahn antigen	...	...	10,390
W. R. antigen	...	...	1,570
Casoni's antigen	...	...	573
Cobra venom solution	...	...	730
R. V. venom solution	...	...	487
<i>E. coli</i> sera	...	...	752
<i>Vi typhi</i> sera	...	...	33
<i>vi</i> antigen	...	...	620
Biological Stains	...	..	18,02,150
Total			22,35,784

DEPARTMENT OF IMMUNOLOGY

During the year under review, a number of changes in the staff and activities of the department took place. The production activities increased due to heavy demands for the products.

The department produced and supplied various anti-sera and toxoids. There was an upward trend in the supply of anti-sera, plain toxoid and adsorbed toxoids.

Staff

Dr. S. S. Rao, the Assistant Director and Shri H. K. Mhatre, Senior Scientific Officer, Class II retired voluntarily, Shri M. E. Kulkarni was appointed as Assistant Director from 1st June 1972. Shri R. C. Kankonkar, Junior Scientific Officer was appointed as Senior Scientific Officer, Class II, from 1st August 1972. Smt. K. S. Gadre was appointed as Junior Scientific Officer from 8th August 1972. Kum. S. T. Deshapande joined the department as Senior technical Assistant from 3rd October 1972, Dr. D. P. Buduk, Senior Veterinary Surgeon, Bombay was appointed as Senior Scientific Officer, Class I (Veterinary) at the Pimpri Stud Farm from 3rd May 1972.

Dr. A. F. B. Standfast, W. H. O. Expert on Biological Standardisation, worked in this department from 14th December 1972 to 6th January 1973. He had a number of discussions with the staff members in connection with the manufacture of Triple Antigen.

The activities of this department were bifurcated during the year from 15th February 1973. All units concerned with the production of bacterial vaccines were attached to the Department of Bacteriology. In consequence, all the staff of Diphtheria and Tetanus Toxin Sections and Toxoid Production Section, responsible for producing toxins and toxoids for production of anti-sera, were transferred to the Department of Bacteriology. The staff producing various components of Triple Antigen, such as Tetanus Toxoid, Diphtheria Toxoid and Pertussis Vaccine as also the staff responsible for production of mixed vaccines such as adsorbed Diphtheria Toxoid, Diphtheria-Tetanus Vaccine and Triple Antigen was transferred to the Department of Bacteriology.

The construction of two new stables at the Pimpri Stud Farm has not yet been completed and is expected to be completed by the end of the next year (1973-74). The dispensary at the Pimpri Farm is being renovated. Additional space by enclosing a portion of the verandah has been created for use as a laboratory. Strength of horses was increased to about 305 at Pimpri and 100 at Bombay, totalling to 405 horses during the year.

Production of Gas Gangrene Antitoxin was restarted as a result of demands received from public hospitals.

Heavy demands poured in for Tetanus Toxoid (Plain) and Tetanus Toxoid (adsorbed) due to scarcity conditions prevailing in the State. Moreover, orders from the Directorate of Health Services also increased tremendously.

In addition, large amounts were required for Bangla Desh and west Bengal.

The local orders for toxoids and anti-sera also increased during the period. Heavy orders were received from the Family Planning Scheme units.

The department was geared up to meet these excess demands and immediate steps were taken to plan increased production.

The following are some of the highlights of research work carried out during the year.

Shri S. V. Gadre completed his work for Ph.D. degree on "Role of phage and Iron in toxin production by *C. Diphtheriae*". Shri P. M. Naik, carried out work on "Role of Adjuvants, Steroids, gonadectomy on Immune Response". Dr. D. P. Buduk will be completing his work on plasma pheresis in horses for his Ph.D. degree. Smt. Sandhya Sathe carried out work in production of diphtheria toxin in casein hydrolysate medium. Shri Vitkar completed his work for M.Sc. degree on "Adjuvants and Immuno-suppressors". Work is also being carried out on snake venoms, production of Tetanus Toxin in peptic digest of meat medium, adjuvants for diphtheria toxoid, production of L and Q toxins from *Cl. perfringens*, production of tetanus toxin in tryptone medium and efficacy of drugs in tetanus and diphtheria.

Production

Name of product			Quantity (Litres)
<i>Tetanus Toxin</i>			
Peptic-digest medium	...	...	834
Protein-free medium	...	...	1948
<i>Tetanus Toxoid</i>			
Peptic-digest medium	...	...	346
Protein-free medium	...	...	1787
<i>Diphtheria Toxin</i>			
Papain-digest medium	...	...	336
Protein-free medium	...	...	927
<i>Diphtheria Toxoid</i>			
Papain-digest medium	...	...	155
Protein-free medium	...	...	927
Adsorbed Tetanus Toxoid	...	...	555
Adsorbed Diphtheria Toxoid	...	...	30
Diphtheria-Tetanus Toxoid	...	...	157
Antivein	...	...	421.5
Tetanus anti-toxin	...	...	1719 M. U.
Diphaheria anti-toxin	...	...	424 M. U.

DEPARTMENT OF IMMUNO-HAEMATOLOGY

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

After the cessation of hostilities in December 1971, the in-flow of blood from all sources suddenly showed a steep fall, as was experienced on earlier occasions. Intensive efforts had to be made to keep up the voluntary blood donation programme.

However, the load of work pertaining to processing of blood collected during the last national emergency, continued to be heavy.

Number of institutions/colleges and factories etc. contacted, 54

Number of institutions/colleges and factories etc. where blood collection sessions were held. 39

Number of voluntary donors enrolled ... 4,011

Blood donors.

Voluntary ... 3,111

Paid ... 3,510

Prisoners ... 2,331

Hospitals ... 4,073

13,025

Blood plasma

Name of product	Packing (ml.)	Produced	Supplied
Dried plasma ..	250	5,927 bottles	2,128 bottles
Human gamma globulin ..	1	3,815 ampoules	3,798 ampoules
Human fibrinogen ..	150	269 bottles	268 bottles
Human fibrinogen ..	10	129 ampoules	129 ampoules
Cryoprecipitate ..	100	24 bottle	24 bottles
Human serum albumin ..		471 grammes	471 grammes
Packed cells ..	150	782 bottles	782 bottles
<i>Tests carried out</i>			
Rh tests ..	..		117
Paternity tests ..	..		3
Coomb's tests ..	..		10
Cross-matching tests ..	..		11
Samples for export ..	..		9

Blood grouping reagents

Name of product	Packing (ml.)	Produced	Supplied
Anti-A	1	11,730 ampoules	11,719 ampoules
Anti-A	5	4,092 "	4,152 "
Anti-B	1	11,645 "	11,703 "
Anti-B	5	3,916 "	3,991 "
Anti-A + B	1	785 "	598 "
Anti-A+B	5	284 "	183 "
Coomb's reagent	1	1,371 "	1,440 "
Anti-D	1	4,250 "	4,199 "
Bovine albumin	1	2,540 "	2,419 "
Bovine albumin (in powder form)		21.25 grammes	21.25 grammes
Anti-A (Lectin)	1	134 ampoules.	20 ampoules
Anti-A (Serum)	1	147 "	58 "
Anti-H (Lectin)	1	193 "	163 "
Number of anti-venine ampoules dried.		. .	68,522 ampoules

DEPARTMENT OF PHARMACOLOGY

During the year under review, the following members of the staff from the Biology Section were transferred to the Drugs Control Laboratory with effect from 31st March 1973.

Shri M. C. Deshpande.	Senior Scientific Officer, Class II.
Smt. L. C. Kashyap.	Junior Scientific Officer.
Shri P. D. Pilankar.	Senior Technical Assistant.
Shri V. M. Chavan.	Laboratory Assistant.
Shri M. B. Jaiswar.	Head Clerk.
Shri A. J. Jagrap.	Laboratory Attendant.
Shri J. D. Kamble.	Do.

As Shri B. B. Jadhav, Junior Technical Assistant, was not in a position to go to the Drug Control Laboratory, the post of Junior Technical Assistant held by him was transferred to Drug Control Laboratory and Shri Jadhav was transferred to Department of Chemotherapy on a similar post with effect from 31st March 1973.

Smt. Shakuntala Kumar, who was working in this department as a Unichem Research Fellow, was appointed to the post of Assistant Research Officer on an I. C. M. R. Enquiry in the Department of Clinical Pathology.

Shri R. S. Joshi was appointed as Unichem Research Fellow. Shri S. R. Chavan and Shri S. T. Nikam, Research Assistants in the C.C.R.I.M.H. Unit in the Department, were appointed as Senior Scientific Officer Class II and Junior Scientific Officer respectively, in the Department of Pharmacology.

Kum. S. E. Bhagwager, Unichem Research Fellow and Shri S. Natarajan Iyer, Senior Technical Assistant, resigned and left the department for better prospects elsewhere. Two private trainees were trained and given opportunity to acquaint themselves with the different techniques of analysis of drugs. The training programme in Drug Analysis was transferred to the Analytical and Control department. However candidates were continued to be trained in bioassays.

Shri H. V. Nerurkar, Junior Technical Assistant, was deputed for training in Control and Communication Service at the Manekji Technical Centre, Bombay, for ten days, under the Civil Defence Scheme.

The department continued testing of drugs under the Drugs and Cosmetics Act and samples of Toddy, Foreign Liquors etc. on behalf of Prohibition and Excise Directorate, under Prohibition Act. The total number of samples tested this year was 3,647 only. The Government Analysis Section, which was added to this department since the inception of the Drugs Act in Maharashtra, was finally transferred to Drugs Control Laboratory established under the Food and Drugs Administration.

Smt. G. V. Aroskar, Senior Technical Assistant and Shri P. D. Pilankar, Senior Technical Assistant, obtained M.Sc. degree in Biochemistry, Kum. U. S. Kushe, Senior Technical Assistant and Kum. N. D. Vaidya, Senior

Technical Assistant, have been registered for the M.Sc. degree in Applied Biology and Organic Chemistry respectively.

Shri S. S. Karkhanis has been promoted to the post of Junior Scientific Officer (Pharmacognosy) and Kum. S. R. Dhond was appointed as Senior Technical Assistant in the post falling vacant, Shri P. Y. Naik and Shri S. S. Mahajani were appointed as Assistant Research Officer and Research Assistant respectively in circuit No. 2 of the Central Council for Research in Indian Medicine and Homeopathy, located at this Instituted.

Government Analysts' Section

The Pharmacology Department was initially being financed by the Indian Research Fund Association. The then Government of Bombay took over the department in 1943. It was then financed mainly by military authorities. The main responsibility of the department was that of drug testing on behalf of the military authorities. After the 2nd World war. The Drug Act, 1940 was enforced. The expertise and facilities for drug testing were almost nonexistent in the country. So in a short period the Assistant Director in charge of the department and his senior colleague were recognised as Government Analyst by Customs, State of Bihar, Orissa, Bengal, C. P. and Berar, Delhi and Ajmer, Merwara, Assam, U. P. Patiala and E. Punjab, Corrg and Kerala. Hundreds of drug samples were analysed and reported. The work load at the department increased tremendously. The authorities decided to discontinue the work from other states as the activities of the state control increased and research in the department suffered. In fact the nucleus could have been possibly used for the development of a laboratory for Indian Pharmacopoea or a regional drug testing laboratory. Haffkine Institute Reorganisation Committee felt that the Institute was not a proper place for Government. Analyst activities. The Institute itself is a production unit, so it was recommended in 1965 that a new Laboratory be established under the Drug Control Authorities by transferring the expert staff from the pharmacology Section. The Laboratory was established in 1968 and the first one of Pharmaceutical Chemistry Section was transferred in August 1968 and Vitamins and Antibiotics section in March 1972. The remaining section that of biologicals was transferred on the last day of the financial year. It is generally agreed upon that the standards of the drugs produced in the state of Maharashtra are of high order. The Government Analyst section shared a big responsibility for maintaining these standards. The number of samples of from Food and Drugs Administration is now very small the number being 94 and consequently the number of samples remaining to be reported was 6. Facilities and help to organise the laboratory are still made available to the sections transferred to Drug Control Administration. The main investigational study taken up this year was on suture materials (except cat-gut). As many as 40 samples were analysed and reported upon. Quite a number of samples were found misbranded. One sample of heparin was found to contain 5 times the amount of active drug as compared to the labelled amount. The number of samples of insulin showing undue reactions still continue to be received. But none of them is shown to be sub-standard.

Prohibition and Excise Section

The department received 796 samples of Toddy for alcohol content and for detection of any adulteration. Three samples were found to be adulterated with cholera hydrate. The content of alcohol in toddy samples varied from 0.1 to 5 per cent. Four hundred and ninety samples of foreign liquors were received for the analysis of alcohol contents. None of the samples failed for the content of alcohol. The department also received 60 samples of Ganja, Bhang, etc. for report on their fitness for human consumption. All the samples were found unfit for human consumption when tested by physical and Chemical methods.

Food Samples

Food Corporation of India reported that imported milo was contaminated with Dhatura Seeds. Twelve samples of the milo grains and milo flour were sent to this Institute for detection of Dhatura alkaloids and for fitness for human consumption. Many of these were found to be contaminated with Dhatura alkaloids by chemical and biological tests.

Commercial Samples

The department continued to receive about 2,000 samples for testing, mainly according to Pharmacopoeia of other official books recommended for standardisation of drug cosmetics. The main feature of the year was that facilities for infrared spectroscopy and chromatographic procedures such as their layer, column, etc. were expanded.

Toxicology and clinical Pharmacology

The department tested and reported upon four samples of a drug formulation for sub-acute toxicity. It also studied samples of antibiotics in human volunteers for their bio-availability. The studies on antibiotics included chloramphenicol tetracycline, erythromycin, ampicillin, etc. One sample of frusemide was studied in human volunteers for its efficacy. It was shown that, in human volunteers, Isokin Syrup prepared by a manufacturer was as equally absorbed as the INH powder.

QUALITY CONTROL DEPARTMENT

The Department, during its rather short existence of less than a decade, was shifted to different premises in the Institute and it has now settled down in the space previously occupied by the Nutrition Department. The main functions are carried out at this site. The department has gradually expanded to a full-fledged department and stringent quality control of drugs is its hall-mark. Owing to limited laboratory space, unification of the varied functions of the department could not be brought about. However, the acquisition of an extra room in the animal house has partially eased the congestion in the departmental Animal Section.

The department has performed its varied duties of great responsibility of examining and certifying the finished products manufactured by the Institute for their quality, purity and strength, thus fulfilling the Drug Control obligations. The additional responsibility of analysing raw material that go into production of biologicals and non-biologicals and disinfectants was efficiently carried out. One thousand one hundred and twenty eight samples of raw materials with 10,000 tests were analysed during the period of the report compared to 800 during the last year, thereby indicating heavy increase in work-load of this department. A lot of intermediary products were tested and buffer saline and normal saline, etc. which are used in the manufacture of sera preparation and vaccines, were received for testing regularly.

Besides routine duties, the department participated in other scientific activities, viz.

- (1) International collaboration with State Serum Institute, Copenhagen, Denmark and other Laboratories, in the study on replacement of the present International Standard for Diphtheria Toxoid (Plain) was availed of. The study was completed on schedule and data submitted to State Serum Institute, Copenhagen.

- (2) Conducted relevant experiment under the guidance and supervision of Dr. A. F. B. Standfast, W. H. O. Consultant, for streamlining the testing of pertussis component of triple vaccine, Dr. Standfast certified that our testing technique was perfect. His stay with us was very enlightening.

- (3) A pertussis Cell was formed with workers from the department and Bacteriology department to carry out certain tests simultaneously to evolve satisfactory methods for the manufacture and standardisation of pertussis vaccine. The work has led to constructive contribution.

- (4) Accelerated temperature studies to investigate into the stability of Diphtheria and Tetanus Antitoxins at 4°C and 37°C at regular intervals were carried out for nine months. It was found that there was marked progressive deterioration at 37°C in both the cases.

- (5) Research on different problems was carried out by the staff of this department. One student was registered for M.Sc. in Microbiology, one in Organic Chemistry and two in Biochemistry.

(6) The department imparted technical training to post-graduate candidates sponsored by, the World Health Organisation, private pharmaceutical firms, M. L. T. Students etc.

Dr. F. L. Saldanha, Assistant Director of the department, was invited by the Chairman of Japanese Toxic Micro-organism Panel, Joint U. S.-Japan Corporation of Development and Utilisation of Natural Resources Japan, to participate in the First International Conference on *Vibrio parahaemolyticus*, to be held at Japan.

The University of Bombay recognised Dr. F. L. Saldanha as a post-graduate guide in Microbiology.

Two posts of Senior Scientific Officers, Class I, sanctioned by the Government about 2 years back, remained vacant during the year.

Smt. A. T. Darnule, Senior Technical Assistant, resigned and Shri P. P. Chaudhary joined the department in her place. Smt. K. D. Walavalkar was transferred to Clinical Pathology Department and Shri P. I. Parekh was appointed in that vacancy.

Dr. F. L. Saldanha charted out and arranged a course of 140 lectures by Scientists of the Institute, phased out during a period of about one year.

The following candidates were given training in Drug Analysis :—

<i>Sponsoring Organisation</i>			Total
World Health Organisation	...	...	2
Private firms	...	...	1
Private Candidates	...	...	14
M. L. T. Students	...	...	12
Grand Total			29

The number of different tests and samples analysed by the department during the period under report are tabulated below :—

TABLE

<i>Products</i>	<i>Tests</i>	<i>Number of samples tested</i>
	<i>Sterility</i>	
Bacterial Vaccines	...	16776
Antirabic Vaccines	...	9186
Parenteral bulk solutions	...	4448
Antitoxins, sera, etc.	...	7796
Miscellaneous	...	880

Pyrogen

Parenteral bulk solutions	...	...	1147
---------------------------	-----	-----	------

Chemical and Physical Examination

Parenteral bulk solutions	...	...	1326
Vitamin preparations	...	...	282
Sulpha drugs	...	...	86
Miscellaneous	...	...	275

Toxicity

Antitoxins, toxoids			
Vaccines etc.	...	...	551

Absence of infective viruses

Viral vaccines	...	...	298
----------------	-----	-----	-----

Potency

Antitoxins	...	...	37
Antivenine	...	...	36
Toxoids	...	...	30

 43,524
Preservatives

Vaccines, antitoxins	...	...	612
----------------------	-----	-----	-----

Free Formaldehyde

Bacterial vaccines, toxoids	...	...	203
-----------------------------	-----	-----	-----

Microbiological assays

Pemon	...	...	90
Vitamin preparations	...	...	108

Raw Materials

Vitamins	...	...	138
Sulpha drugs	...	...	66
Miscellaneous fine chemicals, antiseptics			
Pharmaceutical necessities, etc.			

Miscellaneous tests	...	244
---------------------	-----	-----

 Grand Total ... 45,639

DEPARTMENT OF VIRAL VACCINES

General

During the year under review, the antirabic vaccine production unit, which was housed in the main building of the Haffkine Institute, was transferred to then Department of Viral Vaccines. The production area has been provided with one hot room and two walk in refrigerators.

Smt. P. K. Patel, Senior Technical Assistant, was awarded the degree of M.Sc. (Micro.) for the thesis entitled 'Study of Simian intestinal viruses' by the University of Bombay.

Shri S. V. Pandit, Junior Scientific Officer, submitted his thesis entitled 'Isolation and characterization of rat viruses', for M.Sc. (Micro.) to the University of Bombay.

During the period under report, requests for supply of reference enterovirus antigens and antisera were received from the following :—

- (1) Officer-in-charge, ICMR Virus Unit, Department of Pathology and Bacteriology Medical College, Nagpur-3.
- (2) Officer-in-charge, ICMR Virus Unit, Department of Microbiology, Bangalore Medical College, Bangalore-2.
- (3) Dr. B. Baloya, Department of Microbiology, All India Institute of Medical Science, Ansari Nagar, New Delhi-16.
- (4) Officer-in-charge, ICRM Peripheral Virus Unit, B. J. Medical College, Ahmedabad.

All requests have been met with.

Production

(a) Pilot work on the production of Oral Polio Vaccine has been completed. Production of seed virus for type 1 polio virus has been completed. Approximately, 17 litres of seed virus fluid was produced. These lots are being tested. On completion of all the tests, the seed Virus would be sent to a Reference Laboratory abroad.

(b) *Processing of Oral Polio Vaccine.*—During April 1972 to March 1973, 27,08,235 doses of trivalent Oral Polio Vaccine were distributed.

(c) *Production of Antirabic Vaccine.*—During the period under report 38,37,253 ml. of Antirabic Vaccine for human use and 26,406 ml. of Vaccine for canine prophylaxis were produced.

(d) *Supplies of Vaccines.*—Monthly supplies of Oral Polio Vaccine and Antirabic Vaccine are shown in tables 1 and 2 respectively.

TABLE 1
Supply of Oral Polio Vaccine

Month.	No. of doses.		
April 1972	...	...	2,23,725
May 1972	...	...	2,23,985
June 1972	...	...	2,19,250
July 1972	...	...	2,31,845
August 1972	...	...	2,43,025
September 1972	...	...	2,31,585
October 1972	...	...	2,65,005
November 1972	...	...	2,37,325
December 1972	...	...	2,48,500
January 1973	...	...	2,12,885
February 1973	...	...	1,92,080
March 1973	...	...	1,79,025
Total	...	...	27,08,235

TABLE 2
Supply of Antirabic Vaccine

Month.	No. of doses.	
	Human	Canine.
April 1972	3,55,656	2,496
May 1972	3,71,252	3,216
June 1972	5,17,426	2,976
July 1972	3,05,592	2,580
August 1972	3,47,284	2,100
September 1972	3,44,414	1,704
October 1972	2,00,774	2,004
November 1972	2,98,592	2,418
December 1972	2,12,716	132
January 1973	3,30,134	2,112
February 1973	2,18,519	2,262
March 1973	3,34,894	2,406
Total ...	38.37,253	26,406

DEPARTMENT OF VIROLOGY**General**

On 19th May 1972, the activities and staff of the Antirabic Vaccine Section were transferred to the newly created Department of Viral Vaccines.

Dr. S. M. Desai, Junior Scientific Officer, went on study leave for one year from 10th October 1972, to take up a research appointment at the Argonne Cancer Research Hospital, Chicago, U. S. A.

Dr. (Smt.) Naseema A. Metha, who was on earned leave had to extend it from 18th November 1972 due to personal difficulties.

As a result of these changes and the prevailing scarcity conditions, under which new posts could not be filled up, the usual departmental activities had to be curtailed during the year under review.

Kumari R. Baride, M.Sc., joined the department on 16th August 1972, as a Government of India Research Fellow and undertook studies on biochemical problems related to the work of the department.

Diagnostic work on Rabies

Specimens received for rabies diagnosis, their tests and results are enumerated in Table 1.

TABLE 1

Diagnostic material received and results obtained

Material	Number of samples	Results of microscopy		Results of mouse inoculation			Positive	Negative	Tests in progress
		Positive	Negative	Positive	Negative	Tests in progress			
Dog brains ** ..	9	1	7	1	5	1	1	6	2
Human brains ..	10	2	8	2	2	6	2	2	6
Rabbit brains ..	1		1		1			1	..
Calf brain ..	1		1	1			1		
Cow brain ..	1		1	1				1	
Buffalo milk ..	2		2		2			2	

**One brain specimen was received in a putrified condition and, hence, could not be processed further at all. Another brain specimen showed paralysis in mice by mouse inoculation test and presence of intranuclear inclusions resembling Neri bodies in the brain smears of the paralysed mice. The inclusions were photographed and the report will be released only after the results of a serum virus neutralization test are evaluated.

Considerable difficulty and delay was experienced in the work of viral isolation in general, and diagnosis in particular, owing to non-availability of mice as well as presence of aberrant infection in the mouse colony.

DEPARTMENT OF ZOONOSIS

General ...

Cholera carrier studies were initiated in Kadrabad area of Parbhani Town.

Epidemiological studies in *Amoebiasis* and *Dracunculosis* were started.

Axenic culture of *E. histolytica* is being established in the department.

Plague epidemiological studies were continued in 'D' and 'E' ward areas of Bombay City.

Epidemiological studies in filariasis were carried out in Bhayandar area of Thana district.

Trainees from D. M. L. T. course were shown different parasitological and entomological techniques.

Shri V. B. Babne joined this department as Junior Research Fellow. Shri S. Y. Guirges joined as a post-graduate student on Government of India Cultural Fellowship for 1972-73.

Shri N. E. Vad was awarded Ph.D. degree in Zoology. Smt. L. V. Sarma submitted her synopsis for Ph.D. degree in Microbiology.

Dr. M. V. Sant continued to guide Shri D. S. Chitnis and Shri A. K. Patil for M. S.c. Degree and Sarvashri R. S. Karbhari.

Hussain Khaliefat, K. K. Das, T. U. K. Menon, Smt. S. Cherian, Kumari M. S. Kulkarni, Shri P. B. Dichwalkar and Dr. N. G. Chanderkar for Ph.D. Degrees.

Dr. M. V. Sant, Deputy Director (Research), attended the I.C.M.R. Expert Committee meeting on Cholera as a member of that committee, in September 1972.

The department continued to provide expert guidance in respect of identification of insect pests of medical importance, rodents and snakes and their control. Table 1 details the number of snakes and other items received from outside for identification and advice. Besides attending to the above queries, the department also supplied insects, snakes, rodents and micro-slides of insects to a number of educational and research institutions. The numbers of insects, snakes, rodents and besides supplied is shown in table 2.

Colonies of fleas (*X. Cheopis* and *X. astia*), flies (*M. domestic nebulo*), Cockroaches (*P. americana*), mosquitoes (*Culex P. fatigans*) and rodents (*Tatera indica*), were continued to be maintained for Insecticide and plague studies. Evaluation of commercial insecticide formulations DS-23 and DS-24 was undertaken.

Visitors

Visitors coming to this department were shown the venom extraction technique by actual demonstration and were given detailed information on identification of snakes and first-aid treatment of snake- i

The total number of visiting teams and distinguished visitors who visited this department, is shown in Table 3.

Production

During the period under report 223.71 gms. of snake venoms were processed and 298.29 gms. of venoms were supplied for the manufacture of antivenin and research purposes as shown in Table 4.

Scorpion venoms processed and supplied during the year are shown in Table 5.

The number of snakes and scorpions purchased during the year is shown in Table 6.

TABLE 1
Snakes, rodents, insects received for identification.

Material received	number
Snake	41
Rodents, insects and other queries ...	4
Rodent and insect control queries ...	6

TABLE 2
Insects, snakes rodents, and micro-slides of insects supplied

Material Supplied	Number.
<i>Dead Cobra</i>	6
<i>Dead Russell's viper</i>	7
<i>Dead kraits</i>	8
<i>Dead echis carinatus</i>	13
<i>Dead Non-poisonous snakes</i>	7
<i>Dead Scorpions</i>	8
<i>Snaks skins</i>	16
<i>Cast skins of snakes</i>	6
<i>X. cheopis</i>	6
<i>M. domestica</i>	6
<i>Culex p fatigans</i>	6

TABLE 3
Visitors and visiting teams shown snake venom extraction techniques

Details	No. of teams and demonstrations.
Students from different Colleges ...	57
Visitors from scientific institutions ...	29
Distinguished visitors ...	28
Number of persons seeing the demonstration of snake venom extraction.	5,110

TABLE 4
Snake venoms processed and supplied

Snake Venoms	Processed	Supplied
Krait ...	44.30	51.51
Cobra ...	141.29	109.30
Russell's viper ...	38.12	94.78
Echis ...	—	42.70
Total ...	223.71	298.29

TABLE 5
Scorpion venoms processed and supplied

Scorpion Venoms	Processed	Supplied
Black scorpion (<i>Palamaneus gravimanus</i>)	1.86	12.49
Red scorpion (<i>Buthus tamulus</i>)	0.40	3.74
Total ...	2.26	16.23

TABLE 6
Snakes and scorpions purchased

Snakes and Scorpions	Number
Cobra ...	274
Krait ...	719
R. viper ...	385
Echis ...	80
Non-poisonous snakes ...	75
Red scorpions ...	447
Black Scorpions ...	3,035

ANIMAL FARM

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

Eleven students of Diploma in Medical Laboratory Technology (D.M.L.T.) and seven students of the Medical Laboratory Technicians' Training Course (M. L. T.) were trained in general handling and management of laboratory animals. Sixteen trainees of Laboratory Animals Technicians' Training Course, conducted by the Laboratory Animals Information Service of the Indian Council of Medical Research were provided with facilities for practical work in connection with their course. Kumari A. M. Tilak and Dr. P. L. Chavarkar attended a short-term training course in maintenance and care-taking of laboratory animals during the quarter beginning from October 1972.

The strain of guinea-pigs of Haffkine Institute was found resistant to Tuberculosis. A tuberculosis susceptible strain was obtained from the Tuberculosis Research Centre, Madanapalli, Andhra Pradesh, in October 1972 and is being bred in the Animal Farm. It was decided to use Beagle Dogs for toxicological studies. In order to breed the Beagles, six female dogs and one male Beagle dog were procured from Hoechst Pharmaceuticals, Mulund, Bombay and are being bred at this Institute. The demand for hamsters increased.

In order to meet the increased demand, 30 females and 10 male hamsters were purchased from the Ciba Research Centre Goregaon, Bombay for breeding.

The below-mentioned figures will show the number of different species of laboratory animals bred in the Animal Farm, supplied to the different departments of the Institute and sold to other laboratories during the year under review.

Serial No.	Type of Animal	Production	Supplied to departments	Sold to other Laboratories
1	Albino Mice	2,20,331	1,72,633	5,247
2	Albino Rats	12,344	7,136	3,148
3	Rabbits	3,064	1,666	1,306
4	Guinea-pigs	5,538	4,093	1,117
5	Mansters	1,006	436	27
6	Cotton rats	150	32	
Total		2,42,433	1,85,996	10,845

In addition to the breeding of the above mentioned species of animals, sheep, monkeys, cats, and horses were maintained in the Animal Farm as per the requirements of different departments of the Institute.

LIBRARY SECTION

Re-organisation

In recent years, there has been tremendous output of the published scientific literature, especially in the fields of Chemistry, Biology, Medicine etc. and it is not possible for a specialised research library like the Haffkine Institute Library to give up-to-date service to the researchers, unless it is provided with Reprographic facilities like 'Microfilming', 'Xerox-copying' etc. In view of this, efforts are being made to make these facilities available at this library in the near future.

Taking into consideration the interest of the Institute in the communicable diseases, especially in cholera, plague, poliomyelitis and typhoid, it is proposed to have a reprint library specialised in these important diseases. Reprint library will be a section of the main library. As a first step in this direction, the work of collecting references and getting, wherever possible, reprints on 'Cholera' published during the last five years has been undertaken.

General

This year, three meetings of the Library Committee were held and re-organisation and other related matters were discussed. The members of the Library Committee made useful suggestions from time to time to improve the library services. The library continued to participate in the international exchange programme sponsored by the W. H. O., American Medical Library Association etc. and could procure 26 issues of different journals to complete the sets of the journals. During the year, about ten important but very old and rare publications on plague and cholera were got microfilmed from the INSDOC, Delhi, since the original publications had become very brittle and difficult to handle. It is proposed to get microfilm copies of some more publications during the next year. This year, 29 books were presented to this library by CIBA, Glaxo Laboratories, W. H. O. etc. The library continued to participate in the Inter-library Loan Service. The extension of working hours up to 7-00 p.m. was continued as in the last year.

Library Working

The following figures indicate the working of the library during the year :—

Number of outside readers visiting the library.	5,960
Number of books and periodicals issued to our staff.	1,200
Number of periodicals issued under Inter-Library Loan Service.	349
Books and periodicals obtained for our staff from other libraries.	47

Additions to Library

Numbers of Books added during the year. ...	316
Number of volumes bound ...	502
Number of current periodicals subscribed ...	184
Current periodicals received free of charge ...	115
Number of reports of Scientific institutions received.	45

LIST OF PAPERS PUBLISHED DURING THE YEAR 1972-73

Bhat, A. K. Bhamaria, R. P. Patel (Smt). M. R. Bellare, R. A. and Deliwala C. V.: Chemotherapy of Fungus Infections Part III Alkyl or Aryl Thiosemicarbazones, Acid Hydrazones and Styryl Aryl, of 5 BROMO, and Nitrosalicyldehydes (1972).

Indian Journal of Chemistry, 10, 694-698.

Chatterjee, P. K.; Rao, S. S. and Dutta, N. K.; Attempt to characterise Choleragenic Fraction and the skin permeability factor from *Vibrio* Culture Filtrates as separate identity (1972).

Indian Journal of Medical Research, 60 1168-1574.

Chavan, A. S. and Sant, M. V.: Cultural diagnosis of *Bordetella pertussis* infection (1972).

Journal of Postgraduate Medicine, 18, 68-73.

Darnule (Smt.), A. T. and Colah, R. B. M. : Effect of Vitamin B I on the stability of Folic Acid (1972).

Journal of Institution of Chemists (India), November.

Das, K. K. and Sant, M. V. : Evaluation of the Immunoaffluorescent test in Typhoid (1972).

Journal of Postgraduate Medicine 18, 123-131.

Dave (Smt.), J. K. Fernandes (Smt.), Y. and Sant, M. V.: Urethral Rhinosporidiosis-Report of two cases with a brief review (1973),—

Indian Journal of Medical Sciences 27, 35-36.

Fernandez, J. A.; Bellare, R. A. and Deliwala C. V. : Synthesis and Control Nervous System Activity of New Piperazine Derivatives IV (1972).

Journal of Medicinal Chemistry, 15, 417-419

Gaitonde, B. B. and Nimbkar, A. Y. : Interaction of bretylium and guane-thidine on the relaxations of the rat isolated fundal strip preparation, evoked by indirect stimulation (1973).

British Journal of Pharmacology, 47, 268-271.

Gaitonde, B. B.: Vakil, B. J.: Samuel M. R.: Chabria, N. L. and Kamath, R. A. Pharmacokinetics of Iron Absorption with a New Iron Preparation (1972).

Indian Journal of Medical Research, 60, 1674-168.

Hakim (Kum.), S. M. A. : Baxi A. J. Balkrishnan V. Kulkarni, K. V. Rao, S. S. and Jhala, H. I. : Blood Groups, Secret in Status and ability to teste Phenylthiocarbamide in some Muslim Groups (1973).

Human Heredity, 23.

Hakim (Kum.), S. M. A. Baxi, A. J. Balkrishnan, V. Kulkarni, K. V. Rao, S. S. and Jhala, H. I. Glucose-6-phosphate Dehydrogenase Deficiency and Colour-Vision studies in Indian Muslims (1972).

Humungenerik, 15, 90-92.

Hakim (Kum.), S. M. A., Baxi, A. J. Balkrishnan, V., Kulkarni, K. V., Rao, S. S. and Jhala, H. I. Haptoglobin Transferrin and abnormal Haemoglobin in Indian Muslims (1972).

Indian Journal of Medical Research, 60, 99-703.

Kankonkar, R. C., Rao, S. S., N. E., and Sant, M. V. Efficacy of Haffkine Institute Polyvalent Antivenin against Indian Snake Venoms (1972).

Indian Journal of Medical Research, 60, 512-516

Kulkarni, K. V., Baxi, A. J.; Patankar, V. M. and Das, P. C.:
Studies on Australia Antigen in India (1972).

Vox Sanguinis, 22, 510-518.

Mehta (Smt.), N. A. Nanavati, A. N. D., Jhala, H. I. and Sant; M. V.:
Measles Antibodies in Commercial preparations of Gamma Globulin (1972).
Indian Journal of Medical Research, 60, 661-669

Mehta (Smt.), N. A., Nanvati, A. N. D. Jhala, H. I. and Sant; M. V.:
Seroepidemiology of Measles in Bombay (1972).
Indian Journal of Medical Research 60, 670-73.

Renapurkar, D. M. Bhat, (Knm.), V. M. Menon, T. U. K. and Sant; M. V.:
Some Observation on duration of different stages of life history of *Culex pipiens fatigans*, *Wied* (1972).
Current Science 41, 417-419.

Sant, M. V. Nimbkar, Y. S. and Renapurkar, D. M.: Is Plague Lurking
in Maharashtra ? A Survey in Bhir District (1972).
Indian Journal of Medical Sciences, 26, 480-484.

Shah, (Smt.), Usha, K., Banerjee, K. L., Nanavati, A. N. D. and Metha
(Smt.), N. A.: A test Survey of Measles in a rural community in India (1972).
Bull. W. H. O., 46, 130-138.

Shekawat, D. R.; Sabnis, S. S. and Deliwala, C. V.: Potential Anticancer
Agents IV Schiff Bases from Benzaldehyde Nitrogen Mustards (1972).
Journal of Medicinal Chemistry, 15, 1196-1197.

Following Paper was sent for seminar on development of immunization
services, held by the world health organisation Regional office at new Delhi,
from 17th to 24 July 1972.

Dave, K. H.

Control of Poliomyelitis in Bombay.

LIST OF PAPERS SENT FOR PUBLICATION DURING THE YEAR 1972-73

Advaney, (Kum.), Maya ; Venkatraman (Smt.), A. and Rao, N. R.:
A study of serum Cholesterol levels in a group of population (Sindhia)
using hydrogenated oil as the main dietary fat.
Indian Journal of Nutrition and Dietetics.

Bapat, J. P. and Baxi, A. J.: Quantitative Levels of Glucose-6-Phosphate. Dehydrogenase, Pyruvate Kinase and Gultathione in normal and G-6-PD deficient individuals.

Indian Journal of Medical Research

Bapat, J. P.: Baxi, A. J.: and Kulkarni, K. V.: A rapid Counterimmunelectrophoretic technique for the detection of Australia antigen and alphafoetoprotein on the same slide.

Indian Journal of Medical Research

Chavan, A. S. and Sant, M. V. Immunological surveillance of pertussis in Bombay.

Journal of the Indian Medical Association

Chitnis, D. S.: Dave (Smt.), J. K. and Sant, M. V.: A new simple composite medium for the identification of non-lactose fermenting Enterobacteria

Indian Journal of Pathology and Bacteriology

Dave (Smt.), J. K. and Das, K. K. Immobilisation test as an aid to the rapid detection of *V. Cholera* organisms.

Indian Journal of Microbiology

Dave (Smt.), J. K. Vaidya (Smt.), V. S.; Menon (Kum.), S. and Sant, M. V. Observations on fluorescence Microcopy in the identification of *M. tuberculosis* in Tissue Sections.

Indian Journal of Pathology and Bacteriology

Deoras, P. J. and Vad, N. E.: Lung reduction in Snakes and Toxicity of salivary secretions.

Journal of the University of Bombay

Deoras, P. J. and Vad, N. E.: Vacuum method of venom extraction and differentiation in Toxicity.

Journal of the University of Bombay

Desai, S. M. Sehgal, P. B. Nanavati, A. N. D. and Shirodkar, M. V.: A Rabies-induced serum factor inhibiting Rous Sarcoma Virus in Chicks 1.

Journal of General Virology

Desai, S. M. Sehgal, P. B., Nanavati, A. N. D. and Shirodkar, M. V. A Rabies-induced serum factor inhibiting Rous Sarcoma Virus in Chicks.

Journal of General Virology

Kankonkar (Smt.), Sharda, Gatlwar, W. N. and Sant, M. V. Diagnosis of Filarasis by indirect Haemagglutination test.

Indian Journal of Medical Research

Nanavati, A. N. D.:

Rabies: A review of current problems and trends.

Indian Journal of Medical Sciences.

- Nanavati, A. N. D. and Mehta, (Sma.), N. A.: Measles surveys.
Bull W. H. O.
- Renapurkar, D. M.; Menon, T. U. K.; Bhat (Kum.), V. M. and Sant, M. V.
Tolerance in *Bandicota bengalensis* to Warfarin.
Indian Journal of Experimental Biology
- Rumao, L. M. and Sant, M. V.:
Serology typing of Beta-Hemolytic Streptococci Group-A.
Journal of Postgraduate Medicine.
- Shirgaonkar, (Kum.) G. V. and Sant, M. V.: Biotyping, toxigeny and phage
typing of *Corynebacterium diphtheriae* (Part I).
Indian Journal of Pathology and Bacteriology.
- Shirgaonkar (Kum.) C. V. and Sant, M. V.: Biotyping Toxigeny and
Phage typing of *Corynebacterium diphtheriae* (Part II).
Indian Journal of Pathology and Bacteriology.
- Talwalkar, S. C. and Gaitonde, B. B.: Thyroid function in protein malnutrition.
Journal of Endocrinology.

MAINTENANCE ENGINEERING SECTION

During the year, the Section continued to be fully busy with increased work-load and maintenance of plants and machinery etc.

Due to increased activities of the Institute, the Engineer and Technicians of this Section carried out a number of jobs departmentally in order to keep the production and research activities going smoothly.

The Mechanical Section carried out a number of jobs during the year under review. This has resulted in considerable financial saving as well as saving in time. Uninterrupted service in regard to the maintenance of freeze-drying plant and stand by micro-vacuum pump attached to the above plant has been carried out during the year. In addition to this, various jobs were carried out departmentally such as rectifying the design of the weak section of the imported shaft of 11-ton airconditioning plant for the use of Bacteriology Department, preparing worm gear of gun metal for the bottle rolling machine, welding the glass-lined kettle in the Chemotherapy Department, preparing miniature screw of 64 T. P. I. and 48 T. P. I. of 3/32" diameter, preparing tablet punch for the Tableting Section, designing fabricated inoculation hood for the Polio Department and many other fabrication jobs. Also, the annual hydrolic testing of all the equipment and machinery, which works under pressure as required under the Factories Act, 1948, was carried out.

The Electrical Section played a significant role in maintaining electrical installations, number of electrical connections to the various motors and

wiring for such motors. Further, this Section looked after larger number of jobs like repairing various types of ovens, air-compressors, vacuum pumps, stirrers, pharmaceutical machinery etc.

One incubation room with 37°C temperature for the use of Anti-Rabic Section in the Viral Vaccines Department was devised and put into beneficial use.

Shri M. K. Pitale, Electrical Supervisor, was deputed to the Bhabha Atomic Research Centre, Trombay, for training in the field of repairing electronic equipment at this Institute. His training in this field for a period of 6 months was very useful to the Institute in maintaining electronic equipment.

The air-conditioning Section carried out a number of jobs, such as repairs to deep-freeze, refrigerators, walk-in-coolers, water coolers, bottle coolers, air-conditioning plants, cold rooms etc. for the use of various departments.

It is gratifying to record here that repair and maintenance jobs with regard to multi-stage ultra-low temperature (70°C) deep-freeze in the Polio Department were undertaken. This year, two additional Walk-in-coolers were installed in the Anti-Rabic section of the Department of Viral Vaccine. These installations have helped release considerable congestion in the cold room.

The Carpentry Section also contributed substantially in the year's output. There were heavy demands for carpentry work connected with wooden crates of various sizes for the use of different departments of this Institute. Specially required wooden items for research and production, such as animal despatch cages and crates of different capacities, Pyrex jars, laboratory racks and card punching boards for the use of Time Keepers' Office, repairs to chairs, laboratory tables etc. were prepared.

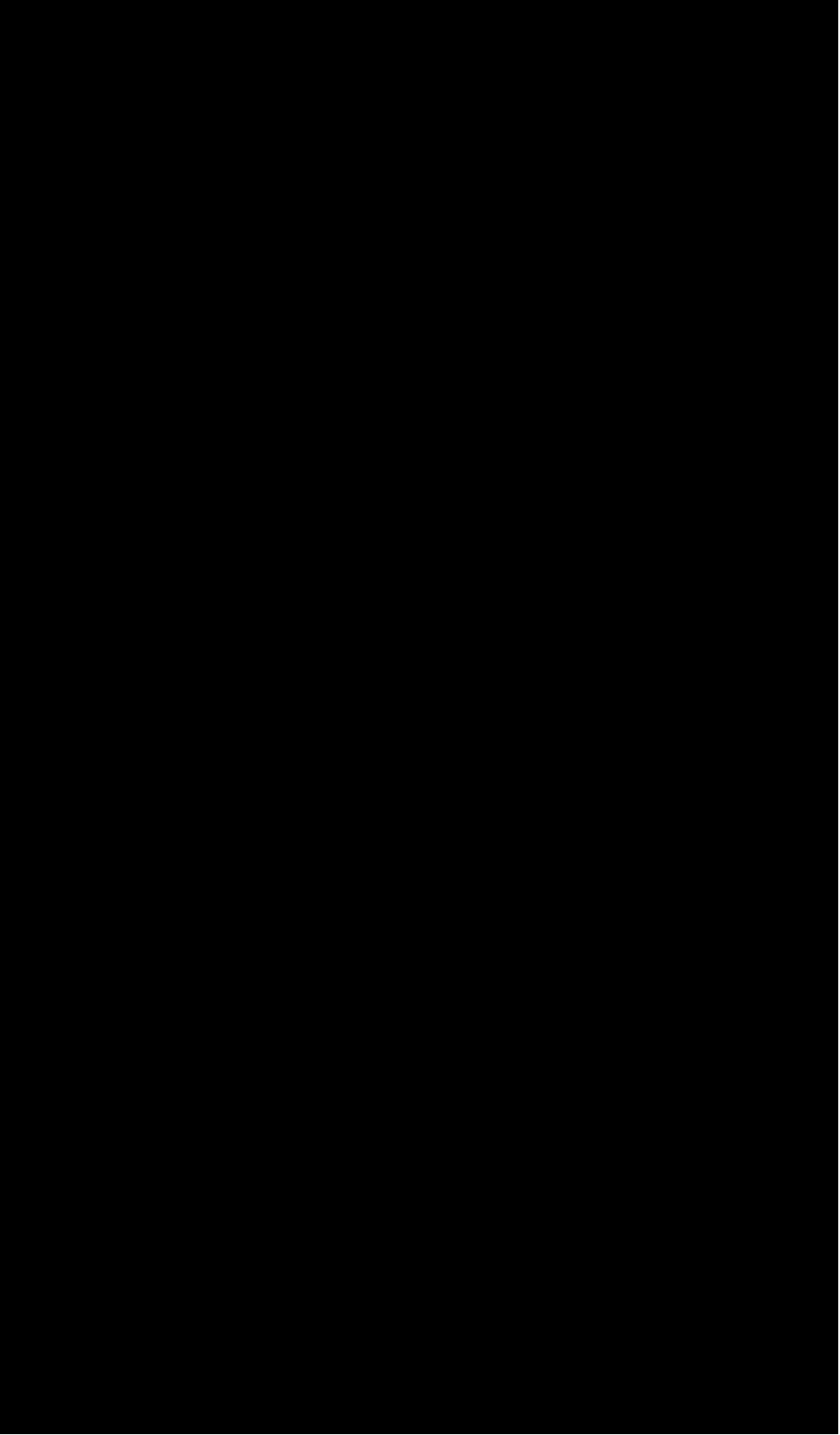
In short, all the Sections played a vital role in the total output of the Maintenance Engineering Section.

CRITICS CLUB

The Club continued its usual activities under the Secretaryship of Dr. H. J. Mansharamani. Of the 41 meetings held during the year under review, 18 were addressed by scientists from outside and 23 were addressed by scientists from within the Institute. The details of the scientific meetings are given below :—

INSTITUTE SPEAKERS

<i>Speaker</i>	<i>Subject</i>
Shri A. V. Muthe	... Role of tissue factor in the extrinsic activation of prothrombin.
Dr. D. M. Renapurkar	... Studies in animal filariasis.
Shri D. S. Chitnis	... A new composite medium for the identification of entero-bacteriaceae.
Dr. S. S. Rao	... Search for drugs effective in experimental tetanus.
Shri Vijay D. Rane	... Effect of dietary carbohydrates and fats on the lipid metabolism.
Kum. P. H. Marker	... Berberine in toxin-induced experimental cholera.
Kum. Kamala Thyagarajan	... Epidemiological survey of Influenza in Bombay.
Shri R. P. Bhamaria	... Submerged culture growth of <i>S. typhi</i> .
Shri V. M. Patankar	... Australia antigen hepatitis and liver function.
Shri R. P. Kini	... Search for drugs effective in experimental tuberculosis.
Smt. L. V. Sharma	... Studies on leptospirosis.
Shri L. M. Rumao	... Incidence of B-haemolytic streptococci in various disease conditions.
Shri S. V. Gadre	... Some fundamental studies in diphtheria toxin production.
Shri V. P. Shinde	... Nutritional requirements and biochemical studies of cholera organisms.
Shri S. R. Chavan	... Bio-availability of drugs INH in syrup form.
Shri V. K. Rao	... Poliomyelitis in Delhi.
Kumari M. Shanta	... Newer methods in diagnosis of plague.



- Dr. Nitya Nand, Scientist In-charge, Medicinal Chemistry Division, Central Drug Research Institute, Lucknow. Some aspects of drug design.
- Dr. E. G. J. Olsen, Consulting Pathologist to the National Heart Hospital, London. Study of the mechanisms of thrombo-embolic phenomenon.
- Dr. Yoshio Sawai, Chief, Division of Tropical Epidemiology, Institute of Medical Science, University of Tokyo, Japan. Studies on the snake venom toxoid.
- Dr. Louis S. Diamond, Department of Health, Education and Welfare, National Institutes of Health, Bethesda, Maryland, U. S. A. Some biological properties of the polyhedral viruses of *Entamoeba histolytica*.
- Dr. A. F. B. Standfast, W. H. O. Consultant, Retired Chief of Bacteriological Department (Production and Research), Lister Institute of Preventive Medicine, Elstree Laboratories, U. K. Some problems in pertussis.
- Dr. E. M. Vaughan Williams, Oxford University, England. Experimental background to the search for a rational basis for therapy with anti-dysrhythmic drugs.
- Dr. Stanley A. Plotkin, Associate Member, Wistar Institute, Associate Professor of Pediatrics, University of Pennsylvania, U. S. A. Newer aspects of viral vaccines.
- Dr. S. M. Merchant, Hon. Pediatrician, B. J. Wadia Hospital for Children, Bombay. Jaundice in the new born.

DISTINGUISHED VISITORS

- Dr. Klaus Dietz ... Division of Research in Epidemiology and Communications Science. W. H. O., Geneva, Switzerland.
- Dr. J. B. Davis ... Tropical Products Institute, England.
- Dr. Julius Axelrod ... Nobel Laureate in Medicine, National Institute of Mental Health, Bethesda, Madison, U. S. A.
- Shri Nizamuddin Ahmed ... Secretary to the Government of Maharashtra, Urban Development Public Health and Housing Department, Bomba .

Dr. Dean L. Moyer	... University of Southern California School of Medicine, Los Angeles, U. S. A.
Dr. B. N. Bhattacharya	... Assistant Director-General of Health Services, Government of India, New Delhi.
Dr. B. J. Vakil	... Hon. Professor of Medicine, Grant Medical College, Bombay.
Dr. W. Gitzels	... Director, Medical Services, Sultanate of OMAN.
Shri Sunil Mukherjee	... Chairman, Medical Education Committee, Government of Bihar, Patna.
Dr. Lala Surajnandan Prasad	... Director/Professor, Paediatrics, P. W. Medical College, Patna.
Dr. Jiwand Singh	... Additional Secretary to Government of Bihar, Department of Health, Patna.
Dr. Y. K. Sinha	... Joint Director of Health Services (Medical Education), Bihar, Patna.
Shri Sam. H. J. Pratt	... Assistant Director, National Police Academy, Mount Abu.
Dr. S. Agarwala	... Medical Adviser, Rallis (India) Limited, Bombay.
Mr. H. J. Higgins	... President, International Veterinary Students' Association, Royal Veterinary College, England.
Shri T. K. Karira	... Chairman, Public Health Committee, Municipal Corporation of Greater Bombay.
Shri T. G. Solanki	... Member, Public Health Committee, Municipal Corporation of Greater Bombay.
Shri G. B. Dutia	... Do.
Dr. H. Smith	... Department of Microbiology, University of Birmingham, England.
Mrs. Kalyagin	... New Delhi Correspondent of Moscow Radio.
Dr. V. Deunaev	... Moscow, Soviet Television.
Mr. N. Kepko	... Producer, Soviet Television, Moscow.
Shri Ajit Panja	... Minister for Health, Government of West Bengal.
Dr. A. Kar	... Director, Drugs Control, West Bengal.
Shri D. N. Saraf	... Divisional Commissioner for Handicraft, Ministry of Foreign Trade, Government of India, New Delhi.

Shri T. C. A. Srinivasan	... Deputy Director, Ministry of Foreign Trade, New Delhi.
Dr. M. S. AL-HAWAL	... I. N. A. S. Tripoli, Libya.
Dr. Mohamed S. Burghikhan	... G. S. Libya Tripoli.
Dr. M. Hamedd Haraga	... I. N. A. S. Tripoli, Libya.
Prof. Michael Davies	... Head of the Department of Medical Ecology at the Hebrew University of Jerusalem.
Mr. Ivan Moskov	... Pharmachin Sofia.
Mr. Ivan Yabukov	... Trade Commissioner of Bulgaria.
Mr. Tinkov	... Deputy Trade Commissioner of Bulgaria.
Dr. R. H. Kirk	... Canberra, Australia.
Dr. N. Saho	... Canberra, Australia.
Dr. Abdul Hai	... Deputy Director, Health Services (Administration), Bangla Desh.
Dr. Hussein	... Deputy Director, Urban Development, Bangla Desh.
Mrs. Rahima Khatom	... Superintendent of Nursing, Bangla Desh.
Dr. G. S. Tawil	... Regional Advisor in Health Laboratory Services, W. H. O. Regional Office for South-East Asia, New Delhi.
Dr. Olsen E. G. J.	... Consulting Pathologist to the National Health Hospitals, London U. K.
Dr. P. R. Nilakanthan	... Joint Director-cum-Officer-Incharge, Regional Station, Indian Veterinary Research Institute, Hebbal, Bangalore.
Dr. Yoshio Sawai	... Institute of Medical Science, University of Tokyo, Japan.
Dr. Manabu Homma	... Department of Pathology, Gunma University Medical School, Gunma, Japan.
Prof. Albert Raymond	... Department of Embryology, Institute Pasteur, France.
Prof. Ivan Assenmacher	... Head, Department of Physiology, University of Montpellier, France.
Dr. M. I. D. Sharma	... National Institute of Communicable Diseases, Delhi.
Prof. V. Ramalingaswami	... Director, All-India Institute of Medical Sciences, New Delhi.
Major-General M. S. Boparai	... Director, Medical Research, Office of D. G. A. F. M. S., Ministry of Defence, New Delhi.

Mr. B. M. S. Riyami	... Chief Pharmacist, Sultanate of Oman.
Prof. Louis S. Diamond	... Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, Maryland, U. S. A.
Dr. M. Lundé	... Do.
Dr. A. F. B. Standfast	... Lister Institute of Preventive Medicine, London.
Dr. Joseph P. Polard	... Director, Biological and Medical Sciences Division, Office of Naval Research, Arlington, Virginia, U. S. A.
Dr. Arthur J. Emery	... Programme Director, Microbiology, Office of Naval Research, Arlington Virginia, U. S. A.
Dr. J. J. Pinajian	... U. S. A. E. C. Scientific Representative-cum-Consul, Bombay.
Dr. Paul Janssen	... Janssen Pharmaceutical, Beerse, Belgium.
Dr. Peter De Vogel	... Unilever Research Laboratory, Saint Denis (Peru).
Dr. Zdenek Sebek	... National Reference Laboratory for Leptospirosis, Jihlava, Czechoslovakia
Dr. Koloman Boda	... Institute of Animal Physiology Slovak Academy of Science, Bratislava.
Dr. Hans Kohl	... Hoechst Pharmaceuticals Ltd.
Dr. Karl H. Teichmann	... Farbwerke Hoechst, Frankfurt.
Shri G. S. Sarnayak	... Minister of State for Urban Development, Public Health and Buildings and Communications, Government of Maharashtra.
Dr. Dziubek Zdzislaw	... Warsaw, Poland.
Dr. D. A. A. Kidd	... May & Baker.
Dr. Stain Erichsen	... Secretary of the International Committee on Laboratory Animals (W. H. O.), Norway.
Dr. J. Murai	... Tokyo Tanabe Co. Ltd.
Dr. S. Shirato	... Tokyo Tanabe Co. Ltd.
Dr. John Goulton	... Medical Adviser, Glaxo International, London.
Shri A. K. Majumdar	... Secretary, Health and Family Planning Department, Government of West Bengal, Calcutta.
Mr. John Bleby	... Director, Medical Research Council, Laboratory Animals Centre, Carshalton, Surrey, U. K.

Dr. Forej Matanuovic	...	Ann Arbor, Michigan, U. S. A.
Dr. E. M. Vaughan Williams	...	Oxford University.
Dr. Shuwakât EL Fatouri	...	Chief of Medical Branch, I. N. A. S., Tripoli, Libya.
Dr. EL Gammi Youssef	...	Director of Medical Stores, I. N. A. S., Tripoli, Libya.
Ali Mohamed Attoushi	...	I. N. A. S., Tripoli, Libya.
Mohammed Mohammed Tumia	...	Director of Tripoli Office, I. N. A. S. Libya.
Dr. H. Bissoonauth	...	Ministry of Health, Mauritius.
Professor Stanley A. Plotkin	...	Associate Member, Wistar Institute, and Assistant Professor of Paedia- trics, University of Pennsylvania, U. S. A.

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 degree and diplomas :—

Post-graduate degress

M.Sc. : Microbiology, Biochemistry, Zoology, Organic Chemistry and Applied Biology, Pharmacology.

Ph.D. : Microbiology, Biochemistry, Zoology Organic Chemistry, Applied Biology, Pharmacology.

M. Pharm. : Pharmacology.

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. (Smt.) A. Venkataraman.
 Dr. K. H. Dave.
 Dr. F. L. Saldanha.
 Dr. M. B. Bhide.
 Dr. S. D. Ambegaonkar.
 Dr. A. J. Baxi.
 Dr. P. Y. Dighe.
 Dr. M. H. Shah.
 Dr. A. N. D. Nanavati.
 Dr. S. S. Sabnis.
 Dr. K. V. Kulkarni.

The Institute is also recognised by the Colleges of Physicians and Surgeons of Bombay for training candidates for (a) F. C. P. S. Examination in Pathology, Bacteriology and Pharmacology and (b) D. P. B. Examination. The following officers of this Institute are recognised as teachers of the College :—

Dr. M. V. Sant.

Dr. (Mrs.) J. K. Dave.

The Institute is also affiliated to the University of Poona for post-graduate training course. 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.

NUMBER OF POST-GRADUATE STUDENTS ON ROLL DURING THE PERIOD FROM 1ST APRIL 1972 TO 31ST MARCH 1973

ORGANIC CHEMISTRY.

M.Sc.

Shri G. V. Joshi.
Shri S. R. Prabhu.
Kum. B. K., Kapadia.
Shri J. K. Dhorda.
Shri V. S. Khot.
Shri R. P. Kini
Shri V. S. Tamore.
Shri P. S. Narse.
Shri D. P. Sule.

Ph.D.

Shri B. N. Palande.
Shri B. G. Khadse.
Shri D. G. Bapat.
Shri K. Y. Mahajan.
Shri D. T. Chaudhari.

MICROBIOLOGY

M.Sc.

Kum. G. V. Shirgaonkar.
Smt. P. K. Patel.
Shri S. V. Pandit.
Shri M. M. Farro.
Shri R. P. Vitkar.
Smt. S. S. Sathe.
Kum. Kamala Thyagarajan.
Smt. A. Gandhi.
Shri D. S. Chitnis.
Smt. E. R. D'souza.
Kum. M. N. Bablad.
Shri A. K. Patil.
Kum. M. Shanta.
Shri S. D. Gajjar.
Kum. A. C. Gupta.
Shri V. R. Gurao.
Shri Sami Yousif Guirges.

Ph.D.

Kum. L. S. Iyer.
Shri K. N. Joishy.
Shri P. K. Chatterji.
Kum. M. S. Kulkarni.
Shri R. S. Karbhari.
Shri Khleifat Husein Ali Mutlag.
Shri K. K. Das.
Smt. Susy Cherian.

BIOCHEMISTRY

M.Sc.

Smt. H. V. Phadnis.
 Kum. G. M. Dalal.
 Shri V. A. Aroskar.
 Smt. Laxmi Jayaram.
 Shri P. D. Pilankar.
 Shri D. V. Pradhan.
 Kum. J. A. Bellare.
 Shri S. S. Karkhanis.
 Shri V. D. Rane.
 Shri R. C. Shah.
 Kum. L. S. Shirsat.
 Shri R. B. Pathare.
 Shri H. K. Soni.
 Smt. M. N. Shah.
 Shri N. V. Wanjara.
 Shri P. M. Kulhalli.
 Shri S. D. Nabar.
 Shri V. D. Sardeshpande.
 Shri M. B. Shinde.
 Shri Y. V. Lele.
 Smt. P. K. Mardikar.

Ph.D.

Shri J. K. Kamath.
 Shri R. C. Kankonkar.
 Shri V. P. Shinde.
 Kum. R. S. Buit.
 Shri V. M. Patankar.
 Shri A. S. Qureshi.
 Shri U. J. Dumaswala.
 Shri M. P. Naik.
 Shri G. S. Chhatwal.
 Shri Syed Raziuddin.

APPLIED BIOLOGY

M.Sc.

Shri R. D. Nabar.
 Kum. U. S. Kushe.

Ph.D.

Dr. D. P. Buduk.
 Dr. H. J. Mansharamani.
 Shri G. R. Fernando.
 Shri S. N. Iyer.
 Dr. N. G. Chanderkar.
 Shri H. J. Kulkarni.
 Shri S. N. Joglekar.
 Kum. V. M. Barsiwala.
 Shri T. Unnikrishnan Menon.
 Dr. (Smt.) D. Chengappa.
 Shri P. R. Dichwalkar.

ZOOLOGY

M.Sc.

Shri T. G. Tare.

Ph.D.

Shri N. E. Wad.

PHARMACOLOGY

Ph.D.

Shri A. Y. Nimbkar.

STUDY AWARDS (DEGREES)

*M.Sc (Biochemistry)**Topics*

- | | | |
|---------------------|-----|--|
| Smt. Laxmi Jayaram | ... | "The role of haptoglobins in liver function under clinical and experimental conditions". |
| Kum. G. M. Dalal | ... | "Biochemical studies in Atherosclerotic heart diseases". |
| Shri V. A. Aroskar | ... | "Studies in cholera toxin". |
| Shri P. D. Pilankar | ... | "Studies on oral hypoglycaemic agents". |

M.Sc. (Organic Chemistry)

- | | | |
|--------------------|-----|---|
| Kum. B. K. Kapadia | ... | "Synthesis and study of piperazine derivatives as anti-amoebic agents". |
|--------------------|-----|---|

M.Sc. (Microbiology)

- | | | |
|-------------------------|-----|--|
| Smt. P. K. Patel | ... | "Studies on Simian intestinal viruses". |
| Kum. Kamala Thyagarajan | ... | "Epidemiology of influenza in Bombay". |
| Shri M. N. Farro | ... | "Incidence of carrier state of rabies virus in normal dogs". |

Ph.D. (Applied Biology)

- | | | |
|---------------------|-----|---|
| Shri G. R. Fernando | ... | "Experimental studies of anti-fertility drugs". |
|---------------------|-----|---|

Ph. D. (Zoology)

- | | | |
|----------------|-----|---------------------------------------|
| Shri N. E. Wad | ... | "Studies on Indian poisonous snakes". |
|----------------|-----|---------------------------------------|

*Trainees admitted for Technicians' Training Course
for the year 1972-73.*

Shri S. D. Irani.
Shri A. M. Choudhari.
Shri J. B. Dhabuwala.
Shri D. I. Danawala.
Kum. P. M. Pardesi.
Kum. N. A. Dhavalikar.
Kum. N. G. Waingankar.

Trainees admitted for the Diploma Course in Medical Laboratory Technology (D. M. L. T.) for the year 1972-73

Shri N. V. Pai.
Kum. S. M. Rao.
Kum. S. R. Pathak.
Kum. N. J. Save.
Kum. M. S. Mukherjee.
Kum. B. K. Khichadia.
Kum. K. T. Joshi.
Kum. N. G. Hadikyal.
Kum. S. A. Doodhwala.
Kum. N. V. Dalvi.
Kum. S. G. Gada.

Short-term trainees during the period during the year 1972-73.

Shri R. K. Savla,
Bombay-64.
Smt. R. S. Patel,
Bombay-18.
Kum. P. S. Pradhan,
Bombay.
Kum. A. P. John,
Bombay-7.
Kum. G. Sabharwala,
Bombay-52.
Shri R. A. Kamal,
Nagpur.
Shri R. R. Karwa,
Bombay.
Dr. V. S. Jadhav,
Bombay.
Shri S. K. Kulkarni,
Bombay-19.
Dr. J. C. Borach,
Gauhati, Assam.
Shri R. G. Baxi,
Bombay.
Shri W. H. Ansari,
Ranchi.
Shri K. M. Desai,
Kalyan.
Shri S. F. Kagalwala,
Bombay-2.
Kum. R. R. Mehta,
Bombay.
Kum. D. N. Patel,
Bombay-1.

Kum. S. Bhatt,
Bombay-58.
Kum. P. G. Kamath,
Bombay-89.
Smt. M. W. Dharwarkar,
Bombay-50.
Dr. N. H. Kamdar,
Bombay-72.
Dr. (Kum.) R. M. Bhatia,
Ulhasnagar-3.
Shri R. D. Kathawala,
Bombay-77.
Kum. U. G. Ayachit,
Thana.
Smt. S. S. Ghokhale,
Bombay-25.
Kum. S. K. Uppal,
Kalyan.
Kum. R. A. Chandok,
Bombay-24.
Kum. P. S. Shembade,
Bombay-22.
Kum. G. M. Udani,
Bombay-7.
Shri K. K. Shenoy,
Kalyan.
Shri M. B. Borkar,
Poona.
Kum. M. C. Shah,
Bombay-31.
Kum. S. Vincent,
Virar.

Shri Y. G. Boxwala,
 Bombay-3.
 Shri J. M. Dabholkar,
 Bombay-12.
 Shri K. K. Varkey,
 Bombay-52.
 Shri J. T. Kantawala,
 Bombay-3.
 Shri H. Balakrishnan,
 Poona-1.
 Dr. P. L. Chavarkar,
 Bombay-22.
 Kum. A. M. Tilak,
 Bombay-71.
 Kum. M. Fernandes,
 Bombay-50.
 Kum. B. G. Ratnani,
 Bombay-9.
 Kum. I. J. Patel,
 Bombay-34.
 Smt. S. S. Savarkar,
 Bombay.
 Kum. P. P. Migatwalla,
 Bombay-7.
 Shri R. M. Joshi,
 Bombay-92.
 Dr. R. K. Jha,
 Heavy Engg. Corpn., Dharwar.
 Kum. S. V. Kothari,
 Bombay-4.

Kum. K. N. Shah,
 Thana.
 Kum. H. I. Bakali,
 Bombay-9.
 Dr. (Smt.) N. R. Pisey,
 Wardha.
 Kum. K. B. Nadkarni,
 Bombay-4.
 Kum. R. R. Bhatte,
 Bombay-28.
 Kum. M. M. Fernandes,
 Bombay-50.
 Kum. A. N. Rao,
 Bombay-1.
 Shri A. K. Chhazed.
 Ratlam (M. P.).
 Kum. V. A. Kumari,
 Vijayawada (A. P.).
 Shri V. R. Ayyar,
 Bombay-57.
 Shri V. H. Sinha,
 Bombay-4.
 Kum. V. I. Patel,
 Deolali.
 Dr. V. S. Bisen,
 Poona-1.

The following fellowships and scholarships were attached to the Institute during the period 1972-73.

Sponsored by	Subject	Name of the Scientist
Government of India Research Training Scholarship Scheme.	Organic Chemistry	Shri K. Y. Mahajan.
	Organic Chemistry	Shri D. T. Choudhari.
	Virology	Kum. R. M. Baride.
	Biochemistry	Kum. P. H. Jhala.
Government of Maharashtra	Organic Chemistry	Shri B. G. Khadse.
	Organic Chemistry	Shri D. T. Choudhari.
	Pharmacology	Shri M. G. Phatak.
	Immunology	Dr. (Smt.) S. R. Kankonkar.
	Organic Chemistry	Shri P. P. Dalvi.
	Organic Chemistry	Shri S. V. Kekatpure.
	Microbiology	Shri S. V. Gadre.
	Pharmacology	Shri S. S. Jadhav.
	Biochemistry	Shri Sayed Raziuddin.
	Biochemistry	Shri H. S. Ved.
	Organic Chemistry	Shri B. R. Colha.
	Organic Chemistry	Shri A. R. Manvi
	Biochemistry	Shri V. D. Rane.
Unichem Laboratories Pvt. Ltd.	Pharmacology	Smt. S. Kumar Bhindey.
	Indigenous Drugs	Kum. S. R. Bhagwagar.
	Pharmacology	Shri S. C. Talvalkar.
	Pharmacology	Shri S. R. Chitnis.
	Indigenous Drugs	Shri S. R. Joshi.
University Grants Commission .. Council of Scientific and Industrial Research, New Delhi.	Organic Chemistry	Shri B. G. Khadse.
	Zoology	Dr. (Smt.) S. R. Kankonkar
Haffkine Institute Diamond Jubilee Research Unit	Microbiology	Smt. N. A. Mehta.
	Biochemistry	Kum. Ranjana S. Bui
	Biochemistry	Shri G. S. Chhatwal.
	Clinical Pathology	Shri T. K. Shetty.
	Zoology	Shri V. B. Bane.
Indian Council of Medical Research.	Microbiology	Shri S. V. Gadre.

FINANCIAL SUMMARY

During the year under review (1972-73), the Institute continued to render its traditional services in the cause of public health of the State and the country. As compared to the sales of Rs. 127 lakhs, which include sales worth Rs. 14.93 lakhs to Employees State Insurance Scheme during the year 1971-72, the sales during 1972-73 came down to Rs. 121.85 lakhs, inclusive of sales of Rs. 11.71 lakhs to employees' State Insurance Scheme, during the year under review.

The reasons for variation are attributable to the various difficulties encountered in the procurement of raw materials due to import restrictions and tight market conditions, and drastic cut in power.

The triform accounts, started in 1966-67, were drawn up for the year 1972-73 as well. The preliminary outstandings against sales as on 31st March 1973 stood at Rs. 35.16 lakhs as against the final figure of Rs. 25.75 lakhs for the year 1971-72.

The financial position as on 31st March 1972 as extracted from the audited records is recast as under :—

Our Position as on 31st March 1972

	1971-72 Rs.	1970-71 Rs.
<i>What we have (Assets)</i>		
1. Land & buildings ...	1,06,22,995	99,20,230
2. Plant & Machinery ...	55,79,983	54,28,642
3. Furniture Fixture and Equip- ments.	15,64,697	15,06,216
4. Motor Vehicles ...	1,74,720	1,92,567
5. Library books ...	8,90,843	8,62,441
6. Live stock (Excluding gifts).	4,95,421	4,26,644
7. Stock & Stores ...	72,48,196	63,67,516
8. Amount to be received ...	24,59,697	26,54,746
9. Cash, transit remittances, etc.	19,23,412	14,89,686
10. H. I. Depreciation Fund Investment Account (with Government).	16,50,000	14,00,000
	3,26,09,964	3,02,48,697
<i>What we owe (Liabilities).</i>		
1. Depreciation Fund ...	32,05,935	30,49,799
2. Amount to be paid ...	16,44,544	13,99,344
3. Miscellaneous deposits ...	1,73,638	1,57,148
4. H. I. Depreciation Fund ...	16,50,000	14,00,000
	66,74,127	60,06,291
<i>Net surplus on production side.</i>		
(after providing for all expenses).	10,65,757	8,65,840
<i>What remains (Assets minus</i>	2,48,70,080	2,33,76,566
<i>Liabilities Plus revenue surplus).</i>		
<i>What we earned (Income).</i>		
1. Sales of biologicals and drugs.	1,16,07,458	1,01,80,167
2. Fees for analytical tests ...	5,78,953	4,82,101
3. Hostel Fees. ...	19,110	17,082
4. Interest on overdue bills, ...	Nil	1,64,962
Total ...	1,22,05,521	1,08,44,312
Value of closing stocks ...	72,48,196	63,67,516
Grand Total ...	1,94,53,717	1,72,11,828

	1971-72 Rs.	1970-71 Rs.
<i>What we spent (Expenditure)</i>		
1. On employees ...	54,57,938	48,59,747
2. On raw materials ...	53,83,464	42,05,536
3. On Purchases and services ...	19,06,862	16,54,932
4. On provision for pension ...	3,63,885	3,57,630
5. On provision for depreciation.	5,03,105	4,84,029
6. On provision for interest on mean Capital.	15,57,594	12,04,722
Total ...	1,51,72,848	1,27,66,539
Value of opening stocks ...	63,56,266	58,54,939
Grand Total ...	2,15,29,114	1,86,21,534
Net Surplus (after providing for all expenses).	(-) 12,22,361	(-) 14,09,706
<i>Break-up of net surplus.</i>		
Production ...	(+) 10,65,757	(+) 8,65,840
Research and Training ...	(-) 19,53,274	(-) 17,67,282
Public Health Service ...	(-) 3,34,844	(-) 5,08,264
	(-) 12,22,361	(-) 14,09,706
The production and sale values (exclusive of sales tax and packing charges, but inclusive of excise duty) for the year 1972-73 is as under.		
	<i>Production</i> Rs.	<i>Sales</i> Rs.
	1,19,99,899	1,18,58,034

INTERNAL AUDIT SECTION

This Section normally audits accounts for at least one month in each quarter. However due to unavoidable circumstances and pressure of other special audit during the year under view, the normal audit work could not be undertaken for some time.

The Accountant General (Outside Audit Department), during the course of test audit of accounts of the Institute for 1971-72 (1st spell), pointed out that the transaction of the whole year may be got checked by the Internal Audit Section. Accordingly, special audit of cash book for the year 1971-1972 was taken up from 1st December 1972 onwards and has since been completed. Thus, the special audit kept the Section busy during the entire year and normal audit could not be taken up.

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 the 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, Football and Volley-ball.

It participated in all the local and inter-departmental tournaments.

The Club arranged inter-department tournaments as also friendly matches with its counterparts in other organizations.

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 started a quarterly publication, for the first time, by name "Haffkine Vrutta" on the eve of the 25th Anniversary of the Independance Day on 14th August 1972 and published 3 issues of the chronicle during the year under report. It 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 wiring faculties scattered over various classes of employees.

It is a pleasure to mention here that the following four members of the staff of this Institute were included in team of Maharashtra for Kabaddi, which participated in All-India Civil Services Kabaddi Championship which were held at New Delhi on 2nd, 3rd and 4th March 1973.

Shri M. B. Raut.

Shri T. S. Kadam.

Shri M. N. Kadam.

Shri J. S. Chaphekar.

Welder-cum-Fitter.

Laboratory Attendant.

Laboratory Assistant.

Laboratory Attendant.

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 cost 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. It would be gratifying to note here that, out of the 60 employees who availed of these facilities, 5 student employees scored more than 50 per cent marks in the S. S. C. examination and First Year to B.A. examination of the Bombay University, respectively.

Awards

With a view to recognising merit in the fields of production and research and to give incentive to the talented workers in the fields, the following annual awards were announced :—

(1) Five cash incentive awards of Rs. 1,000, 500, 250, 100 and 50 for meritorious work in the field of production and research.

(2) Four cash incentive awards of Rs. 500, 250, 100 and 50 to Class III ministerial staff, Class III technical staff for efficiency in work.

(3) Five cash awards of Rs. 250, 150, 100, 50 and 25 to 'Best Workers' in the Institute.

Merit certificates to all the awardees will be presented in August 1973.

Training

(a) *Civil Defence Unit*.—Classes on Civil Defence Basic Course were conducted at this Institute twice during the year.

The first course commenced from 29th August 1972 to 15th September 1972. The course was attended by 24 employees and 23 employees were declared as successful.

The second course commenced from 17th January 1973 to 8th February 1973 and 28 employees attended the classes, out of which 21 were declared as successful.

The following members of Staff have undergone training under the various Civil Defence Courses sponsored by the Civil Defence Staff College, Bombay, as per details given below :—

Shri H. V. Nerurkar, Junior Technical Assistant, Department of Pharmacology :—

(i) Civil Defence Basic Course No. 74 from 2nd October 1972 to 9th October 1972.

(ii) Civil Defence Control and Communication Course No. 25 from 17th February 1973 to 27th February 1973.

Shri R. M. Ghag, Laboratory Assistant, Animal House :—

(i) Civil Defence Special First-aid Course from 19th June 1972 to 27th June 1972.

(ii) Civil Defence Basic Course from 2nd August 1972 to 9th August 1972.

(iii) Civil Defence Instructors' Course from 16th August 1972 to 4th September 1972.

Shri Y. V. Thakre, Assistant Director (Administration) :—

Civil Defence Basic Industrial Course No. 5 from 2nd March 1973 to 13th March 1973.

(b) *Diploma Course in Trade Unionism*.—One Class III employee was deputed for 9 months' Diploma Course in Trade Unionism, conducted by the Bombay Labour Institute.

Other Activities

(a) *Chawl Committee*.—There is a Chawl Committee headed by the Business Manager as Chairman, to look after the various complaints and problems of about 300 families of the employees residing in the existing chawls in the compound.

Out of the four new chawls (A, B, C and D) consisting of 192 tenements which were under construction for the residential use of staff, construction of 3 chawls was completed during the year under report and tenements were allotted to nearly 140 members of the staff and rooms in the fourth one, which is nearing completion, will be allotted soon.

(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 the 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 has recommended to the Government to appoint a pay Commission for revision of pay scales of the staff of Haffkine Institute. Discussions were arranged with Minister of Health and State Minister for Health. Final orders in this respect are still awaited from the Government.

Labour Welfare Committee.

Moreover, 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 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 to obtain advice from the Presidents of both the Unions. The Committee consists of the Director as Chairman and the Deputy Director (Production), Deputy Director (Research), Assistant Director (Administration) and Presidents of the Class III and Class IV Unions as members and the Labour Welfare Officer as the Convenor. 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 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, arrangements for opening Sewing Class for dependant female members of the staff are in progress.

Miscellaneous.

(1) An amount of Rs. 2,672 was collected from members of the staff of the Institute and contributed to the Chief Minister's Draught Relief Fund.

(2) Three employees opened Cumulative Time Deposit Accounts during the year under report. Total number of members of C. T. D. accounts stood at 662 as on 31st March 1973.

(3) Ambedkar Jayanti and Buddha Jayanti celebrations were also spontaneously organised by some of the employees of the Institute at Bombay and Pimpri Stud Farm, in which the surrounding inhabitants also participated with great enthusiasm.

HAFFKINE INSTITUTE CO-OPERATIVE CREDIT SOCIETY LTD.

The Society continued satisfactory progress during the year. The following was the position as on 30th June 1972 :—

	1971	1972
	Rs.	Rs.
(1) Membership ...	924	962
(2) Loans advanced to members ...	6,29,240	8,01,321
(3) Share Capital ...	5,72,651	6,59,580
(4) Net profits ...	54,350	53,393

2. The Society carried out the following types of business :—

(i) Advancing loans to members for various purposes and also emergency loans and payment of insurance premia.

(ii) running a staff canteen,

(iii) running a grain shop.

DISPENSARY

The Institute dispensary continued to serve the needs of its staff and their dependants as hithertofore.

New premises were provided, which are more spacious and better suited than the old premises.

A total of about 38,358 patients attended the dispensary for treatment.

About 250 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 checkups.

Family Planning Programme has been started with the administration of contraceptive tablets. About 75 women are taking these tablets regularly. About 200 children were immunised with Polio and Triple Antigen.

Cases requiring expert guidance were referred to the teaching hospitals.

The Departments of Blood Bank and Clinical in Pathology co-operated carrying out routine investigation tests.

