



सत्यमेव जयते

GOVERNMENT OF MAHARASHTRA

ANNUAL REPORT
OF THE
HAFFKINE INSTITUTE
FOR THE YEAR
1970-71

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CONTENTS

PART I

	PAGE
GENERAL	1

PART II

RESEARCH	15
------------------	----

A. Progressive research in—

Antifertility drugs	15
Cholera	16
Diphtheria	21
Enteroviruses	24
Plague	25
Use of antigens labelled with radio-active isotopes in serological epidemiology	29

Departmental research in—

Analytical and Control Department	31
Department of Bacteriology	31
Department of Blood Bank	43
Department of Chemotherapy	49
Department of Clinical Pathology	55
Department of Immunology	57
Department of Biochemistry	60
Department of Pharmacology	69
Department of Virology	74
Department of Zoonosis	80

PART III

ROUTINE ACTIVITIES	83
Analytical and Control Department	85
Department of Bacteriology	87
Department of Blood Bank	90

CONTENTS

					PAGE
Department of Chemotherapy	..	..	..	..	92
Department Clinical Pathology	..	..	..	..	94
Department of Immunology ..	..	..	..	..	98
Department of Biochemistry ..	..	..	..	..	99
Department of Pharmacology	..	..	..	..	103
Department of Virology ..	..	..	..	..	105
Department of Zoonosis ..	..	..	..	..	107
Animal Farm ..	..	..	..	..	109
Library Section ..	..	..	..	..	110
Maintenance Engineering and Carpentry Section	..	..	..	..	115

(A)

STAFF OF THE INSTITUTE

Director

Dr. N. K. Dutta, D.Sc. (Oxon.), D.Phil. (Oxon), M.B.B.S. (Cal.), F.N.A.,
F.A.M.S. (from 6-5-1968).

Deputy Directors

Dr. C. V. Deliwala, M.Sc. (Bom.), Ph.D. (Bom.),
Assistant Director, In-charge, Chemotherapy Department (upto 18-8-1970).
Deputy Director (Research), Division of Pharmacology and Pharmaceutical
Chemistry (from 19-8-1970).

Dr. M. V. Sant, M.D. (Bom.), F.C.P.S. (Bom.), D.E. (Prague),
Assistant Director, Incharge, Clinical Pathology (upto 18-8-1970), Deputy
Director (Research), Division of Microbiology, (from 19-8-1970).
(Additional charge of Assistant Director, In-charge, Department of
Zoonosis);

Assistant Directors

Dr. A. N. D. Nanavati, M.D. (Bom.),
Assistant Director, In-charge, Department of Virology.

Dr. (Smt.) Alamela Venkatraman, B.A. (Madras), M.Sc. (Madras), Ph.D.
(Madras).
Assistant Director, In-charge, Department of Biochemistry.

Dr. S. S. Rao, M.Sc. (Banaras), Ph.D. (Toronto),
Assistant Director, In-charge, Department of Immunology.

Dr. K. V. Kulkarni, M.B.B.S. (Bom.),
Assistant Director, In-charge, Department of Blood Bank.

Shri Y. S. Nimbkar, M.Sc. (Bom.), Dip. Bact. (Manchester),
Assistant Director, In-charge, Department of Bacteriology.

Dr. R. B. M. Colah, M.Sc. Ph.D. (Bom.),
Assistant Director, In-charge, Department of Analytical and Control,
(Retired from 29-8-1970).

Shri K. G. Ananthanarayanan,
Assistant Director, In-charge, Department of Analytical and Control (from
29-8-1970).

(B)

Dr. M. B. Bhide, M.D. (Bom.),

Assistant Director, In-charge, Department of Pharmacology.

Dr. K. H. Dave, M.D.,

Assistant Director, In-charge, Department of Viral Vaccines (from 22-8-1970).

Heads of Sections

Dr. A. E. Kulkarni, G.B.V.C. (Bom.),

Senior Scientific Officer, (Class I), In-charge, Animal Farm.

Administrative and Auxiliary Section

Shri T. V. Ranganathan, B.A. (Madras), Business Manager,

Additional charge of Assistant Director, In-charge, (Administration).

Shri P. K. Godbole, B.A., B.Com.,

Cost Accounts Officer.

Shri M. T. Jagatiani, B.A.,

Stores Officer (from 1-1-1970 to 31-5-1970), Material Manager from 1-6-1970).

Shri B. V. Gosavi,

Accounts Officer.

Shri A. R. Dhavalikar, G.D.C.,

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Assistant Engineer (Maintenance).

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Public Relation Officer.

Shri N. R. Rao, M.A.,

Statistician.

Shri V. R. Kadgadkai,

Personal Assistant to the Director.

Shri V. K. Gogate, B.A.,

Special Officer (Recoveries),

Shri M. M. Manohar, B.Sc. (Poona), Dip. Lib.(Bom.),

Librarian.

PART I

GENERAL

GENERAL

Haffkine Institute is one of the major organisations in India devoted to advanced medical researches. It is situated in the middle of the City of Bombay and covers an area of thirty acres of land. At one time, it was the residence of the Governors of Bombay. It is under the administrative control of the Government of Maharashtra.

The Institute was first established in 1896 as the Bombay Bacteriological Laboratory under the Directorship of Dr. W. M. Haffkine. Since its inception, the Institute has made extensive development in the fields of research, teaching training and manufacture of essential biologicals and other products. The Institute has now ten technical Departments backed by Administrative Departments. There are service Departments like library, maintenance engineering, animal farm etc. About 1,400 personnel work here. The Institute has also a wing at Pimpri (near Poona), where part of the Immunology Department is located.

The members of the two Boards, namely, Haffkine Institute State Advisor Board and Haffkine Institute Research Advisory Board, act in the advisory capacity.

The primary function of the Institute is to conduct researches in the medical and allied fields. The nucleus of research has been built on communicable diseases with reference to their causation, prevention and cure. It is an important training ground for building research and technical manpower of the State. It is a noted centre for post-graduate studies in microbiology, virology, pathology, biochemistry, pharmacology, medicinal chemistry, applied biology etc. It admits 67 students for M.Sc., or Ph.D. It runs two training courses for science graduates, each one of 9 months' duration, besides short-term training assignments.

The Institute renders public health laboratory services, as it is concerned with the investigations of communicable diseases in the State. At a short notice, the Institute sends expert team to any part of the State for on-the-spot investigations. It tests drugs and other clinical specimens that need expert opinion. Epidemiological studies on communicable diseases form an important part of the manifold activities of the Institute.

The Institute manufactures important biologicals like antitoxins, vaccines, antivenine, lyophilized blood plasma, gamma globulin, diagnostic reagents etc. The products of the Institute are mainly supplied to the Government of Maharashtra, Government of India, State Governments, Armed Forces, Employees State Insurance Agencies, etc. The biologicals, which are required for emergency are available day and night at the Institute.

RESEARCH

The Institute's latest programme has been to co-ordinate the research work between the scientists of ours, the Public Health Department, Government of Maharashtra, and the Department of Preventive and Social Medicine in the medical colleges of the State. In this way, it has been possible to co-ordinate activities on those problems, which require active support from the field and the laboratory. The Institute has been greatly benefited by such an approach.

While making the new plague vaccine which has been developed in the light of research work carried out here, the organisms are grown at 37°C at which they produce the major protective antigenic fraction-I much better than that at 28°C which is the optimum temperature for the growth of *P. pestis*. Normally, *P. Pestis* grows very poorly at 37°C in commonly used media including casein hydrolysate. The new plague vaccine was prepared by growing a virulent strain of *P. pestis* at 37°C in liquid casein hydrolysate fortified with vitamins and salts. Calcium and magnesium salts were added as they were found to be essential for growth at 37°C. The cultures were grown in Haffkine flasks on a rotary shaker at about 100 revolutions per min. for 3 days. The organisms were inactivated with 0.07 per cent (V/V) formalin and preserved with 0.0015 per cent phenyl mercuric nitrate. The potency of these vaccines was assayed according to the I. P. requirements by an active mouse protection test. The test showed that it was as effective as the standard plague vaccine.

The clinical (on volunteers) and serological studies of the old as well as the new vaccines mentioned above were undertaken by Dr. Karl F. Meyer, George Willams Hooper Foundation, San Francisco, U.S.A. The results suggest that the antibody titres from serum collections made on the 6th, 15th, and 30th day after booster inoculation impressively support the conclusion that the new Haffkine vaccine reinoculated in small doses, elicits antibody titres considered indicative of the high immunogenic (protective) efficacy of the antigen. This high percentage is rarely attained with other killed plague vaccine causing relatively moderate side effects.

When the mice were inoculated with the Haffkine Institute old vaccine, the PD₅₀ value was almost identical to that of the new vaccine (0.00168 v. 0.00160), the antigen proved non-immunogenic as previously reported. It is apparent that the old type Haffkine vaccine is immunogenically deficient. It is interesting to note that the mouse protection test conducted independently at the Haffkine Institute and at the Hooper Foundation failed to disclose this deficiency. It seems that mouse protection test (PD₅₀) is not sensitive enough to distinguish between a vaccine giving high antigenic reaction in human being to one which is deficient.

It appears that the new type of killed Haffkine plague vaccine is a highly immunogenic preparation, provided it is administered in a two-dose schedule at least two months apart.

Perhaps, the time has come when the time-honoured custom of diagnosing a case of cholera depended only when classical or El tor vibrios are found in the stool, should be modified. Clinical cholera should also be considered as those diarrhoeal cases, where besides the above organisms, the pathogenic strain of NAG-vibrios could be identified from the stools. Recently, 4 strains of NAG-vibrios, stated to have caused acute gastroenteritis in Sudan and Czechoslovakia have been studied. These strains caused experimental cholera in infant rabbits and characteristic fluid accumulation in the isolated loops of the intestine. The reactions are indistinguishable from those caused by El tor or classical cholera vibrios. Vaccines prepared from these pathogenic NAG strains protected mice from homologous challenge, but not against classical Ogawa or Inaba vibrios. Similar results have been seen in the passive immunization test in infant rabbits.

Live parenteral bacterial vaccines as a method of immunisation against plague has been in use in some parts of the world. The process of freeze-drying of vaccines is a complex one, because the number of organisms surviving the process depends upon a variety of factors. The problem is being investigated in order to find the feasibility of having live plague vaccine. The results show that commonly used suspending media, gum acacia plus nutrient broth with different concentrations of glucose, sodium glutamate, starch, lactose, skimmed milk etc., cause very poor survival rate. One of the factors which affect the survival of the bacteria is the rate of evacuation used during the process of drying.

Biological products are now a days sterilised either by heat or chemicals or gas. The new era of sterilisation by irradiation is not far away from the realm of realities. While experiments are going on in many countries, the Institute has taken steps to explore the possibility of the use of irradiation in sterilizing pharmaceutical products. The first product that has been taken up is the cholera vaccine. It is very interesting to observe that the irradiated cultures are much more potent than the usual formalin killed and phenyl mercuric nitrate preserved vaccines.

In the manufacture of antitoxin in the process that has been adopted here it is necessary to digest the antisera with pepsin. It seems that the residual pepsin was found to be the principal cause of deterioration of antisera during their storage in cold. It has been possible to prepare for the first time active insoluble pepsin which has the advantage of preventing deterioration of the antibody during storage. The other advantages of the insoluble pepsin are that (i) it does not contaminate the system and can be removed completely by centrifugation and filtration, (ii) the reaction can be stopped at any stage by simply separating it, (iii) the enzyme could be used over again if still active, and (iv) there would be no antigens contributed by pepsin which may be responsible for sensitization in some patients.

It is now widely accepted that the presence of Australia antigen (Au) in the sera of persons indicates that such persons have suffered from infective hepatitis or serum hepatitis. In India, both infective and serum hepatitis are widely

prevalent, and the Institute which is running a blood bank, should take special interest in finding out the presence of the Australia antigen in the sera of blood donors. A very large number of sera obtained from patients who suffered from infectious hepatitis or serum hepatitis were studied. It was observed, that 10 per cent of the cases, which gave history of hepatitis, the sera showed positive reaction to Australia antigen. The sera of about 5 per cent of the professional donors also showed positive to Australia antigen. This indicates the potential danger in transfusing blood from donor to recipient.

There are very few places in India where the genetic characters in some endogamous groups in the Indian population are being systematically studied. This particular study has two important aspects. The one which is of immediate importance to this Institute is to find out the genetic defects in the blood of donors. Such persons should serve as donors. The second important aspect is the anthropological delineation of the origin of various groups in the population. Presently, 4 groups of Indian Muslims have been studied to find out the differences, if any, amongst them. The groups studied are Khojas, Bohras, Moplas and Misgars. The study involving 8 genetic loci including blood groups, abnormal haemoglobins G-6-PD deficiency, colour blindness, ability to taste phenylthiocarbamide (P.T.C.), etc., has revealed that these groups apparently do not show phenotypic variation with the genetical distance, thereby indicating the vast divergence between Khojas and Bohras on the one hand and Moplas and Misgars on the other.

As a result of investigation carried out here, berberine, and alkaloid from the Indian medicinal plant, *Berberis aristata* Linn., has been introduced in the market as an anticholera and antidiarrhoeal drug. This compound, either singly or in combination with other substances, is being used clinically for the last 4 years. Recent work has provided further evidence that berberine is effective in the treatment of experimental cholera induced by cholera toxin in the baby rabbits. Biochemical studies on the mode of action of some drugs like berberine, furazolidone, chloromycetin, ampicillin and alcohol on the two strains of *V. cholerae* using labelled compounds like amino acids sugars and nucleotides, have yielded interesting results. The alkaloid berberine, in addition to increasing the lag phase, seems to cause a sort of membrane damage and detoxification of the toxin. The synthesis of the macro molecules are affected. Furazolidone and chloromycetin have more or less similar effect on the two strains—both increase the lag period, inhibit the protein but increase the RNA synthesis. Ampicillin and alcohol are observed to cause lysis of the cells and the synthesis of all macro molecules are lowered.

A laboratory study of the influence of carbohydrates on dietary fats and lipid metabolism in the albino rat suggests that a commonly consumed disaccharide like sucrose does not only enhance the hypercholesterolemic effect of a saturated dietary fat like coconut oil but definitely counteracts the hypocholesterolemic effect of an unsaturated dietary fat like safflower seed oil. Marked alterations were observed in tissue lipids and fatty acid components in the presence of increased amounts of sucrose in the diet.

Currently there is great interest in the study of serum haptoglobin levels in relation to their genetic patterns. This is of great diagnostic value. It is found that haptoglobin levels are extremely low or absent in cirrhosis of liver, and in infectious hepatitis. In such cases, the patterns are mostly Hp 0-0 or Hp 2-1. High values are found in nephritis in pulmonary tuberculosis, and in some post operative or infected patients.

For the past few years, the Chemotherapy Department has been working on synthetic compounds with possible oral antidiabetic action. In this effort, a large number of compounds were synthesized, some of which showed good potency. One of these was N^1 -benzenesulfonyl- N^1 isopropyl urea (R-94) which was very potent but was found to be some what toxic clinically. Attention was next diverted towards 1, 2, 4-(H)-triazoles, and a number of compounds were synthesised and screened. Out of these two, namely, 5-*p*-chlorophenyl-4-ethyl-4H-1, 2,4-triazole-3-thiol and 4-ethyl-1-5-*p*-sulfamoyl-phenyl-4H1, 2, 4-triazole-3-thiol produced substantial blood sugar reduction (53 per cent and 45 per cent respectively) in normal as well as alloxan treated rats. Preliminary acute toxicity study revealed that these compounds have very low order of toxicity. 5-*p*-chlorophenyl 4-ethyl-4H-1, 2-4-triazole-3-thiol has been now prepared in larger quantity and its detailed toxicity study is being carried out. A number of enquiries from different parts of the world were received for exploitation of the product for commercial use.

Sooner or later, the Public Health Department has to embark upon mass immunization with oral polio vaccine. Prior to it, it is necessary to understand the immunogenic status of the children in the urban as well as in rural areas. The Institute has done substantial work in the urban areas. During the last year, a team of 5 workers was stationed at the Primary Health Centre, Sirur (Poona District) and had undertaken study in four villages in that Taluka. Out of 1,316 children population (below 6 years), 921 were under study. Although the entire data are not yet available, it seems the incidence of paralytic poliomyelitis in these villages during the last 6 years was 1 case per 8,000 population per year, as compared to cases of paralytic poliomyelitis in an urban area like Bombay, where it is approximately 1 per 10,000 population per annum.

It has also been observed that 20 children in this rural area continue to excrete polio viruses in their stools. All the three types of the polio-viruses were encountered with slight predominance of Type II. There was no immunization programme in Sirur Taluka before.

It was pointed out in the last year's report that the incidence of poliomyelitis among the vaccinated children is a disturbing feature. During the last year 82 cases were recorded with history of paralytic illness inspite of immunisation with oral polio vaccine in the past. Complete epidemiological and virological studies could be done in 70 cases out of which inadequate immunization was detected in 37, while 33 individuals had received all the 3 doses of the oral polio vaccine. Most of the children who had paralytic illness were below the age of three.

The result of one of the investigations which has attracted attention of the Scientist working in different parts of the globe is the inhibition of viral oncogenesis by non-oncogenic viruses. In this study a non-oncogenic rhabdovirus (rabies virus) produced blockade of tumour induction by a strain of *Rous Sarcoma* virus (Bryan Master Standard), an avian sub-group A leukovirus, in the subcutaneous wing web tissue of a high percentage of 5 to 9 day old white Leghorn chickens. The effect was transient ; chicks showing complete sarcoma blockade were found to be susceptible upon subsequent challenge. The blockade effect was demonstrated as an increase in the latent period, the absence of R.S.V. induced tumour, and absence of anti-RSV neutralizing antibody in tumour blocked chicks. The mechanism(s) underlying the effect is (are) not fully understood. However, the effect was reversed by hydrocortisone treatment and actinomycin D. The effect was strain specific, RV failing to block Schmidt-Ruppin strain of R.S.V. While interferon activity may partially explain the mechanism of action, it is possible that more than one type of mechanism is involved. For this work Dr. S. M. Desai, Department of Virology, has been awarded the Kanolkar Prize for the year 1970.

The Institute has worked upon the trial of measles vaccine at the request of the Indian Council of Medical Research, New Delhi. The analysis shows that the seroconversion obtained in Phase I of the trial, wherein blood samples were drawn before, and one month after the vaccine administration was very satisfactory, as measured by the neutralization test. Ninety-nine per cent of the children receiving the vaccine showed four-fold and higher rise in titre, none of the children receiving placebo, showed a rise in titre. The complement fixing antibodies were developed in 101 out of 120 vaccines (84.16 per cent). The follow up of these cases after one year for measles or measles-like illness showed an incidence of 2.5 per cent in vaccinated and 9 per cent in the placebo.

Another survey which was undertaken in a rural area near Aurangabad, in co-operation with the Department of Preventive and Social Medicine, Aurangabad Medical College, showed interesting results. The survey covered 564 families (comprising of 3,621 individuals) in two villages served by the Rural Health Unit at Paithan, 40 miles from Aurangabad (Maharashtra State). Approximately 44 per cent of all the children below 5 years of age had suffered from measles, the incidence rising gradually upto 80 per cent of the children between 4 to 5 years of age. The duration of the illness in 80.2 per cent was from 1 to 15 days. Complications and prolonged illness were relatively rare. It was also observed that 90 per cent of children exposed to measles (family contact) gets the disease, while 10 per cent escaped. It appears that the history of the occurrence of measles is a reliable index of the incidence of the disease in a given population.

The Department of Zoonosis has embarked upon a study on the epidemiology of filaria for the first time. In this venture, the Joint Director of Health Services, Government of Maharashtra and the Medical Officer, Thana Municipality have participated. Every house covering a population of 1,61,000 were numbered. Every 10th household was surveyed by collecting blood for the detection of micro-filaria. Out of the 9,632 slides so far examined, 382 was found positive to micro-

filaria. Simultaneously, the entomological survey of the same area has been undertaken. The density of the vector specimen (of mosquitoes) *C. fatigen* has been determined. The mosquitoes were dissected with a view to find out their infective rate. This epidemiological study is expected to help the public health Department to control and eradicate filariasis from Thana area as their first step.

INSTITUTE NEWS

The World Health Organisation Combined Course in Epidemiology, which was held at the National Institute of Communicable Disease, included a programme of the participants' studies at the Haffkine Institute. About 12 Epidemiologists drawn from different parts of the world, came to the Haffkine Institute to take part in the lectures, discussions, demonstrations, organised at the Institute. The team was led by Dr. S. C. Pal of the National Institute of Communicable Diseases.

The Fourth Training Course in Cholera Control, organised by the World Health Organization and the State Public Health Department, in collaboration with the Haffkine Institute, was held here from January, 18th to 23rd, 1971. Many eminent cholera experts gave lectures to the participants, the medical officers from the districts and Public Health Department.

Shri S. M. Desai, Junior Scientific Officer, Department of Virology, was awarded the Kanolkar Prize for his distinguished paper on "Sarcome blockade *in vivo*: Rabies Rous System in chickens".

Dr. K. V. Kulkarni, Assistant Director in Charge Department of Blood Bank, has been invited to present a paper at the 2nd Meeting of the Asian Pacific Division of the International Society of Haematology at Melbourne, Australia.

Dr. N. K. Dutta, Director, has been appointed as the member of the Board of Visitors of the Faculty of Medicine of the Baroda University, for a period of 3 years. He was also appointed as member of the Editorial Board of the Indian Journal for Experimental Biology of the Council of Scientific and Industrial Research for a period of 3 years. Dr. Dutta has been invited to deliver the Captain Narendra Nath Dutta Memorial Lecture and also Sir Anil Ratan Sorkar Oration at Calcutta.

Dr. N. K. Dutta was deputed to attend the 22nd Annual Conference of the Indian Pharmaceutical Congress held at Calcutta.

Dr. A. N. D. Nanavati, Assistant Director in-Charge, Department of Virology, attended the National Conference of Infectious Diseases at Aurangabad, Maharashtra State.

Dr. M. B. Bhide and Dr. S. Mukherjee were deputed for training in medical uses of radioisotopes at the Bhabha Atomic Research Centre for a period of 5 weeks.

Dr. F. L. Saldanha, who was deputed for an year's study in U.S.A. under W. H. O. Fellowship returned to India.

Two posts of Dy. Directors, one for the Division of Microbiology and another for the Division of Pharmacology and Pharmaceutical Chemistry, have been created. In the former Dr. M. V. Sant has been appointed and in the latter Dr. C. V. Deliwala. Both of them are senior-most Assistant Directors of the Haffkine Institute.

Mohd. Khleifat Hussain of Jordan continued to work at this Institute under a W. H. O. Scholarship.

The Institute participated in two exhibitions, one was the Yeotmal Agro-Industrial Exhibition organised by the Zilla Parishad, Yeotmal; another Agro-Industrial Exhibition was held in Aurangabad and this was organized by the Director of Publicity, Government of Maharashtra.

The Institute continued to attract grants from different sources. The International Atomic Energy Agency sanctioned a grant of 4,660\$ (U.S.) for a joint project between the Haffkine Institute and Dr. Oscar Felsenfeld, Chief, Division of Communicable Diseases, Tulane School of Public Health and Tropical Medicine, U.S.A. on the "Use of antigens labelled with radioisotopes in serological epidemiology". Dr. N. K. Dutta, the Director received a grant of \$3,000 for his study on cholera from the World Health Organisation.

The I. C. M. R. Scheme for the study of antileprosy drugs was sanctioned under Dr. M. B. Bhide in the year 1971-72.

Many of the grants previously sanctioned by the I.C.M.R., Council of Scientific and Industrial Research, Government of India, Government of Maharashtra, Bhabha Atomic Research Central Defence Department, etc. continued for the year.

The Institute offers facilities to the members of the staff as well as outside candidates to continue their studies in various subjects and simultaneously, encourage them to take Master's or Doctorate Degrees of the Bombay or other universities. At present, there are 14 recognised teachers at the Institute. The subjects covered by them are microbiology, biochemistry, organic chemistry, applied biology, pathology, bacteriology and pharmacology and zoology.

In this year 3 post graduate students qualified for Ph.D. degree and 9 for the M.Sc. degree of the Bombay University.

The Institute conducts two major training courses, each of 9 months' duration. Out of the 9 students admitted in the Diploma in Medical Laboratory Technology Course (Bombay University), 8 qualified for it. Seven science graduate successfully completed the Technicians Training Course and were awarded certificates by the Institute.

During the year 1970-71, 60 persons were trained in short-term training courses in different fields of drug analysis, standardization of anti-toxins and poisons, preparation of vaccines, clinical pathology, blood bank technology, etc.

Many distinguished scientists and dignitaries numbering 83 visited the Institute during the year. Several eminent scientists were invited to address the members of the Critics Club of the Institute.

During this year, far reaching changes have been brought about by re-organizing the departments and sections. The Blood Bank Department was shifted to a new place, thereby removing the congregation of blood donors near the main entrance of the Institute. As a result of the shifting of the canteen at the central place within the compound of the Haffkine Institute a good amount of space adjoining the library has been vacated. This space was effectively modified to accommodate new reading room for the library which was a much-needed requirement. The Analytical and Control Department, which was spread out in the different parts of the Institute, has been consolidated into one place and housed where it was previously occupied by the Nutrition section of the Department of Nutrition and Biochemistry.

The original Department of Nutrition, Government of Maharashtra, was amalgamated in 1962 with the Department of Biochemistry at the Haffkine Institute. Today, nutrition has become an applied problem. As a result of this, the Nutrition Section of the Department of Nutrition and Biochemistry was transferred under the Director of Health Services. The laboratory with all its equipment, books, furniture, etc. has been shifted at the Public Health Laboratory at Nagpur.

Considerable amount of staff amenities have been provided during the year. In Bombay, residential quarters for 192 employees and their families are under construction. At Pimpri, the construction of the Class III and Class IV quarters has been completed, as a result of which almost every member of the staff there has been provided with good quarters.

The number of female workers in the Institute has greatly increased during recent years. It is necessary to provide them with resting room during off hours. It has been possible to do so during the year under review.

The Sports Club Pavilion, which was under construction, was ready for occupation during the year. In this building indoor badminton court, room for playing table tennis, housing the library, offices, etc. have been provided. A circulating library has been initiated.

The members of the Sports Club are very fortunate in having Hon'ble Health Minister Dr. Rafiq Zakaria as the Chief Guest at the Haffkine Institute Sports Club Prize Distribution Ceremony. Begum Zakaria was kind enough to give away the prizes to the successful contestants.

A full-time post of Medical Officer for looking after the dispensary and health of the workers of the Institute and their families was sanctioned by Government. A new lady Medical Officer has recently joined her duties. Thus, a long-felt need of the workers has been fulfilled.

Although, there is a full-time Medical Officer's post at Pimpri, unfortunately it has not been possible to fill it up, due to the paucity of doctors. The health of the workers is being looked after by a part-time Medical Officer there.

The Director and the senior members of the staff were greatly benefited by the advice and guidance of the Haffkine Institute State Advisory Board and the Haffkine Institute Research Advisory Board. They wish to thank all the members for taking keen 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 at Sachivalaya, without which many of the changes brought about in the Institute could not have been done.

The Heads of Departments and senior members of the staff significantly contributed to the different activities, be it in the field of research, production, training and teaching. Without their active co-operation which the Director received, it would not have been possible to keep up the tempo of the work.

It should be realised that the Institute is dependant on the integrity, conscientiousness, drive and skill of the individual members of the staff and I am happy to say that all the members contributed to the best of their ability. The Director takes this opportunity to thank them for the continued support he has received from the administrative, scientific and non-scientific workers of the Institute.

PART II

RESEARCH

A. PROGRESSIVE RESEARCH

B. DEPARTMENTAL RESEARCH

A. PROGRESSIVE RESEARCH

ANTIFERTILITY DRUGS

VIDR-2GD, a constituent isolated from the seeds of Wild Banana were found as an antifertility agent in mice, rat, guinea pigs and hamsters. The antifertility action appears to be between fertilization of ovum and implantation of the zygote. In case of rabbits and rats critical dose levels are required to inhibit pregnancy to a significant extent.

Since VIDR-2GD is not a pure substance, further chemical and biological experiments were carried out to characterise it and also to locate and characterise any other antifertility constituents present in it. The isolation of the active principle is being done in collaboration with Dr. S. K. Bhattachary of the Indian Institute of Technology, Bombay. Forty-two fractions (see Table 1) from the seeds have been isolated and tested for their antifertility action in mice. Out of these, a relatively pure substance AJA 31 showed 10 per cent effectiveness in mice when administered orally in a dose of 1.0 mg per day during pre-implementation period. Further work is proceeding with the other constituents that have been received.

TABLE 1

Anti-fertility activity of fractions isolated from Banakadali

Animals: Mice

Drugs		Dose (mg)	Total No. of animals	No. of pregnant animals	No. of young ones	
AJA	7	1.0 for 10 days	8	1	6	normal.
"	8	"	5	0	0	
"	9	"	5	0	0	
"	10	"	5	0	0	
"	11	"	5	0	0	
"	23	"	5	0	0	
"	24	"	5	1	6	normal.
"	26	"	5	0	0	
"	31	"	10	0	0	
"	32	"	5	2	9, 7	normal.
"	33	"	5	5	4, 7, 8, 9, 12	normal.
"	34	"	5	3	8, 7, 7	normal.
"	35	"	5	4	7, 6, 4, 9	normal.
"	36	"	5	3	7, 7, 8	normal.
"	38	"	5	3	5, 8, 9	normal.
"	39	"	5	4	5, 7, 6, 6	normal.
"	40	"	5	2	6, 9	normal.
"	42	"	5	2	6, 8	normal.

CHOLERA

Berberine in toxin induced experimental cholera

In our earlier reports, it has been shown that berberine is as effective as chloramphenicol or tetracycline in controlling experimental cholera in the infant rabbits, due to the infection of the cholera vibrios. Our present study relates to the action of berberine on cholera in the infant rabbits following the administration of a single fatal dose of choleraogenic toxin. The results showed that if the treatment had begun 24 hours before the administration of the toxin, then a number of animals could be saved from death or the life of the remaining animals could be prolonged as compared to the control untreated animals.

In vitro studies by many investigators have shown that berberine inhibits the growth of enteric bacteria, namely, *Shigella*, *dysenteriae*, *E. coli*, *Staphylococcus aureus*, besides *V. cholerae*, though it is not as potent as chloramphenicol or tetracycline.

Choleraic symptoms induced in infant rabbits following the administration of the toxin was a severe disease. Under the condition of the experiments, the animals succumbed to the disease in course of 18 hours. If the benefit of any drug is to be assessed on such a model, then it has to be administered well before the toxin is given. If the drug could prevent or prolong the life of the animal, then it would not be unreasonable to assume that it would be useful clinically where the toxin formation in the intestine is progressive and gradual, being dependent on the multiplication of the vibrios. Pre-treatment with berberine could save infant rabbits from the adverse effects of choleraogenic toxin and could save about 70 per cent of them. It has been shown earlier by Bhide and his co-workers that the level of berberine in the blood of infant rabbits reached a good concentration 8 hours following the administration of it and the drug was found in the blood even after 72 hours. For this reason, pre-treatment was necessary as mentioned before, so that appropriate concentration of the drug reached the blood before the toxin reached the site of action.

Summing up the results, it may be pointed out that those animals which lived, diarrhoea was controlled within 40 hours and the severity of the symptoms was reduced in those cases where there was prolongation of life.

The mechanism by which berberine controls diarrhoeal symptoms, be it in infected or toxin administered rabbits, still remains speculative. It has been shown by Modak and his co-workers that the protein and lipid synthesis were stimulated by berberine to the extent of $2\frac{1}{2}$ times more than the control. Since cholera toxins (endo as well as exo) are known to contain both protein and lipid components, the formation of toxin in the presence of berberine could be hampered. Analysis of fatty acid composition (lipid components) of *V. cholerae* in the presence of berberine revealed that the alkaloid caused disturbance in the relative proportion of various fatty acids, presumably due to biosynthetic disturbances. High turnover of proteins also suggests the

possible formation of heterogenous proteins which was degraded. It might be that berberine did not allow the formation of active and intact toxins according to them.

There are a few pharmacological properties of berberine such as depression of the intestinal peristalsis, removal of inflammatory congestion of internal surface of intestine which may promote recovery from diarrhoea.

While the anti-bacterial effect of berberine on *Vibrio cholerae* could partly be responsible for its action in clinical cases, the real action of the drug as to how it arrests the severe diarrhoeal symptoms could not be fully explained. Here it is shown that berberine is effective in experimental cholera when toxin was administered. It is not only effective against diarrhoeal symptoms caused by *V. cholerae* and related organisms, but also by other intestinal organisms like *Shigellae*, *Pseudomonas*, *E. coli*, *Proteus*, parasites and protozoa. Action of berberine was also not due to the interaction between the cholera toxin and the drug. It appears, therefore, that the host plays an important part in the recovery from diarrhoea when medicated with berberine through varied mechanisms, some of which are mentioned above.

Purification of cholera toxin as a prelude to the preparation of toxoid

It has been recognised now that for protection against cholera both anti-bacterial as well as anti-toxic immunity is necessary. The anti-bacterial immunity is shown by the usual type of vaccines grown on agar but this vaccine may not elicit protection against cholera toxin. Our idea was to study the anti-genicity of the cholera toxin or toxoid prepared out of it. In our previous work we have found that the cholera toxin retains its antigenicity after treating with formalin. Toxoid thus prepared was also compared with toxin and agar grown vaccine for its antigenicity by using passive protection test, intestinal loop challenge and also by determining the agglutination titre of the antisera. These findings are now further confirmed by determining the vibriocidal titre of the antisera.

Determination of the vibriocidal titre

Adult rabbits were immunised by giving two injections each of 2 ml. of the toxin, toxoid and the vaccine intra-muscularly at an interval of fifteen days. The animals were bled a week after the second injection and serum was collected for carrying out the vibriocidal test. Estimates were made by comparison with controls of the proportion of the original inoculum which remained viable and formed colonies under these conditions. Inhibition is considered significant if 50 per cent of the bacteria were killed and the titre was regarded as the highest dilution of serum which killed 50 per cent or more of the starting population. Controls consisted of the mixtures of the same amount of bacterial suspension. Dilutions were made using separate pipettes for each transfer.

Serum of rabbits immunized with toxin or toxoid gave a vibriocidal titre of 10^6 , while the serum of rabbits immunized with vaccine gave a vibriocidal titre of 10^{-5} .

Purification of cholera toxin

Choleraogenic toxin was prepared by using synthetic medium of Finkelstie and Lankford (Jr. Imm. 1966, 96, 440). This toxin which produces typical diarrhoea followed by death when one ml. of it was injected intraintestinal to 10 days old infant rabbits was concentrated by using polyethylene glycol (Carbowax). The toxin was purified by different methods like starch block electrophoresis and column chromatography on Sephadex gel.

The fractions were tested for their choleraogenic property in infant rabbits and skin permeability factor in adult rabbits. The results are given below. (Table 2).

TABLE 2

Fr. No.	Choleraogenicity	Skin permeability factor	O, D, at 280 m, u.
—10	Nil	.. Nil	.. 0.45
— 9	Nil	.. Nil	.. 0.29
— 8	Nil	.. Nil	.. 0.33
— 7	Nil	.. Nil	.. 1.2
— 6	Nil	.. Nil	.. 0.75
— 5	Nil	.. Nil	.. 0.65
— 4	Nil	.. Nil	.. 0.25
— 3	Positive	.. Positive	.. 2.0
— 2	Positive	.. Positive	.. 2.0
— 1	Positive	.. Positive	.. 1.5
+ 1	Nil	.. Nil	.. 1.1
+ 2	Nil	.. Nil	.. 1.0
+ 3	Nil	.. Nil	.. 0.74
+ 4	Nil	.. Nil	.. 0.48
+ 5	Nil	.. Nil	.. 0.43
+ 6	Nil	.. Nil	.. 0.55
+ 7	Nil	.. Nil	.. 0.40
+ 8	Nil	.. Nil	.. 0.30
+ 9	Nil	.. Nil	.. 0.55
+10	Nil	.. Nil	.. 0.8

The diffusion of each of the fractions obtained in starch block electrophoresis was carried out after concentrating the sample using polyethylene glycol (carbowax). The results are given in the Table 3.

TABLE 3

Well No.	Content of the well	No. of Lines			
		Major	Medium	Faint	Total
1	Toxin	4		1	5
2	Fraction No.—1 ..	2		1	3
3	Fraction No.—2 ..	1		1	2
4	Fraction No.—3 ..	1		1	2

The following are the findings of experiments designed to purify cholera-genic toxin:—

(i) Fractions eluted from starch block electrophoresis when tested for cholera-genic property and skin permeability factor only—1, —2 and —3 gave both the reactions.

(ii) When these fractions were tested along with crude toxin by gel diffusion test to assess the amount of purification achieved, crude toxin gave five precipitin lines fraction—1 gave 3 precipitin lines while —2 and —3 gave 2 precipitin lines.

(iii) Cholera-genic factor and permeability factor are present in the same fractions and since these fractions gave 2 distinct precipitin lines by gel diffusion it may be possible that they are two different factors having very close electrophoretic mobility.

Studies on Non-Agglutinable *Vibrios* which had caused choleraic symptoms

In the laboratory animals, NAG produces cholera-like reactions which are indistinguishable from those produced by *El. tor* or classical cholera vibrios.

In the last few years, a good amount of work has been done in the field of cholera vaccines in search of a better one which might give a higher degree of protection and for a longer duration.

Experimentally, a vaccine gives good protection against homologous challenge but in the case of heterologous challenge, the protection that could be afforded by any given vaccine is unpredictable, at least in the case of cholera.

How far the present day vaccines would be effective against pathogenic strains of NAG would be an interesting study.

Through the courtesy of the W. H. O., four strains of NAG from Sudan and Czechoslovakia, which had caused severe "gastroenteritis", were received. The Czechoslovakian strain produced explosive outbreak of gastroenteritis in a group of young men. These patients had diarrhoeal symptoms and also quarter of them vomitted. The Sudanese strain caused acute choleraic diarrhoea. None of these strains caused death in the patients.

Pathogenicity and antigenic characters of the four NAG vibrios have been studied.

The infant rabbits became sick and the diarrhoea had begun at the end of 13 to 17 hours following an intraintestinal inoculation of the NAG strains. The diarrhoea progressively increased and ultimately the animals died within 24 hours. The animals were dehydrated. On opening of the abdomen, the large intestine displayed fluid accumulation. This fluid looked like rice water. The *ante-mortem* and *post-mortem* pictures were similar to that produced by classical Inaba or Ogawa Vibrios in baby rabbits.

All the four strains produced characteristic accumulation of fluid in the isolated loops of adult rabbits. The control loops injected with normal saline evoked no reaction.

The LD₅₀ of the NAG strains 280-13026 (Sudan), 281-13080 (Sudan), 282-13031 (Sudan) and 16/66-10125 (Czechoslovakia) was 7.2×10^2 , 4.4×10^2 and 44 cells respectively in mice. Under similar condition, Ogawa 5321 and Inaba 569 B were virulent. Their LD₅₀ was 3.5 and 2.1 cells respectively.

Active mouse protection results showed that when the animals were immunized with NAG live or killed vaccines and challenged with homologous NAG strains, they were protected against the infection. On the other hand, when the animals were challenged with heterologous cultures of *V. cholerae* Inaba 569B, or Ogawa 5321, they died within 24 hours. Similarly, when the animals immunized with cholera-El.tor vaccine (Haffkine) were challenged with *V. cholerae* Inaba 569B or Ogawa 5321 culture, the animals were almost completely protected. With the heterologous challenge culture of NAG 280-13026 (Sudan) or 16/66-10125 (Czechoslovakia) all the animals succumbed to the disease even when the mice were immunized with a high dose of Vibrios.

Passive protection test in infant rabbits using hyperimmune antisera raised in adult rabbits against living vibrios showed that NAG 280-13026 and 16/66-10125 culture antisera gave significant protection to animals against homologous challenge. The same antisera failed to do so against heterologous challenge by *V. cholerae* Inaba 569 B or Ogawa 5321.

It is also observed that antisera raised against *V. cholerae* Inaba 569 B and Ogawa 5321 which gave significant protection against homologous challenge did not give any protection when challenged with NAG 280-13026 (Sudan) or 16/66-10125 (Czechoslovakia) culture.

The toxicity studies have indicated that NAG killed vaccines were non-toxic in mice and guinea pigs. The live vaccines caused death in all the mice and guinea pigs. These animals died within 24 hours.

The experimental findings demonstrated that all the four strains are pathogenic to infant rabbits producing severe diarrhoeal and other characteristic symptoms which are encountered in these animals when infected with classical or El tor vibrios. All these four strains produced characteristic fluid accumulation in the isolated loops of the adult rabbits.

The virulence test carried out in mice showed that the LD₅₀ of NAG strains 280-13026 (Sudan), 281-13080 (Sudan), 282-13031 (Sudan) and 16/66-10125 (Czechoslovakia) was less than those of Ogawa 5321 and Inaba 569B. This might indicate their less pathogenicity in human beings. They may be less effective in causing death. Clinically, it has been seen that the Czechoslovakia strain, although it produced explosive gastroenteritis, did not cause any death. It could be argued that prompt treatment might have averted the fatality.

The mouse protection test carried out with live and killed vaccines showed that vaccines made from NAG vibrios were protective against homologous change but not when challenge was with Inaba 569 B or Ogawa 5321.

Cholera-El Tor Vaccine (Haffkine) is a quadrivalent vaccine used currently in the prophylaxis against cholera. This vaccine could protect animals against a challenge with an extremely virulent *V. cholerae* 569B or Ogawa 5321. But using an immunizing dose of 15×10^7 cells which was 125 times more than the organisms contained in the protective dose of standard vaccine, the animals could not be protected against an infection with NAG 280-13026 or 16/66-10125 strain, even though these strains were less virulent than Ogawa 5321. Moreover, as mentioned earlier, NAG vibrios could be responsible for outbreaks of cholera-like diseases. If such a situation arises, the present Cholera-El tor vaccine (Haffkine) is likely to be infective against NAG infection.

Perhaps, the time has come when the time-honoured custom of diagnosing a case of cholera only when classical or El tor vibrios are found in the stool should be modified. Clinical cholera are those diarrhoeal cases where, besides the above organisms, the pathogenic strains of NAG could be identified in the stools.

DIPHTHERIA

Cellular composition of *C. diphtheriae*

The earlier studies on the cellular composition and fatty acid pattern were carried out using the PW-8 strain which is used for toxin production at this Institute. This strain belongs to the 'Gravis' type and epidemiological studies have shown that the rate of incidence of diphtheria in the affected population is more of the Mitis type (Gravis 39 per cent, Mitis 57 per cent and Intermedius 4 per cent). These three strains vary greatly in their virulence

nd capacity to produce toxin (data from Clinical Pathology Department). In the present study, the three strains were included along with PW-8 to get comparative idea of their biochemical and metabolic patterns.

(i) The organisms were grown in Wadsworth medium for 4 days at 34° C in Roux bottles. Analysis for proteins, RNA, DNA and total lipids were carried out on the washed cell-mass after drying. Table 4 gives the figures for the gross analysis.

TABLE 4
Cellular composition of the 3 strains

Strain	Total nitrogen g. %	Protein g %	R.N.A. g. %	D.N.A. g. %	Total lipids g. %
PW-8	10.8	46.9	16.2	3.2	16.4
Gravis	10.6	44.2	15.6	2.9	17.1
Mitis	10.2	40.7	14.1	3.1	14.8
Intermedius	9.8	40.6	12.4	3.4	10.5

(ii) *Fatty acid analysis.*—The total lipids were extracted and processed for fatty acid analysis by G.L.C.

TABLE 5
Fatty acid pattern of total lipids of the 4 strains

Fatty acid	PW-8	Gravis Percentage	Mitis Percentage	Intermedius Percentage
Lower than C ₆ : 0	1.4	1.95	4.74	trace
C ₆ : 0	0.6	trace	trace	5.6
C ₈ : 0	1.5	trace	0.51	6.0
C ₁₀ : 0	trace	trace	0.94	trace
C ₁₂ : 0	trace	trace	1.88	2.5
C ₁₄ : 0	1.6	2.14	2.82	trace
C ₁₆ : 0	1.8	1.71		3.0
C ₁₆ : 0	44.2	43.96	41.0	29.25
C ₁₈ : 1	46.0	47.24	32.2	48.75
C ₁₈ : 0	4.0	3.78	1.25	5.25
C ₁₈ : 1	1.4	trace	16.9	trace

As is seen from the Table 5, the Intermedius strain differs considerably from the other three. Palmitic acid constitutes a major portion of both the saturated and unsaturated fatty acids. It is interesting to observe that the Mitis strain

contains a high amount of $C_{18} : 1$ (oleic) as compared to the other three. The maximum chain length is upto only $C_{18} : 0$ and none of the strains were found to contain polyunsaturated fatty acids.

Phage typing of *C. diphtheriae*

A set of 24 diphtheria-phages were obtained from the WHO International Reference Centre in Roumania. Of these, 21 phages were found to be viable. By adopting the recommended procedures of phage typing, a total of 80 strains isolated locally were tested for phase typing. These 80 strains were from a total of 11 strains isolated from 550 throat swabs.

Out of the 80 strains, 50 strains were found to be lysosensitive to phage 19 only. Nine strains showed a phage pattern and 21 were found to be untypable. Broadly, the results are in agreement with the Roumanian workers.

Enzyme studies in *C. diphtheriae*

Transaminases such as oxalacetate transaminase and pyruvate transaminase are some of the key enzymes in protein synthesis in animals and microorganisms alike. To find out the efficiency of transaminase reactions in *C. diphtheriae*, these enzymes were estimated from the cells grown on Wadsworth medium for three days at 34°C . The results are shown in Table 6.

TABLE 6

Transaminase activity in different strain of C. diphtheriae

Strain				Oxal acetate transaminase pyruvic acid unit/hr./mg. of supernatant protein	Pyruvate transaminase pyruvic acid unit/hr./mg. of supernatant protein
PW-8	..	..	..	143	3
Gravis	..	..	..	160	2
Mitis	..	..	..	83	10
Intermedius	..	..	..	42	Nil

PW-8 and gravis strains show higher levels of oxal acetate transaminase activity while the pyruvate transaminase activity is practically nil.

ENTEROVIRUSES

The Enterovirus Unit in the past had concentrated its activity in urban areas. It is now considered essential that the data obtained in urban areas be compared with similar studies in rural areas. For this 4 villages in Sirur Taluka (Poona District) have been selected. All the 4 villages consist of stationary agricultural community. Work was started in February 1970. A team of 5 workers was stationed at the Primary Health Centre, Sirur. Data and materials were collected by house to house visits. Collection of materials for enterovirus survey and survey of antibody pattern against poliovirus has been completed.

For the collection of blood samples for serological studies difficulties are often encountered in the field in terms of non-cooperation of the participants and inadequate facilities for collection of blood by venepuncture. The unit has now standardised a simpler technique in which peripheral capillary whole blood could be collected on filter papers which can be subsequently used in the laboratory for antibody estimations. In Sirur, wherever difficulties were experienced in collecting blood by venepuncture, fingerprick technique was used. As a result, refusals from the participants were comparatively much fewer.

The total population covered (4 villages) was 12,000. The children under study were 921. One thousand three hundred and sixteen samples of stools and 204 specimens of blood were collected. The children aged from 6 months to 6 years were under investigation. The results are given below :

Viruses identified so far were Polio type—1,4; Polio type-2,9; Poliotype-3,7; Coxsackie A-1,1; Coxsackie A-9,2; Coxsackie B-1,1; Coxsackie B-2,4; Coxsackie B-3,2; Echo-1,2; Echo-2,1; Echo-3,1; Echo-4,1; Echo-5,1; Echo-6,2; Echo-7,22; Echo-9,9; Echo 12,1; Echo-13,4; Echo-19, 10; Echo20,1.

Marker studies on poliovirus isolates

During the enterovirus survey in the child population of Sirur Taluka, a total of 20 children out of 921 were found to excrete polio virus. All the three types of polio virus were encountered with a slight predominance of type 2 polio virus (Polio 1-4, Polio 2-9 and Polio 3-7). There has been no immunization programme in Sirur Taluka so far.

Of the 20 poliovirus isolated from the children of Sirur, 8 strains (Polio 1-3 and Polio 3-5), have been studied so far by genetic marker test. Study on reproductive capacity at higher temperature (40° C) has been performed on the 8 isolates. All the 3 strains of polio virus type-1 are found to be of an intermediate character. Of the 5 strains of polio virustype 3, 3 are found to have wild character, 1 strain of intermediate type and 1 strain belonged to vaccine type.

Paralytic poliomyelitis in Sirur

While surveying the 4 villages for enterovirus study attempt was made to find out the incidence of paralytic poliomyelitis in the local population. During the last 6 years (1965 to 1970) 9 cases of paralytic polio were recorded by the team.

PLAGUE

Investigation in susceptibility of rodent population in Maharashtra to *P. pestis* and related serological studies.

The continued absence of plague in the State of Maharashtra apparently indicates three possibilities :—

(i) The natural foci have become inactive or have died out or (ii) the majority of rodents have become genetically resistant to infection with *P. pestis* or (iii) the plague infection is present among the rodents but does not become apparent due to the change in environmental factors.

The existence of one of these factors or combination of these factors can be elucidated by continuous study of the rodent population of the areas of natural foci or their neighbourhood. With this end in view, the work of studying the susceptibility of rodents in different regions of the State of Maharashtra started in 1965 was continued in the Nasik District and some areas of the city of Bombay during the period under review.

The rats from different areas were collected and transported to the Institute where they were quarantined 2-3 weeks before they were taken up for challenge. Approximately equal number of male and female rats from different areas were challenged with a fixed dose of approximately 5,000 organisms of the virulent *P. pestis* strain 195/P as before, and the susceptibility was determined. In addition to this, some of the rats from the same area were collected and these serum-pools were subjected to the haemagglutination test using purified Fraction I antigen. In the city of Bombay the rats belonging to the species other than *R. rattus*, viz. *R. norvegicus* and *B. bengalensis* were also collected and their susceptibility status determined.

The rats from 3 places in Nasik District showed an uniformly high resistance varying from 70 to 78 per cent which was observed in some other places of the same District. The rats from the B ward of city of Bombay have shown some interesting results. The 2 species, viz. *R. rattus* and *R. norvegicus* which are known to be resistant in the past have shown a uniformly high resistance varying between 83-93 per cent in all the 3 samples taken at the interval of about 5 weeks. However, the *B. bengalensis* which is known to be a very susceptible species showed a resistance of 66·7 per cent in the sample collected in the month of May. As the sample size was considered small, the tests were repeated in June and end of July using 100 rats of the same species. The sample in June showed a resistance of 34·2 per cent while that in July showed a resistance of 20 per cent. The first 2 samples showed—comparatively very high resistance than the third which was nearer to the normal. Even if the results of 3 tests be combined, the resistance on a sample of 200 rats is 30 per cent which is higher than that obtained normally with this species. It seems that there is enough indication to show that resistance of *B. bengalensis* to plague in certain areas of the city is increasing. The rats of the same species from the Ward C show a very low resistance of 12 per cent as usual. The other 2 species from

Ward C have the high resistance of about 90 per cent. Thus the increased resistance of *B. bengalensis* in the city again poses a problem whether it is genetic or is it increasing due to some latent infection among rats.

The rats from Marathwada area were collected again with a view to confirm the results obtained in earlier years. They showed a uniformly high resistance of 53 to 59 per cent. The resistance observed in these rats was of the same order as in past.

The sera from rats of Nasik District were also collected and tested by HA. test. All the sera showed a titre less than 1 : 5 indicating absence of any antibody of Fraction I of *P. pestis* and the resistance observed in the rats in Nasik District is genetic in nature and there is probably no latent plague infection present in this area.

New Plague vaccine

The plague vaccine is being manufactured at this Institute according to the method which is in existence for the last 25 years. The method consists of growing a virulent strain of *P. pestis* in a liquid casein hydrolysate medium at 32°C in a stationary culture flask, using 1 litre of medium in a 3-litre culture flask. The cultures are incubated for 14 days. These organisms are killed with 0.07 per cent (V/V) formalin. Phenyl mercuric nitrate is added as a preservative in a final concentration of 0.0015 per cent. The potency of the vaccine is assayed according to Indian Pharmacopoeial (I. P.) requirements (by an active mouse protection test).

While making the new plague vaccine which has been developed in the light of research work carried out here, the organisms are grown at 37°C at which they produce the major protective antigen fraction 1 much better than that at 28°C, which is the optimum temperature for the growth of *P. pestis*. Normally, *P. pestis* grows very poorly at 37°C in commonly used media including casein hydrolysate. The new plague vaccine is prepared by growing a virulent strain of *P. pestis* at 37°C in liquid casein hydrolysate fortified with vitamins and salts. Calcium and magnesium salts are added as they are found to be essential for growth at 37°C. The cultures are grown in Haffkine flasks on a rotary shaker at about 100 revolutions per minute for 3 days. The organisms are inactivated with 0.07 per cent (V/V) formalin and preserved with 0.0015 per cent phenyl mercuric nitrate. The potency of these vaccines assayed according to the I.P. requirements by an active mouse protection test showed that it is as effective as the standard plague vaccine. The clinical (on volunteers) and serological studies of the old as well as the new vaccines mentioned above were undertaken by Dr. Karl F. Meyer, George Williams Hooper Foundation, University of California, San Francisco, U.S.A. The results now available suggest that the antibody titres from serum collections made on the 6th, 15th and 30th day after booster inoculation impressively support the conclusion that the new Haffkine vaccine (Lot 27-2), reinoculated in small doses, elicits antibody titres considered indicative of the high immunogenic (protective) efficacy of the antigen. This high percentage is rarely attained with other killed plague vaccine causing relatively moderate side-effects.

When the mice were inoculated with the Haffkine Institute old vaccine (lot 27-7), the PD50 value was almost identical to that of the new vaccine (0.00168 *versus* 0.00160). It seems that the mouse protection test (PD50) is not sensitive enough to distinguish between a vaccine giving high antigenic reaction in human beings to one which is deficient.

Dr. Meyer concluded "until additional clinical trials have been conducted on human subjects with different lots of Haffkine vaccine, I am fully prepared to state that I consider new type of killed Haffkine plague vaccine to be a highly immunogenic preparation, provided it is administered in a two-dose schedule at least two months apart".

TABLE 7
Susceptibility of rats to P. Pestis

Place of collection	Rats challenged	Mortality (within 15 days)	Rats positive to plague			Resistance (per cent)
			Male	Female	Total	
Khuldabad ..	50	22	13	7	20	58
Mhaismal ..	30	14	7	7	14	53
Kazipura ..	40	19	5	13	18	59
Takli ..	50	22	10	12	32	56

TABLE 8

The continued susceptibility of the different species of the rats from B. Ward (Bombay City) carried out at three different times

Place of collection				Rats challenged	Mortality (within 15 days)	Rats found positive to plague	Resistance (per cent)
B. Ward ..	..	..	..	100	12	11	89
<i>R. rattus.</i>							
B. Ward ..	..	..	..	92	13	13	87
<i>R. norvegicus.</i>							
B. Ward ..	..	..	..	214	149	149	30
<i>B. bengalensis.</i>							

TABLE 9

Susceptibility of rats (Rattus rattus) to P. pestis

Place of collection			Date of challenge	Rats challenged	Mortality (within 15 days)	Rats positive to plague	Resistance (per cent)
Yeola	..	..	4ht April 1970	50	11	11	78
Nandgaon	..	..	4th April 1970	20	6	6	70
Musalgaon	..	..	4th April 1970	30	7	7	77
B. Ward (Bombay) <i>R. rattus</i> .	..	..	20th May 1970	24	4	4	83
B. Ward (Bombay) <i>R. norvegicus</i> .	..	..	20th May 1970	24	3	3	88
B. Ward (Bombay) <i>B. bengalensis</i> .	..	..	20th May 1970	24	8	8	67
B. Ward (Bombay) <i>R. rattus</i> .	..	..	26th June 1970	50	4	3	94
B. Ward (Bombay) <i>R. norvegicus</i> .	..	..	26th June 1970	50	8	7	86
B. Ward (Bombay) <i>B. bengalensis</i> .	..	..	26th June 1970	76	50	50	34
Thana Road <i>R. rattus</i>	..	..	31st July 1970	36	4	4	90
Thana Road <i>R. norvegicus</i>	..	..	31st July 1970	18	2	2	90
Thana Road <i>B. bengalensis</i>	..	..	31st July 1970	14	11	11	21
B. Ward (Bombay) <i>B. bengalensis</i> .	..	..	22nd October 1970.	100	80	80	20
C. Ward (Bombay) <i>R. rattus</i> .	..	..	22nd October 1970.	50	6	6	88
C. Ward (Bombay) <i>R. norvegicus</i> .	..	..	22nd October 1970.	50	4	4	92
C. Ward (Bombay) <i>B. bengalensis</i> .	..	..	22nd October 1970.	50	44	44	12

USE OF ANTIGENS LABELLED WITH RADIOACTIVE ISOTOPES IN SEROLOGICAL EPIDEMIOLOGY

The aim of the investigations has been to develop rapid, short and simple methods for the detection of antibodies in the sera of persons and/or animals during epidemiological surveillance operations when large numbers of blood samples have to be investigated. A prerequisite to the studies was that the methods developed should be feasible for use in tropical climates, and that in preparation thereto attempts should be made to examine sera from as many parts of the world as possible, so that it can be used universally. A large number of the sera are collected and jointly investigated with Dr. Oscar Felsenfeld of Tulane Research Centre, U.S.A.

The results of the tests are gratifying when data obtained by routine serological methods were compared with that of a radioactive labelled antigen. It appears that in cholera both antitoxic and antibacterial antibodies are of importance. The determination of toxin neutralisation requires either the use of laboratory animals or neutralisation in tissue culture. Radioactive isotope labelled toxoid co-precipitated with antigamma rabbit serum, however, gave comparable results in 91 to 93 percent of the investigated sera. Crude vibrio extract, labelled with C-14, Cr-51, I-125 yielded results in agreement with the vibriocidal tests in 93 to 94 percent of the examined sera. It is planned to study more sera.

While surveying for typhoid immunity, attention was focussed on IgG-class antibodies. Due to circumstances beyond our control, a sufficient number of convalescent sera has yet to be collected. The same holds true to brucellosis.

The possible differential-diagnostic value of mycobacterial antigens was studied. A large number of sera from patients with tuberculosis and/or with leprosy were collected. Two approaches are being used : Tuberculo-protein is one antigen, arabinomannan the other. Both are isolated from *M. tuberculosis*. The sera are absorbed with various strains of *Norcardia brasiliensis* which has a very active common antigen with *M. leprae*. The passive haemagglutination test yields conflicting results but the titres can be increased by internally labelled antigens. This is done with C-14 (for the polysaccharide). The precipitin test with arabinomannan appears valuable. The study covers 120 cases of tuberculosis, 50 cases of leprosy, 6 patients with throat diseases and 40 ne ative controls.

The field of virology has been invaded. Vaccinia was selected first being a large virus. Both C-14 and Cr-51 were used to label Copenhagen strain of vaccinia grown in monkey kidney tissue cell monolayers. The antigen was used in the co-precipitation test and the results compared with those of the neutralization test. The sera of 12 children were collected before and 1, 2, 6, 9 and 12 months after primary vaccination. Further groups of 12 each consisted of children and adolescents immunised 2, 2½, 3, 4 and 5 years ago respectively. The radioisotope precipitation test remained positive for 30 to 36 months after vaccination, whereas the neutralisation test became negative 17 to 23 months after immunisation. Epidemiological evidence shows that immunity after vaccination against small-pox last for about 3 years. The radioisotope test is, therefore, indicative of immunity superior to the best routinely used serological method. It is also technically the only best feasible for use in mass surveys for immunity against small-pox.

Sera from patients, convalescents, immunised and not immunised individuals were collected for the study of diphtheria, and immunised and not immunised persons for the investigation of immunity to tetanus. The goal is two-fold. First it is hoped to establish the proportion of susceptible individuals. Secondly, to explore the dangers of hyperimmunisation against tetanus, that may result in Arthus-type reactions. Preliminary tests, comparing the passive haemagglutination test with formal and tannic acid treated cells and toxoid as an antigen, with radioactive labelled toxin (Wyeth) in the co-precipitation test, indicate that unwarranted tetanus toxoid injections can be avoided if and when a 2 hour period is allowed to perform the rapid test with radioactive isotopes labelled antigens. The test shows little fluctuation and a small variance around the minimal protective concentration (0.01 antitoxin units per ml serum) with tetanus toxin or toxoid, but a somewhat broader spectrum with diphtheria toxoid. Additional sera are being collected in Parel area in Bombay, so that data on larger number of blood samples can be compared with the findings made principally in a poor area of Louisiana, U.S.A.

In connection with the studies of toxins, attempt is being made to improve the determination of enterotoxins and their antibodies in the bowels. The number of examinations is small, but all have been worked up individually which is time-consuming. The use of protease inhibitors to prevent the degradation of the immunoglobulins is being made. Small amounts of Class IgG, IgA and IgM antibodies could be demonstrated with the aid of radioactive isotope labelled reagents. C-14 and Cr-51 were mostly used. Staphylo-enterotoxin and clostridial toxins were the principal goals of the studies. It is understandable that human material is not readily available from such patients.

B. DEPARTMENTAL RESEARCH

ANALYTICAL AND CONTROL DEPARTMENT

Stability of folic acid

Investigation on stability of folic acid at pH 7.0 was carried out at 37° C, 45° C and 121° C, individually, in the presence of thiamine HCl, destruction-products of thiamine-HCl, and separated moieties of thiamine-HCl. Niacin amide was used to accelerate the destruction of thiamine HCl. The results revealed that thiazole moiety and the hydrogen sulphide liberated during the destruction of thiamine-HCl was responsible to destroy folic acid. Further study on folic acid stability at 45° C in presence of certain metallic salts showed that copper sulphate and cobalt chloride could destroy folic acid while manganese sulphate and sodium salt of ethylene diamine tetra acetic acid had no effect on folic acid. Study on effect of sunlight on folic acid kept in amber coloured glass containers and bottles covered with cellophane of different colours in the presence of other vitamins, minerals and thiazole and pyrimidine moieties (each taken individually), unravelled that niacinamide, vitamin C, $MnSO_4$, Na-EDTA and both the moieties of thiamine HCl destroyed folic acid. Light passing through the cellophane had decreasing effect with the colour in order : blue, green and red.

Effect of light on Vitamins

Studies on destruction of vitamins by light are more concentrated on U. V. rays. Investigation is afoot on the effect of different regions of the spectrum of light using different dyes or combination of dyes as filters. Vitamins, individually and in combinations, at different pH and concentrations are being studied. Investigations, so far have revealed that riboflavin gets destroyed in the violet, blue and green regions while yellow, orange and red rays showed no effect. Riboflavin when sealed in nitrogen, destruction is inhibited. Niacinamide was found to remain stable even after 100 hrs-exposure to sunlight at different pH, but nicotinic acid showed slight deterioration under identical conditions. Vitamin C remained quite stable when exposed to light at different pH.

DEPARTMENT OF BACTERIOLOGY

The studies on the development of submerged culture methods for the growth of *S. typhi* were continued. A new twin unit fermentor was obtained and the conditions for growth of the organism are now being studied with the help of this new unit.

The study of storage stability of typhoid vaccines at high altitudes was continued. The investigations revealed that formalin-killed and phenyl-mercuric nitrate preserved, as well as heat-killed and phenol-preserved T.A.B. vaccines do not show any deterioration in the potency up to 15 months when stored in frozen condition. Both the vaccines also show no loss of potency

on repeated freezing and thawing. However, the heat-killed and phenol-preserved vaccine shows variation in physical properties and becomes granular on repeated freezing and thawing. Studies on susceptibility of rats from different areas of Maharashtra to *P. pestis* continued.

Development of submerged and continuous culture methods for the growth of microorganisms

The development of a complete casein hydrolysate medium fortified with tryptophane, sodium B-glycerophosphate, Glycerol and yeast extract for the growth of *S. typhi* was reported last year. It was also shown that this medium had superior growth properties than synthetic and semi-synthetic media studied using stationary and shaken culture methods. These studies were extended to the use of this medium in submerged culture methods.

The experiments were initially started with a chain fermentor. Using complete casein hydrolysate medium in 5 litre quantities in a 15 litre culture vessel experiments were carried out to determine the optimum conditions for growth of *S. typhi* with respect to the rate of aeration and agitation.

The effect of variation in the rate of aeration was studied by varying the air-flow rates from 5 litres per minute to 13 litres per minute with a constant rate of agitation. The results presented in the Table 10 showed that growth increased with the increase in the rate of aeration. Increase in air-flow rate beyond 12 litres per minute did not increase the growth significantly. The antigenic composition remained unaffected by variation in the air flow rate within the range used.

The effect of agitation was studied by increasing the speed of the stirrer from 470 r.p.m. to 600 r.p.m., keeping the rate of aeration constant at 10 litres per minute. It would be apparent from the results presented in the Table 11 that the growth increased by about 1.5 times when the rate of agitation was increased from 470 r.p.m. to 600 r.p.m. which was about 8 to 9 times the growth obtained in a stationary culture. The antigenic pattern remained the same at all the levels of agitation.

Though the results obtained with this chain type fermentor were encouraging, this unit had two deficiencies viz., (a) the speed could be changed only to three levels with a minimum of 470 r.p.m., (b) it had a less efficient system of aeration with only a single point air sparger. A more modern New Brunswick type win fermentor unit was obtained during the year. This unit has a more efficient aeration system with a fountain type sparger and a stirrer with a variable speed, in the range of 200 r.p.m. to 800 r.p.m. Preliminary experiments were carried out to study the effect of aeration and agitation on the growth of *S. typhi* in this new unit.

The effect of variation in the rate of aeration alone, without any agitation as studied by varying the air-flow rate from 2 litres per minute to 10 litres per minute. Similarly, the effect of agitation was studied by varying the

speed of the stirrer from 200 r.p.m. to 440 r.p.m. keeping the air flow rate constant at 5 litres per minute. The results of these experiments presented in the Table 12 showed that the growth obtained at the air-flow rate of 10 litres per minute without agitation was 1·5 times of that obtained at an air-flow rate of 2 litres per minute and was ten times of that obtained in a culture without aeration. However the production of Vi and H antigen in these cultures was unsatisfactory. The agitation of cultures affected the growth considerably. When the speed of the stirrer was varied in the range of 200 to 440 r.p.m. at a constant aeration rate of air flow of 5 litres per minute, the growth was 13 to 14 times more than that obtained in a stationary culture. The production of H and O antigens appeared to be normal but the production of VI antigen was not satisfactory.

These results obtained with this fermentor unit showed that a substantial increase in growth could be obtained by submerged culture methods but more detailed studies are necessary for working out the optimum conditions for growth and antigen production. After the methods for the growth and antigen production are standardized vaccines prepared from cultures grown under submerged conditions will be studied for their immunogenicity and toxicity.

TABLE 10

*Effect of Aeration on the growth and antigenicity of S. typhi grown in fermentor**

Air supplied in litres per min.		Antigenic response				No. of cells/ml medium
		H	Vi	O		
5·0	..	..	1 : 128	1 : 32	1 : 256	9 × 10 ⁸
7·5	..	..	1 : 256	1 : 32	1 : 256	10 × 10 ⁸
10·0	..	..	1 : 64	1 : 32	1 : 256	12 × 10 ⁸
12·0	..	..	1 : 64	1 : 32	1 : 512	12·5 × 10 ⁸
13·0	..	..	1 : 32	1 : 32	1 : 256	12 × 10 ⁸
Stationary	..	..	1 : 128	1 : 32	1 : 256	1·5 × 10 ⁹

*The cultures were agitated at the rate of 470 r.p.m.

TABLE 11

*Effect of agitation on the growth and antigenicity of S. typhi grown in fermentor**

Agitation rate r.p.m.	Antigenic responses			Growth No. of cells/ml. medium
	H	Vi	O	
470	1 : 64	1 : 32	1 : 256	10×10^9
520	1 : 128	1 : 64	1 : 256	13×10^9
600	1 : 64	1 : 32	1 : 256	14×10^9
Stationary	1 : 128	1 : 16	1 : 256	1.5×10^9

*The cultures were aerated with 10.0 litres of air per min.

TABLE 12

Effect of aeration and agitation on the growth and antigenic composition of S. typhi grown in the fermentor

Volume of air lit./m.	Rate of agitation in r.p.m.	Antigenic response			Growth No. of cells/ml. of the culture
		H	Vi	O	
2.0	0	1 : 32	<1 : 32	1 : 256	10×10^9
10.0	0	1 : 32	<1 : 32	1 : 256	15×10^9
(Stationary)	0	1 : 128	<1 : 32	1 : 256	1.4×10^9
5	200	1 : 128	<1 : 32	1 : 256	21×10^9
5	320	1 : 128	<1 : 32	1 : 256	25×10^9
5	440	1 : 128	<1 : 32	1 : 256	26×10^9
(Stationary)	0	1 : 128	<1 : 32	1 : 256	1.5×10^9

Lyophilization of bacterial cultures

The use of live parenteral bacterial vaccines for mass immunization is confronted with the difficulty of maintaining the required number of organisms per dose in a live state till it is administered to the population at risk. Freeze drying is one technique which has been successfully applied to this purpose as in the case of B. C. G. vaccine. It has been reported earlier that the suspending medium plays a very important role in the survival of organisms during lyophilization. A mixture of sodium glutamate and lactose gave the highest rate of survival after lyophilization. Further studies were carried out, using different combinations of sodium glutamate and lactose. It was observed that a mixture containing 6 per cent sodium-glutamate and 6 per cent lactose gave the maximum survival rate of 5 to 8 per cent which was the best so far achieved in the small scale "Cryochem" lyophilization machine.

The preparation of live parenteral cholera vaccine is also under investigation in the department. It was, therefore, considered desirable to extend these studies to *V. cholerae* also and see if optimum conditions to obtain high survival rates in the case of two avirulent strains of *V. cholerae* could be worked out. These investigations included (a) the effect of freezing on the survival rate, (b) the effect of suspending medium on the survival of organisms after freeze drying, and (c) the effect of bacterial concentration on the survival.

The two strains, viz., *V. cholerae* C₁₄S₅ and *V. eltor* water 6 were grown on nutrient agar and suspended in appropriate media and frozen at -30°C. The percentage of organisms surviving immediately after freezing and after lyophilization, was determined. The results are summarized in Tables 13 and 14.

It will be seen that very significant destruction of organisms took place after freezing when glucose alone or in combination with gum acacia was used as a suspending medium, the survival rate being only 0.35 per cent. In other suspending media such as gum acacia + nutrient broth or mixtures of sodium glutamate and lactose or skimmed milk the survival rate varied between 20 to 40 per cent for both the strains. This survival rate after freezing is fairly low as compared to *P. pestis* which gave survival rate of approximately 60 per cent in some of the media.

The survival rates on lyophilization for both the strains were very low varying from 0.0001 to 0.1 per cent in different media. Highest rate was found when 10 per cent skimmed milk was used as a suspending medium. The rate of survival was very low as compared to 5 to 8 per cent obtained with *P. pestis*. These low rates of survival indicate that *V. cholerae* is a delicate organism and some other factors such as temperature of freezing, rate of evacuation etc. may be involved which are under investigation.

TABLE 13

Percentage survival of V. eltor Water 6 after freezing and freeze drying.

Suspending medium	After freezing (per cent)	After lyophilization (per cent)
5 per cent gum acacia + nutrient broth	Not tested	0.002524
12 per cent lactose + sodium glutamate in 3 per cent, 6 per cent or 12 per cent concentration.	20 (approx.)	0.0034
6 per cent lactose + sodium glutamate in 3 per cent, 6 per cent or 12 per cent concentration.	40	0.025
3 per cent lactose + sodium glutamate in 3 per cent, or per cent concentration.	20	0.001
10 per cent swimméd milk; 12 per cent lactose; 6 per cent starch; 6 per cent lactose, 20 per cent sodium glutamate.	25	0.1
10 per cent ucose; 7.5 per cent glucose + 5 per cent gum acacia.	0.35	0.0003

TABLE 14

Percentage survival of V. Cholerae C₁₄S₅ (Ogawa) after freezing and freeze-drying

Suspending medium	Survival after freezing (per cent)	Survival after lyophilization (per cent)
5 per cent gum acacia ÷ nutrient broth	Not tested ..	0.000047
12 per cent lactose + sodium glutamate in 3 per cent, 6 per cent or 12 per cent concentration.	60	0.0008
6 per cent lactose + sodium glutamate in 3 per cent., 6 per cent or 12 per cent concentration.	30	0.00015
3 per cent lactose + sodium glutamate in 3 per cent, 6 per cent or 12 per cent concentration.	Not tested ..	0.005
10 per cent glucose; 20 per cent sodium glutamate; 12 per cent lactose.	12	0.009
per cent starch; 6 per cent dextrin	10	0.05
7.5 per cent glucose + 5 per cent gum acacia ..	0.175	0.0001

Radiation killed cholera vaccine

The recent field trials conducted in our country as well as in some other countries showed that the conventional cholera vaccines offered very little protection of short duration. This may be attributed to the intrinsic quality of the vibrio which may possess only low protective quality or it is possible that the protective antigen may be partially destroyed due to the chemicals used for inactivation of the vibrios. Therefore, it would be interesting to study if a method other than use of chemicals is employed for inactivation of the organisms, so that the protective power of the organisms would be better preserved and may yield a more potent vaccine. With this end in view, the studies on use of radiations for killing the vibrios are undertaken.

Roux bottles containing casein hydrolysate agar were inoculated with an 18 hour old culture of the strain Inaba 569 B. The growth was harvested after 24 hours incubation at 37° C and tested for purity. The pure harvest was then diluted with 0·02 M phosphate buffered saline to contain 60×10^9 organisms per ml. Then 15 ml. of the suspension was distributed into each of the six 30 ml. screw capped culture tubes. Five of these tubes were then exposed to a 60 Co source for periods varying from 5 minutes to two hours. The suspensions exposed for 1 hour or 2 hours were found to be sterile, however those exposed for shorter periods were not completely inactivated.

The suspension from the sixth tube was killed by addition of 0·3 per cent formalin and kept at 37° C. for 4 days. The sterile suspension from the tubes exposed for 1 hour and 2 hours and the formalin killed suspension were then diluted to contain 6×10^9 organisms per ml. with phosphate buffered saline containing 0·0015 per cent phenyl mercuric nitrate. The three vaccines were then tested for their antigenicity by active mouse protection test. The results of one test carried out so far are given below :—

TABLE 15

Vaccine				ED50	Relative potency
Inaba 569 B irradiated for 1 hour	..	..	..	0·00004	2·75
Inaba 569 B irradiated for 2 hours	..	..	..	0·00004	2·75
Inaba 569 B formalin killed	..	..	..	0·00011	1

It can be seen from the results that both the vaccines prepared from irradiated cultures are much more potent than the usual formalin killed and phenyl mercuric nitrate reserved vaccine.

Dynamics of disinfection

The studies in dynamics of disinfection of formalin and phenyl mercuric nitrate (p. m. n.) in respect of *S. typhi* and *S. paratyphi B*. reported earlier were continued. The effect of the two disinfectants on the two abovementioned organisms at a low temperature (4° C.) and at pH 7·5 was investigated.

Culture suspensions containing about 8×10^9 organisms per ml. were prepared for both the organisms and were cooled to the temperature of 4° C. The disinfectants were added in required concentration and the cultures maintained at 4° C. and at a pH level of 7·5. The cultures were sampled after different intervals of time and the number of organisms surviving and the time taken for complete sterilization of the culture were determined.

The results showed that *S. typhi* (strain Ty2) was killed with a concentration of 20 mg./100 ml. (p. m. n.) in 510 min. while *S. paratyphi B* required 3420 min. The concentration coefficient of (p. m. n.) in case of *S. typhi* was found to be about 1 while that of *S. paratyphi B*. was about 0·5. 0·2 per cent formalin killed the *S. typhi* in 1620 min. while it took 2400 min. to inactivate the *S. paratyphi B*. at the same concentration. The concentration coefficient of formalin for *S. typhi* was found to be 1·4 while that for *S. paratyphi B*. was 1·9. In the case of disinfection of *S. typhi* with formalin at 4° C., a peculiar phenomenon was observed that when a sample of reaction mixture was plated after neutralization of formalin with sodium meta bisulphite, the plates were crowded with large number of colonies. However, when a tenfold dilution of the same culture was plated very few colonies were obtained and a further tenfold dilution failed to show any colony. However, when the same tenfold dilutions when subcultured in nutrient broth treated with 5 per cent charcoal, these subcultures showed growth. Use of nutrient agar treated with 0·5 per cent charcoal did not improve the growth on agar plates. This peculiar phenomenon observed only with *S. typhi* when treated with formalin at 4° C. prevented the determination of number of organisms surviving at different intervals after treatment. Therefore, in this case only the death time was determined by subculturing the reaction mixture in nutrient broth treated with charcoal and concentration coefficient and the temperature coefficient were determined with the help of the death time. This peculiar growth property was considered to be attributable to the diluent, viz. buffered saline. In order to verify this point buffered saline was replaced by 0·5 per cent peptone water and nutrient broth but no improvement in the growth from diluted suspension was observed.

The death time and the concentration coefficient of formalin at 4° C. with *S. typhi* is shown in the Table 16.

TABLE 16

The effect of formalin and phenyl mercuric nitrate (p.m.n.) on S. typhi and S. Paratyphi B at pH 7.5 at 4° C.

Bacteria	Disinfectant used	Conc. per cent of the disinfectant	Death in min.	Mean conc. exponent
<i>S. typhi</i>	Formalin	0.2 (V/V)	1620	..
		0.1 „	4800	1.45
		0.05 „	102120	..
	p.m.n.	0.02 (W/V)	510	..
		0.01 „	840	1.04
		0.05 „	1800	..
<i>S. paratyphi B</i>	Formalin	0.2 (V/V)	2400	..
		0.1 „	9480	1.09
		0.05 „	34800	..
	p.m.n.	0.02 (W/V)	3420	..
		0.01 „	5400	0.050
		0.005 „	6600	..

Storage stability of typhoid vaccine at high altitudes

The investigations on the effect of storage of vaccines under frozen conditions or the effect of repeated freezing and thawing on the protective efficacy of vaccines were continued. It was reported last year that no deterioration in potency of a formalized and phenyl mercuric nitrate (p.m.n.) preserved vaccine was observed when stored at 35° C for 1 year as shown by mouse protection test. Under similar conditions, the heat killed and phenol preserve vaccine was shown to be unaffected for two weeks in which period the test were completed. The agglutinin production was also unaltered. The potency of both the types of vaccines remained unaffected by repeated freezing and

thawing for six times. However, the heat killed and phenol preserved vaccine showed some granulation after third thaw which increased on further repetition of the freezing and thawing process.

In addition to the two vaccines (i) formalin killed and p.m.n. preserved (ii) heat killed and phenol preserved a third vaccine : phenol killed and phenol preserved was also included in the study. The results presented in the Table 17 show that none of the three vaccines showed any loss in potency when tested by active mouse protection test, after storage at -35° C for a period of 15 months, 12 months and 12 months respectively. The physical appearance of the vaccine (i) remained unaffected till the end of 15 months. However, vaccine (ii) started showing a little granular appearance after storage of 6 months which increased through passage of time. The vaccine (iii) showed marked granulation after a storage period of 3 months and was completely granulated at the end of 12 months.

The question arose whether the immunogenicity and toxicity remained with the granules or with the supernatant in the vaccines which showed granulation on repeated freezing and thawing. For this purpose a sample of heat killed and phenol preserved vaccine after 6th thaw was centrifuged and the supernatant was separated. The granules were washed thrice with buffered saline (pH 7.5) and finally suspended to original volume in buffered saline containing 0.5 per cent phenol. The supernatant and the suspension containing the granules, were separately tested for potency by active mouse protection test along with the whole vaccine of the same batch preserved at 4° C. The results presented in the Table 18 show that the cell free supernatant contained almost all the protective activity of the whole vaccine while the granules showed activity of less than 5 per cent of that of the whole vaccine. The variations in the relative potency observed specially with the phenol killed and phenol preserved vaccine might be due to uneven distribution of granules in different samples taken for test.

The results of abnormal toxicity tests carried out showed that the supernatant containing all the protective activity was non toxic while the animals inoculated with the suspension of the residue showed paralysis of the hind legs and shivering for about 30 to 4 min. after which the animals recovered and remained normal. However, this reaction is indicative of some abnormal toxicity and the cause for the same is being investigated.

The agglutinin response of vaccines stored at -35° C. for 6 months was compared with that of one stored at 4° C. The results presented in Table 19 show that the agglutinin response to the vaccine stored at -35° C was as good or even slightly better than that obtained with the same vaccine stored at 4° C.

TABLE 17

Potency tests of T.A.B. and Typhoid vaccines stored at— 35°C for different periods

Periods of Storage	Storage temperature in C°	Formalin killed and p.m.n. preserved T. A. B. Vaccine		Heat killed and phenol preserved T.A.B. Vaccine		Phenol killed and preserved typhoid vaccine	
		Mean* ED ₅₀	Relative potency**	Mean ED ₅₀	Relative* potency*	Mean ED ₅₀ *	Relative potency**
2 days	.. 4 —35	0.0131 0.0127	1.03			0.00763 0.01031	0.74
2 weeks	.. 4 —35	0.0145 0.0104	1.39	0.0174 0.0150	1.16	0.0146 0.0129	1.13
3 months	.. 4 —35	0.0188 0.0172	1.09	0.0049 0.0037	1.30	0.0037 0.0019	1.90
6 months	.. 4 —35	0.0164 0.0123	1.33	0.0041 0.0041	1	0.0060 0.0105	0.57
9 months	.. 4 —35	0.0148 0.0118	1.25	0.0042 0.0034	1.23		
12 months	.. 4 —35	0.0148 0.0147	1.008	0.0043 0.0044	0.99	0.0048 0.0024	2.00
15 months	.. 4 —35	0.0116 0.0076	1.59				

*Mean of 3 tests **Relative potency: $\frac{\text{ED}_{50} \text{ of vaccine stored at } 4^{\circ} \text{ C.}}{\text{ED}_{50} \text{ of vaccine stored at } -35^{\circ} \text{ C.}}$

TABLE 18

Potency tests on supernatant and residue of heat killed and phenol preserved B. A. T. vaccine subjected to repeated freezing and thawing

ED ₅₀ of whole vaccine stored at 4° C.		Residue of repeatedly frozen and thawed vaccine		Supernatant of repeatedly frozen and thawed vaccine	
		ED ₅₀	Relative potency	ED ₅₀	Relative potency
0.0041		0.08	0.0522	0.0042	0.99
0.0033		0.08	0.0420	0.0035	0.95
0.0035		0.08	0.0442	0.0039	0.88

Relative potency: $\frac{\text{ED}_{50} \text{ of whole vaccine stored at } 4^{\circ} \text{ C.}}{\text{ED}_{50} \text{ of residue or supernatant potency of whole vaccine is taken as } 1}$

TABLE 19

Agglutinin response in rabbits immunised with T.A.B. vaccine stored at 4°C and at —35° C.

Exp. No.	Vaccine	Storage temperature and period	*Geometric mean agglutination titres of the rabbit sera														
			Preimmunization					After 1 dose of vaccine					After 2 doses of vaccine				
			Vi	O	H	PA	PB	Vi	O	H	PA	PB	Vi	O	H	PA	PB
1	T. A. B. 13B	—35° C for 6 months.	20	16	31	10	10						201	1613	1613	1613	508
	T. A. B. 13B	4°C	10	10	10	10	10						40	640	806	320	640
2	T. A. B. 13B	—35° C for 6 months.	10	10	20	10	10	160	640	1016	402	201	202	2560	2032	806	507
	T. A. B. 13B	4°C	10	10	10	10	10	20	254	403	80	115	32	806	806	355	640

*Mean of three rabbit sera

BLOOD BANK DEPARTMENT

Studies on genetical characters in some endogamous groups in Indian Population

This work is undertaken to study the distribution of genetical character in the endogamous groups of population in the country. The data so collected will be a useful addition to ethnical data of the population in the country. The data available in this country is very scarce.

The study comprises in determination of bloodgroups of ABO, Rh (CDEe) MNSs Duffy, Kidd, Kell Diego groups together with other genetic markers viz. colour-blindness, ability to taste phenylthiocarbamide, abnormal haemoglobins and G-6 PD deficiency. The serum groups studied are haptoglobin and transferrins. These results were statistically analysed and compared with that of similar studies in other areas.

So far the study on these lines had been conducted on 3 endogamous groups from Gujarat area (Audich Brahmins, Lad Vania and Visa Oswal) and on group from Maharashtra (Mahars) one and from Kerala (Syrian Christians). These studies have revealed some interesting similarities between Audich Brahmins and Syrian Christians.

The present study was on 5 endogamous groups of Indian Muslims viz. two Shia groups: Dawoodi Boharas and Ismaili Khojas. The former samples were collected in Bombay and the latter at Poona. Both the groups originated in Saurashtra and Gujarat. Two Sunni Muslim groups: Moplahs and the Misgars originated in Kerala and Ahmednagar respectively and samples were collected from there. The fifth group of the mixed Muslims were from Bombay and did not have any subjects belonging to the other four endogamous groups. The results of the ABO, MN, Rh, Duffy and Kidd are given in the following Tables respectively :

TABLE 20
Phenotype percentage (ABO)

	O	A1	A2	B	A1B	A2B	Total No.
Bohras	.. 34.6	23.1	1.5	30.8	8.5	1.5	130
Khojas	.. 37.4	20.7	2.3	33.3	5.4	0.9	222
Misgar	.. 41.8	18.3	3.9	27.5	7.8	0.6	153
Moplah	.. 42.5	20.4	2.2	32.7	3.2	0.0	186
Mixed	.. 36.0	18.0	3.2	30.4	10.4	2.0	250

TABLE 21

Phenotype percentage (MN)

			M	MN	N	Total No.
Bohra	..	..	36.2	50.0	13.8	130
Khoja	..	..	39.6	48.2	12.2	222
Misgar	..	..	34.6	49.7	15.7	153
Mixed	..	..	33.3	49.0	17.7	249

TABLE 22

Rh allelic frequencies

			R2	r	Ro	R'	R ⁺
Bohra	..	..	10.4	32.2	2.4	2.1	52.9
Khoja	..	..	8.8	26.5	2.5	1.6	60.5
Misgar	..	..	9.2	22.9	6.6	2.4	49.1
Moplah	..	..	11.6	24.0	6.7	1.1	56.7
Mixed	..	..	11.4	26.0	4.0	3.5	55.1

TABLE 23

Duffy phenotype

					Fy (a+)	Fy (a—)
Bohra	..	..	..	..	62.3	37.7
Khoja	..	..	..	..	65.3	34.7
Mixed	..	..	..	..	68.0	32.0

TABLE 24

Kidd phenotype

					Jk(a+)	Jk(a—)
Bohra	..	..	..	..	62.3	37.7
Khoja	..	..	..	..	65.3	34.7
Mixed	..	..	..	..	68.0	32.0

It was observed that the phenotypic frequencies of all the blood groups did not show any significant difference. It has also been observed that all the samples tested were K negative, K positive, Kp^o negative and Kp^o positive. Not a single Diego (a positive) was detected.

The study of serum groups haptoglobins and transferrins showed that the frequency of Hp gene was low and varied from 0.2-0.29. The difference was not significant. All the samples were Tf CC. About 2 per cent of Khojas and mixed Muslims and 1 per cent of the Misgars were G-6 PD deficient. Only 2 samples of the Khoja group had haemoglobin D along with haemoglobin A.

All the subjects tested for the ability to taste phenylthiocarbamide and it was observed that about 40 per cent to 50 per cent of people were non-tasters. The difference was not significant. A study of colour blindness in all the five groups showed that not a single Bohra or Mophla had this defect whereas about 2 per cent of people belonging to Khoja, Misgar and the mixed Muslim group were found to be red-green deficient.

Ge typing

Many serum proteins in human have shown to be polymorphic systems under genetic control. Some of these hereditary gamma globulin types and haptoglobins are well-known.

Jan Hirschfield showed another group of α -2 globulins to exhibit polymorphism in 1959. He termed them Gc (Group Specific) components. The Ge proteins were shown to be controlled by two allelic genes Gc¹ and Gc². Clinical and physiological role of these proteins is unknown.

Gc studies in Marathi speaking groups, of Maharashtra showed a frequency of Gc¹ 0.275 P in a sample of 110. Only previous study reported from U.S.A show a Gc¹ frequency of 0.305. Our findings are in agreement with this study.

Studies on human immunoglobulins

Immunoglobulins in diseases (a) Immunoglobulins were determined qualitatively on starch-gel and immuno electrophoretic technique using non-specific antisera in cases of multiple myeloma liver cirrhosis, nephrosis recurrent bacterial and viral infections. The following results were obtained. Out of 4 cases of multiple myeloma 2 were IgG myeloma 'IgA' and showing 'IgM' paraprotein.

Hypergammaglobulinemia (10 cases) which is a common feature in liver cirrhosis was found to be mainly due to increase in IgG content detected by starch-gel technique. It was observed that IgG gave a sharp and concentrated recipiting line in immunoelectrophoretic study using antihuman whole serum s compared to normal.

Three out of 6 cases of recurrent baterial and/or viral infection from K.E.M. ospital, Bombay and B. J. Hospital, Poona, for children showed gross ypogammaglobulinemia by starch-gel studies. No quantitation was carried out... In 2 cases besides IgG reduction, there was also a decrease in IgM.

(b) *Studies on human immunoglobulins.*—The gamma globulin prepared in his Institute was tested for the presence of IgG, IgA and IgM (qualitatively) by el diffusion technique using the specific antisera obtained through the courtesy f Dr. H. W. Krijnen of Central Blood Transfusion Laboratory, Amsterdam. Some batches showed presence of traces of IgA and IgM but the major portion was IgG.

Ten batches of the gamma globulin were tested for immunoglobulins by the World Health Organisation International Reference Centre, Lausanne. The report from that laboratory confirmed our findings.

The gamma globulin was further purified by preparative agar electrophoresis to obtain IgG in pure form. Its purity was tested by immunoelectrophoresis and gel diffusion techniques. The IgG so prepared was quite homogenous.

The isolation of immunoglobulin (IgG, I.gA, IgM is being done for the preparation of specific antisera. The DEAE cellulose chromatographic method has been used to isolate immunoelectrophoretically pure IgG.

A good quantity of plasma was collected from a case of multiple myeloma, and on analysis, this was found to contain mostly IgG fraction. The other immunoglobulins have been found to be absent. This plasma was processed for isolation of pure IgG by starch-block preparative electrophoresis. This fraction is being utilised for preparation of specific antiserum (IgG).

Studies on specific immunoglobulins

Recent trend in immunotherapy is to use homologous hyperimmune sera in cases of infective conditions like tetanus, small-pox, diphtheria etc. The main advantage of such prepration is the avoidance of sensitization and the resulting of anaphylactic reactions, (which at times are fatal) following the administration of geterologous serum like antisera prepared in horses.

The pilot study to prepare anti-tetanus hyperimmune immunoglobulin sera by immunizing human donors is in progress. For this purpose the prisoners in different prisons are being immunised with tetanus toxoid injections and their blood tested after each injection. They are bled when the antibody titre is high.

A total of 73 prisoners were injected in 4 batches with aluminium-phosphate adsorbed tetanus toxoid. Each dose was 0.5 ml., 10 I.U./ml. Relevant details are presented down below :—

Batch I.	No. of volunteers.	..	..	24
	No. of injections		..	2 each
Interval between injections	..	..	..	8 weeks.
Average titre of antitetanus antibody	..	..	..	2.1 I.U./ml.
Batch II.	No. of volunteers	..	..	14
	No. of injections	..	..	4 each
Interval between injection	..	..	..	4 weeks
Average titre	..	..	..	6.5 I. U/ml.
Batch III.	No. of volunteers	..	..	20
	No. of injections	..	..	4 each
Interval between injections	..	..	..	15.20 days
Average titre of antitetanus			..	15-20 days
Average titre of antitetanus	..	..	..	6.2 I.U./ml.
Batch IV.	No. of volunteers	..	..	15
	No. of injections		..	3 each
Interval between injections	..	..	..	1 month
Average of antitetanus antibody			..	5.5 I-U/ml.

The blood from donors showing high antibody titre (over 5.0 I.U./ml.) has been separated and is being processed further into immunoglobulin fraction by Cohn's cold ethanol fractionation method.

Cryoprecipitates from plasma for the treatment of haemophilia

The recent advance in treatment of haemophilia is the use of "Cryoprecipitates" prepared from the fresh human plasma. They show greater concentration of A.H.G. than fibrinogen (Cohn fraction I) prepared from fresh human plasma and do not contain other contaminants. Also the

can be prepared in ordinary blood bank set up. Cryoprecipitates are being prepared in Haffkine Institute Blood Bank. The product is also being lyophilised to facilitate storage and transport.

The product has been clinically used in a large number of cases. There was very good response (anti-haemophilic globulin level rose from 15 per cent to 85 per cent after *normal* administration of these cryoprecipitates).

Australia antigen

Owing to the importance of 'Au' antigen in larger context of the infectious hepatitis in this country, a study was undertaken to assess the status of 'Au' antigen in voluntary and professional blood donors and also in patients with hepatitis.

The serum samples investigated in the present study were obtained from the healthy voluntary donors as controls and infective hepatitis or liver cirrhosis cases from hospitals. Serum samples from professional donors were collected in the Blood Bank Department, Haffkine Institute.

The antibody No. JD/ED used in the present study was supplied by Dr. P.C. Das of the Blood Transfusion Centre in Edinburgh, Scotland. The genetic typing Au (1) or Au (2) was not available.

The results of the work are reported in the following Table 25 :—

TABLE 25

Incidence of Au antigen in various diseases and controls.

				Nos. examined	Au No.	+ ve % $\frac{1}{2}$
Control	..	..	..	132	1	0.75
Hepatitis (mixed)	..	..	..	215	21	10.00
Cirrhosis	..	..	..	40	0	0.00
Professional donors	..	..	..	36	2	5.50

Studies on biochemical aberrations in disease conditions

G-6-P-D deficiency.—Thirty cases (20 males and 10 females) of neonatal jaundice were studied of which 3 were deficient in G-6-P-D and 1 case out of 8 haemolytic anaemias.

Glutathione.—Facilities were created this year, for glutathione determination of red cells. Reduced glutathione is important in maintenance of red cells integrity. Low levels indicate increased red cell sensitivity and subsequent destruction. Four cases of hemolytic anaemia referred from K.E.M. Hospital gave low values (27-32 mg %/normal 40 mg. %) . It is proposed to continue the service to help diagnosis of the deficiency of the enzyme glutathione reductase in hemolytic anaemias.

Studies on serum proteins

Haptoglobulins.—Forty-three cases of liver diseases, 4 cases of neonatal jaundice and 8 of haemolytic anaemias were studied for Haptoglobulins. Haptoglobulins were reduced by more than 50%-20 mg. (normal 60 mg. %) in cases of confirmed cirrhosis and very low value (10 mg %) were obtained in haemolytic anaemias. 3 cases of infantile cirrhosis showed values between 25-30 mg % (normal 50 mg. %).

DEPARTMENT OF CHEMOTHERAPY

Chemotherapy of Cancer

The work in this area began when we observed certain heterocyclic moieties studied by us in the broader field of chemotherapy as offering possibility of being modelled into antitumor agents. From among the earliest compounds sent to Cancer Chemotherapy National Service Centre (CCNSC), Bethesda, many were found to be active in L 1210, Walker carcinosarcoma and Dunning leukemia and having a low order of toxicity.

The basic approach in the design of such compounds was either to incorporate a physiologically active molecule into nitrogen mustards so that the resultant products would have an efficient biological carrier to take the drug at the target cells or in the alternative to mask the general cytotoxic action of the nitrogen mustards by such groups as would render the resultant product sufficiently protected, i.e. to have latent activity which would be activated only at the target cells or tissues as a result of the enzymatic action.

The work has been directed primarily towards the synthesis of different Schiff bases of nitrogen mustards which would liberate the alkylating agents at the site of action and secondly to introduce thiazole and related heterocyclic ring systems to act as biological carriers. Out of a large number of compounds synthesised, so far over 150 have been screened by the CCNSC against several experimental tumor systems and also for their toxicity.

The work done and the reports received during the period under review has shown certain structural features that contribute either to better activity or reduced toxicity. The Table 26 summarises the findings obtained during the year under review on the activity of compounds synthesised here. It can now be concluded that the replacement of the methoxy group by ethoxy group has produced more active and less toxic compounds. Also, the introduction of the hydroxy group has rendered the resultant product more soluble without

adversely affecting the activity. It is further seen that the dimethyl-thiazole moiety has potentiating effect as far as activity against L 1210, lymphoid leukemia and a dosage as low as 3 mg/kg has been found to be active while at a dose level of 11 mg/kg the survival time has been doubled, i.e. T/C 200. with a dose of 25 mg/kg, 2 cures (complete reversal of tumor) out of 6 animals has been registered. These findings are proposed to be applied for the further synthetic programme. Another direction specifically attempted was to introduce additional functional groupings in the more active compounds from this series in order to improve their solubility pattern.

The Institute has in hand a few new compounds with favourably high activity in the experimental tumor system which the CCNSC considers as clinically predictable. It is felt that these compounds now merit further study particularly for haematinic response and if found satisfactory then clinical evaluation.

TABLE 26
Lymphoid leukemia

Compound (1)	Minimum effective dose mg/kg (2)	Toxicity data HNTD or LD ₁₀ (3)	Status (4)
4-[4-bis (2-chloroethyl) amino] 3-methoxybenzylidene] amino-2, 6-dichlorophenol, NSC-116245.	10.0	HNTD 150 mg/kg	Activity confirmed.
4-[p-] [4- [bis(2-chloroethyl) amino] -3-methoxybenzylidene] amino] phenyle-2, 5-dimethyl thiazole. NSC-115488.	3.0 (2 cures out of 6 animals treated at 25 mg/kg)	HNTD 25 mg/kg	Activity confirmed.
N-[4[bis (2-chloroethyl) amino] -3-methoxybenzylidene]-2-naphthylamine. NSC-121878.	2.0	HNTD 60 mg/kg	Activity confirmed.
N, N-bis (2-chloroethyl)-4-[N-(3, 5-dibromophenyl) formimidoyl]-o-anisidine. NSC-112909.	5.25	HNTD 100 mg/kg	Activity confirmed.
2-Benzyl-4-[p-] [4-[bis (2-chloroethyl) amino]-3-methoxybenzylidene] amino] phenyl]-5-methyl thiazole. NSC-113449.	13	HNTD 45 mg/kg LD ₁₀ 42 mg/kg	Activity confirmed.
4-[N-(m-bromophenyl) formimidoyl]-N, N-bis [2-chloroethyl]-o-anisidine NSC-103397.	5.0	HNTD 75 mg/kg LD 16.5 mg/kg	Activity confirmed.
4 [3-] [4-]bis (2-chloroethyl) amino] -3-methoxybenzylidene] amino] -4-methoxyphenyl]-2-phenyl thiazole. NSC-107639.	2.2	HNTD 44.4 mg/kg LD ₁₀ 40 mg/kg	Activity confirmed. Activity not activity

*HNTD highest non-toxic dose in mice.

LD₁₀ toxicity in rats.

Chemotherapy of tuberculosis and leprosy

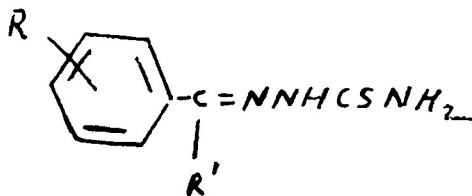
Antitubercular activity of thiosemicarbazones.—It is now universally acknowledged that in order to have an effective therapy of tuberculosis with reduced chances of development of resistance and minimum number relapses, it is essential to use a combination of two or more antitubercular drugs. While streptomycin and isonicotinic acid hydrazide are the most powerful and primary drugs of choice, the secondary drugs leave much to be desired although a wide range of such drugs are at present available. Most of these have undesirable side effects and even now there is a dire need for better substances, i. e. those having lower toxicity than the ones at present available and with less serious side effects. Among the widely used secondary drugs thiacetazone needs special mention. This compound was studied as early as in 1946 and was found to have *in vitro* activity at 2 ug/ml and also active in experimental tuberculosis of laboratory animals in which it was very well tolerated. However, when tried in human being it was found to be too toxic even at such a low dosage as 300 mg/day and was accordingly then discarded. In recent years, it has been revived again since at a still lower dosage of 150 mg/day it is reasonably tolerated and is effective. It is, therefore, now in clinical use.

Thiacetazone is a thiosemicarbazone obtained by reacting p-acetamino benzaldehyde with thiosemicarbazide. While the maximum tolerated dose of thiacetazone in animals is about 5 g/kg per day, in the human beings the maximum tolerated dose is as low as 0.005 g/kg/day. This wide variation in its toxicity between laboratory animals and human beings may be due to the cleavage of the azomethine linkage to liberate the aldehyde. If ketones are used instead, the breakage of bond could be less easy and one might expect better tolerance in human beings. Secondly, investigation of groups other than aminophenyl, acetaminophenyl or nitrophenyl was considered worthwhile in order to reduce the toxicity since most of the work in that period was on compounds containing aminophenyl acetaminophenyl or nitrophenyl on the basis of the activity of sulfonamides and sulphones like DDS which were supposed to act by virtue of competitive inhibition of PABA utilization.

The preparation of a few such model compounds from ketones were taken up *viz.* substituted acetophenones for possible use as antitubercular and anti-leprosy drugs. So far they have only been tested for their *in vitro* antitubercular activity. The following Table 27 gives their structure and *in vitro* activity :

TABLE 27

In vitro antitubercular activity (H₃₇ Rv-strain)



R				R	mcg/ml
4-Cl	..	..	..	.. CH ₃	0.2
4-F	..	..	..	.. CH ₃	2.0
4-CH ₃	..	..	..	.. CH ₃	0.2
4-OCH ₃	..	..	..	.. CH ₃	0.2
3,4-(OCH ₃) ₂	..	..	..	.. CH ₃	0.2
3,4,5-OCH ₃) ₂	..	..	..	.. CH ₃	2.0
3,4,5-(OCH ₃) ₃	..	..	..	.. H	2.0
4-CH ₃ CONH (Thiacetazone)	..	..	..	.. H	2.0

This class of compounds appears to be much more active than thiacetazone *in vitro*. A few compounds of this class have also been mentioned in the published literature to have good *in vitro* anti-tubercular activity. However, no data concerning their *in vivo* activity or toxicity to human being is recorded, the prior work being done in a period when thiacetazone was discarded as too toxic and the advent of substances like pyrazinamide, cycloserine etc. had eclipsed the class of substances called thiosemicarbazones. In retrospect, it is felt that some of the compounds obtained from the aryl ketones and semicarbazide being of very good activity—far more active than thiacetazone and being easy to produce—may be studied further particularly for long term toxicity in different species of animals and if results be encouraging then in human beings for possible use in tuberculosis and leprosy.

Synthetic of 4, 6-Diamino-1, 2-dihydro-2, 2-dimethyl-1-1-substituted benzamido-S-triazine

A series of 1-substituted benzamido-2,2-dimethyl-1,2-dihydrotriazines have been prepared by the cyclization of requisite substituted benzoic acid hydrazides, dicyandiamide and hydrochloric acid. They were obtained as hydrochlorides from the reaction mixture and were highly soluble in water. These compounds have been screened for *in vitro* antibacterial (including antitubercular) antifungal and antiamoebic activity. No significant activity was detected.

Synthesis of 7-amino-5, 5-dimethyl-3-aryl-5, 6-dihydro-s-triazolo-(4, 3-a) s-triazines

The above benzamidodihydrotriazines (bases) when heated above 200° C in an oil-bath were found to melt and then resolidify within a short while to give the isomeric 4-amino-6-benzoylhydrazino-2,2-dimethyl-1,2-dihydro-s-

triazine which lost a molecule of water to give a triazolotriazine. These compounds have also been tested for *in vitro* antibacterial (including anti-tubercular), antifungal and antiamoebic activity. No compound with significant activity was found.

Both the above two series of compounds may possibly possess antitumor activity and accordingly a few representative compounds are being sent to CCNSC for screening.

Hypoglycaemic agents

Synthesis and study of 4H-1,2,4-triazole derivatives as possible hypoglycaemic agents.—It has been reported earlier that two compounds viz. 5-*p*-chlorophenyl-4-ethyl-4H-1,2,4-triazole-3-thiol and 5-*p*-sulphamoylphenyl-4-ethyl-4H-1,2,4-triazole-3-thiol as possessing very highly potent and prolonged hypoglycaemic activity in normal and alloxan diabetic rats. A single dose of 25 mg/kg of either of these compounds reduced the blood sugar level by 45 to 53% and the period required for the level to come to normal values exceeded 36 hours. Considerable interest has been shown in these compounds by many workers all over the world. Further work on these compounds, particularly the detailed pharmacological and toxicological studies for the purpose of their preclinical evaluation, is being pursued by the Pharmacology Department of this Institute.

Synthesis and study of 2, 5-Disubstituted-1, 3, 4-thiadiazoles and Oxadiazoles as possible hypoglycaemic agents

As an extension of the above earlier work other allied series, viz. thiadiazole and oxadiazoles have been synthesised and evaluation of their hypoglycaemic activity has been carried out in normal and alloxan diabetic rats in collaboration with the Department of Pharmacology of the Institute. Out of the 75 compounds 48 compounds showed low to moderate activity (upto 10-20% lowering) and 12 compounds showed good activity (25 percent lowering or more). The most active compound in this series was 2-(3',4',5'-trimethoxyphenyl)-5-phenylamino-1,3,4-oxadiazole (38.7 percent at 7 hr. and 40.9% at 24 hr.). However, although a number of compounds among them have shown good activity, none was found as potent as the earlier two compounds of the triazole series. A few isoxazole derivatives of different structures have been reported in the literature to possess hypoglycaemic activity but these have been found to develop resistance very soon. In view of these reported findings the representative compounds from oxadiazole and thiadiazole series were subjected to prolonged administration to find out possible drug resistance, if any. The few active compounds from these series were also found to produce resistance.

Antiamoebic and antifilarial substances

It is well-known that compounds containing piperazine ring are active against micro-filaria. A few piperazine derivatives synthesised here have also shown activity *in vitro* against *E. histolytica*. The work carried out during the period under review comprises three different series as given below :

Synthesis and study of N-Methyl, N⁴-(2 (alkyl substituted, p-chlorobenzyl) amino) ethyl piperazines as antiamoebic agents

We have reported earlier that R-1396 (N¹-methyl-N⁴[(p-chlorobenzhydryl) amino]ethyl-piperazine is active *in vitro* against *E. histolytica* at a concentration as low as 1.0 mg/ml i. e. almost nearing the range of M. I. C. of emetine. With a view to study the structure activity relationship we have now effected modifications in the molecule of R-1396 and a series of compounds have been synthesised and studied for their *in vitro* activity against *E. histolytica*., The results indicate that when one of the phenyl rings of the benzhydryl moiety is substituted by a lower alkyl group, viz. methyl, ethyl, propyl etc. the activity was slightly reduced in the following order Ph₂, C₇H₇, C₂H₅, CH₃ which would suggest that the lengthening of the alkyl group may increase activity. Work in the preparation of such compounds is in progress. Another study was directed to the effect of the number and position of halogens in the aryl portion on the *in vitro* activity. It is found that no substantial role was played by the position or number of halogens.

Synthesis and study of Acyl derivatives of N¹-Methyl, N⁴-[B Greek alphabet beta (p-chlorobenzhydryl) amino] ethyl piperazine as antiamoebic agents

During the period under review the compound R-1396 has also been studied for *in vivo* activity in intestinal and hepatic amoebiasis in golden hamsters in the Department of Pharmacology. It was found that this compound shows activity at a much higher dose level than what was expected from the *in vitro* results. The compound being highly water-soluble i.e. hydrophilic in nature, it was conjectured that it may either not be absorbed or get eliminated rapidly. Accordingly, the incorporation of lipophylic acyl groups such as acetyl, propionyl, butyryl, hexanoyl and *n*-palmitoyl have been effected into the molecule of R-1396 and these newly synthesised compounds are now awaiting *in vivo* studies in hamsters.

Another derivative in this series, wherein the methyl substituent on the piperazine group of R-1396 has been replaced by beta hydroxyethyl (HO-CH₂-CH₂), has also been prepared and found to possess almost the same *in vitro* activity and lower toxicity than the parent compound. This substance also awaits *in vivo* study.

Synthesis and study of -1[2 (p-substituted piperazinyl) ethyl]-2-methyl-5-nitroimidazoles as antiparasitic agents

1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole, a drug originally developed as very effective in the treatment of trichomoniasis has recently been shown to be very potent antiamoebic agent and is being now used very widely by the clinicians. Since piperazine ring has afforded in our laboratory series of compounds having good *in vitro* antiamoebic activity, we were tempted to incorporate piperazine ring with nitroimidazole molecule. Hence 1-(2-chloroethyl)-2-methyl-5-nitroimidazole was condensed with different monosubstituted piperazines. The incorporation of the piperazine was found, however, to reduce or totally eliminate the activity of the resultant compounds, when studied for *in vitro* antiamoebic and antitrichomonal activity.

Synthesis and study of 2-(N-substituted piperaziny)-3-p-nitrophenyl-1:3-propanediols

Considerable work has been done to modify the molecule of chlroamphenicol a broad spectrum antibiotic. While the changes introduced have, in general only reduced the activity, the replacement of the dichloroacetyl group b other acyl or aroyl groups have shown some interesting observations. Since the piperazine has not so far been introduced into the chloramphenicol mole cule one was tempted to replace the dichloracetamido group by substitute piperazines. In view of the known stereospecificity of the chloramphenicol, only the D(—) *threo* was used i.e. the stereospecific isomer of the 2-amino-3-*p* nitrophenyl 1:3-propanediol for condensation with N-alkyl-N-aryl-, o N-dichloroacetyl-, B-dibromodiethylamine to obtain D(—) *threo* 2 (N-alkyl-aryl-or dichloroacetyl-piperaziny)-3-*p*-nitrophenyl-1:3-propanediols. So far, a number of compounds were prepared and characterised. These are awaiting screening for their antimicrobial activity.

DEPARTMENT OF CLINICAL PATHOLOGY

Incidence of Brucellosis in the State of Maharashtra

Epidemiological survey of some States of the country has shown varying incidence of brucellosis in man and animals. Incidence among cattle has been reported as high as 20 per cent in Orissa and Madras, while in human being the figures fluctuate from 0 to 3 per cent.

In the State of Maharashtra the figures of Directorate of Disease Investigation Section, Department of Animal Husbandry Poona, show that during 1967 to 1970, 6,334 sera of cattle, sheep, goat and pigs of different farms show 6 to 17 per cent positive agglutination test to *Brucella abortus* antigen.

A total of 30 pig sera from Regional Pig Breeding Station, Borivli, were collected. These animals were suspected to be suffering from brucellosis and 27 of them were seropositive. Cultures in 11 cases were tried but proved abortive. According to the Director of the Institute, this disease in the pigs may have been imported from outside.

During the period from September 1970 to February 1971, 85 sera samples of prisoners from different parts of Maharashtra were screened but none were positive. One case suffering from pyrexia of unknown origin gave a positive serological test but this case could not be traced further.

This study has only touched a fringe of the problem. It has not taken into account the people coming in contact with the animal population and efforts are being made in that direction.

Studies in diphtheria

The immunological study was carried out by estimating the anti-toxin level in 50 normal persons, 50 patients, 50 carriers and 10 schick positive and 10 schick negative children.

It was observed that the anti-toxin level is higher in carriers ranging from 0.12 to 12 units per ml., while in 74 per cent normal persons and 70 per cent patients, it was lower than 0.01/unit per ml. According to Schick antitoxin 0.03 units per ml. or more is the protective level.

Studies in Leptospirosis

A total of 567 human sera samples and 90 animal sera have been screened. These sera were subjected to microscopic agglutination test, sensitised Erythrocyte lysis test and haemagglutination test. The human sera were from rice field workers, suspected leptospiral cases, blood donors and also prisoners from different places, (Nasik, Thana, Yawada Visapur) of Maharashtra. The animal sera were from sheep, goat, bullock, swine, rats and dogs. Out of these of the human sera and 15 of the animal sera showed leptospiral agglutination. Out of 7 human positive sera, two of the sera were from prisoners, (Nasik and Thana) one from rice field workers, two from suspected leptospiral cases and the remaining two were from normal blood donors. They were positive for all the three tests in more than 1/100 dilution. The serotypes were found to be *L. pomona*, *L. ballum*, and *L. icterohaemorrhagiae*. Out of 15 positive animal sera 4 were from stray dogs, 6 were from aborted cases of sheep, cattle and goats and 5 of the sera were from rats. The highest titre was found to be 1/1000 among stray dogs. Other sera were reactive at 1/100 or 1/300 dilution. The serotypes were found to be *L. canicola*, *L. pomona*, *L. ballum* and *L. icterohaemorrhagiae*. The work is in progress.

Studies on mycophages and drug resistance in mycobacteria.

In continuation of the work reported earlier the strains of mycobacteria were tested for their sensitivity to a new antitubercular antibiotic rifampicin. About 50 per cent of the strains were found to be resistant to this antibiotic in a concentration of 20 µg/ml or more. The test was repeated by using dimethyl sulfoxide (DMSO) and bacitracin. However, no change in the antibiotic-resistance-pattern was observed due to these chemicals. Preliminary experiments to study the transfer of rifampicin sensitivity by recombination was attempted.

Phage-typing of all the 44 strains of mycobacteria is completed. Phage types D12, D29 and GS4 were found to be prevalent in Bombay. Most of the phages were found to be heterospecific in their action. Chemical composition of the cell wall of mycobacteria both before and after treatment with DMSO was estimated. DMSO treatment reduced the phosphorus content of the cell wall. Six lysogenic strains were successfully induced from these strains after ultraviolet treatment. Both the temperate phages and the lysogenic strains were purified by Haye's technique. Preliminary experiments with transduction were found to transduce chromogenic marker. However, the repeated experiments revealed drug resistance transductants. These resistance clones are under analysis. About 249 lymph glands from tubercular lymphadenitis cases were cultured for the isolation of atypical mycobacteria. More than 10 strains of atypical mycobacteria were isolated.

DEPARTMENT OF IMMUNOLOGY

Stability of purified antitoxic sera

The work reported previously indicated that addition of pepsin to pepsin-digested and purified antisera greatly accelerated the loss of antibody activity during storage in cold. These results indicated that the deterioration of peptic digested antisera may be due to residual pepsin, since none of the other agents tested such as antiseptics (phenol, merthiolate, para-chloro-meta-cresol) or presence of amino acids (lysine or glutamic acid) had any effect on the deterioration. Further, heating of pepsin digested antisera at 60°C for 1/2 to 1 hr. rendered antisera more stable.

In the light of the above results it was possible to modify the pepsin digestion process to reduce the quantity to residual pepsin in the final sera to make it more stable during storage. One method was to wash the antibody precipitate with 50 per cent ammonium sulphate solution in which pepsin is soluble and antibodies are insoluble. A second possible method is the use of insoluble pepsin. A process is being worked out to prepare such insoluble active pepsin for this purpose.

Insolubilisation of pepsin

It has already been reported last year that it was possible to prepare insoluble and active pepsin by co-polymerization of pepsin and gelatin in the presence of ethylchloroformate. The product was stable in wet condition but lost its activity when dried. It could be preserved in wet condition in cold for over 6 months, without significant loss in activity. The activity of insolubilised pepsin for digestion of horse antitoxic plasma was about 1/12th the activity of soluble pepsin, when the activity was measured at 25°C for 3 hrs. at pH 3.5. When the enzyme activity was measured by Anson's procedure using haemoglobin as substrate, the activity of insolubilised pepsin was 1/25th that of soluble pepsin. Experiments are now under way to insolubilise pepsin using gluteraldehyde.

Fractionation of Russell's viper venom and isolation of biologically important constituents

Russell's viper venom was separated using starch gel electrophoresis. About 9 bands were obtained, 8 on the positive side and 1 on the negative side. The starch gel electrophoresis method was improved by incorporating 8 molar urea in the citrate buffer at pH 8.6, ionic strength 0.05. By this procedure Russell's viper venom showed 12 distinct bands, 7 on the negative side and 5 on the positive side. The enzyme and toxic activities of these separated bands is being studied.

Studies in tetanus toxin production

The results of work using semi-synthetic medium based on acid hydrolysate of casein reported before, has been applied to improve production in the Massachusetts medium and also in the peptic digest of meat medium. Supple

entation of these media with vitamins and KCl has improved toxin yield from 15 to 20 LF/ml in the Massachusetts medium, to 30 to 40 LF/ml. The yields in the peptic digest of meat medium have also improved.

Effect of adjuvants and immunosuppressors on production of antibodies to diphtheria toxoid

The work on adjuvants and immunosuppressors for diphtheria toxoid as continued in guinea pigs. It is known that antisera can act as an adjuvant or as an immunosuppressor depending on the relative concentration of antibody and its antigen. When guinea pigs were immunised with 3 injections of diphtheria toxoid each of 10 Lf, at intervals of 15 days, highest antibody titre of 6.1 I.U./ml was attained after 37 days. When 10 Lf of diphtheria toxoid as mixed with 1 I.U. of horse antitoxin and injected as above, antibody production increased to 14.2 I.U./ml after 37 days. When diphtheria toxoid 10 Lf was mixed with 5 I.U. of antitoxin, antibody titre attained after 37 days was 20.6 I.U./ml. When diphtheria toxoid 10 Lf was mixed with 10 I.U. of antitoxin, antibody titre went upto 31.4 I.U./ml. after 37 days. However when 10 Lf of diphtheria toxoid was mixed with 20 I.U. of antitoxin, antibody titre was only 0.01 I.U./ml. after 37 days, which indicated immunosuppression. These results indicate that diphtheria toxoid in neutral or antigen excess region is more antigenic than diphtheria toxoid alone. But diphtheria toxoid with excess antisera acts as immunosuppressor.

Screening of immunosuppressors and adjuvants using sheep red blood cells (SRBC) in mice

Five anti-cancer compounds belonging to the group of nitrogen mustards synthesised in the Chemotherapy Department of the Institute were tested in mice both for primary and secondary antibody response against sheep R.B.C. used as antigen. The results were compared with the immunosuppressing effects of the well known anti-cancer compound cyclophosphamide which also belongs to nitrogen mustard series. Three of the compounds were found to have more or less same activity as cyclophosphamide, while 2 showed less immunosuppression than cyclophosphamide both in primary and secondary response.

Preparation of horse antidog lymphocyte sera

The work carried out in collaboration with the department of Surgery at the K.E.M. Hospital under Dr. P. K. Sen had previously shown that horse anti-dog lymphocyte sera prepared using dog spleen lymphocytes was potent. The side reactions such as anaemia could be prevented by proper absorption with dog R.B.C. Local reactions, which were common with anti-lymphocyte sera, when injected by sub-cutaneous route, were avoided by injecting the sera intraperitoneally. The skin transplants from another dog carried out in the occipital region, were not rejected for over 3 months in the dogs treated with A.L.S. Efforts are being made to prepare antilymphocyte sera using lymphocytes obtained by canulation of thoracic duct of dog.

Treatment of leukemia by active and/or passive immunization

Active immunisation of leukemia patients with W.B.C. tagged with dinitro fluoro benzene was continued in 7 patients. All the patients including one case of acute leukemia have shown improvement. One case showed remission after 9 months but recovered very quickly after treatment with chemotherapeutics. Active immunisation with tagged W.B.Cs. is being continued in this patient.

Immunoglobulins in Indian water buffalo

Two buffalos at the Bombay Veterinary College were immunised with tetanus toxoid. Later, immunisation was changed over to tetanus toxin. The antibody titre came upto about 100 I.U./ml. in one buffalo and 150 I.U./ml. in the second buffalo. The antisera was used to prepare floccules with tetanus toxin. The floccules were thoroughly washed. The washed floccules were used for immunising rabbits for production of anti-buffalo immunoglobulin serum. This serum would be a specific reagent to detect immunoglobulins because the washed antigen antibody floccules used for preparing rabbit antisera would contain only immunoglobulins and no other serum proteins from horse serum. The anti-floccule serum will be used to detect immunoglobulin pattern in colostrum, milk and other secretions of the buffalo.

Studies in diphtheria toxin production in acid hydrolysed casein medium

The previous work has shown that maltose, which is to be imported, could be replaced by mixture of sodium acetate and glycerol, without any decrease in toxin production. Attempts were made to see if casamino acids (acid hydrolysed casein, Difco) could be replaced by other nitrogen sources. Preliminary results have shown that casamino acids could not be replaced even partially with urea or ammonium sulphate or ammonium acetate or sodium nitrate or ammonium nitrate.

The iron in the medium is known to inhibit diphtheria toxin production. However, its effects could be partially nullified by adding suitable increased amounts of calcium or phosphates or both. The favourable effect was probably due to precipitation of iron as iron phosphate.

A simple synthetic medium for the study of diphtheria toxin production

A purely synthetic medium was evolved with *C. diphtheriae* strain Weissensee G-5 which could give 50-55 Lf/ml. of toxin after 5 days. The medium consisted of 5 amino acids (glutamic acid, cystine, valine, isoleucine and methionine) 4 vitamins (nicotinic acid, B-alanine, thiamine and pyridoxine) salts and maltose. This medium was used to study the metabolism of *C. diphtheriae*.

The sulphur metabolism of the organism was investigated by replacing cystine in the above medium with thioglycollate, sodium sulphide, thiosulphate or extra methionine. Thioglycollate was as good as cystine followed by sulphide and thiosulphate. The other compounds containing sulphur could not be utilised for the synthesis of cystine.

Methionine was also not converted into cystine or *vice versa*. The results indicate that the organism is probably able to obtain cystine with the help of hydrogen sulphide and serine mediated by the enzyme, serinesulphhydrase.

The carbon metabolism was investigated. The organism could utilise in place of maltose the following sources of carbon : sucrose, lactose, cellobiose, acetate, succinate, fumarate and malate. Glucose and fructose inhibited growth due to acid formation. Citrate also was inhibitory to growth probably because of its chelating ability for essential ions such as magnesium and calcium. Since it could be reversed by adding extra magnesium and calcium. The work on the role of iron in inhibiting toxin production will be carried out using this medium.

Role of phage in induction of toxin

Thirteen phages and 3 host strains of diphtheria (1180,304 and 3148) were received from Dr. Alice Saragea, Head of Diphtheria Laboratory, Rumania. It was found that phage No. 14 and 15 individually did not lyse the diphtheria strain PW 8. There was also no lysis of PW 8 strain when the two phages were mixed. However, when a recombinant of phage No. 14 and 15 obtained by superinfecting the diphtheria strain 304 (14) was found to be virulent and lysed the PW 8 strain though the individual phages (14 and 15) did not lyse PW 8 strain.

Phage No. 15 which is non-toxinogenic was able to transduce the code for toxin production from the toxinogenic strain 3418 to the non-toxinogenic strain 304. It was found that phage No. 3 was related to phage P as the diphtheria strain C7 lysogenised either with 3 or P became resistant to the two phages. Phage No. 3 gave clear plaques with strain PW 8 but not when it was lysogenised with phage P.

DEPARTMENT OF BIOCHEMISTRY

Growth requirements of microorganisms

Studies on the nutritional requirements of V. cholerae and P. pestis.—Earlier work on the amino acid analysis of casein hydrolysate (acid digested) and defatted peanut cake hydrolysate (acid digested) had shown that while the casein hydrolysate was completely free from tryptophane and deficient in phenylalanine, the peanut hydrolysate was deficient in tryptophane, phenylalanine and methionine.

With casein hydrolysate as the standard for growth-experiments for *V. cholerae* and *P. pestis* it was found that while *V. cholerae* could grow well in the peanut hydrolysate, the *Pasteurella* organism showed very poor growth under the same conditions. Addition of the deficient amino acids to the peanut hydrolysate did not, however, improve the growth of *P. pestis*. It was of interest to note that in the case of the casein hydrolysate, the addition of tryptophane while inhibiting the growth of *V. cholerae* improved (doubled) the growth of *P. pestis*.

The growth of the two organisms were also compared in a simpler glucose salt medium (standard media for *E. coli*) with and without added supplements-amino acids and nucleic acid precursors. It was found that the amino acid supplementation was necessary for the growth of *V. cholerae* while *P. pestis* could not grow at all in the simpler medium.

Since acid digestion destroys tryptophane of proteins, enzymatic hydrolysis (papain and trypsin) was tried. While the growth of *V. cholerae* was unaffected, *P. pestis* could grow much better (50% more) in the enzyme hydrolysed casein but not in the peanut cake hydrolysate.

Since it was necessary to study the growth of the two organisms in isolated protein hydrolysates free from carbohydrates and any other impurities present in the whole defatted cakes, work was undertaken to isolate the pure protein fractions from the (a) peanut cake (defatted), (b) sesame cake (defatted), (c) safflower cake (defatted), (d) gawar protein and (e) gluten (BDH.)

The isolated proteins were hydrolysed with dilute HCl under pressure at 120° C and used directly for growth experiments after decolorisation and adjustment to pH to 7.5. By this procedure of hydrolysis, the amino nitrogen is lower (10–15%) than complete hydrolysis with H₂SO₄ + HCl (casein only).

Table 28 presents the data for the growth of *V. cholerae* and *P. pestis* in hydrolysates of the pure proteins. While all the other proteins gave fairly good growth for *V. cholerae*, safflower seed protein showed only 50-60% growth.

TABLE 28
Growth of organisms in terms of optical density

Hydrolysates (135 mg. N/100 ml.)	Optical density	
	<i>V. Cholerae</i> (72° hrs. at 37 C)	<i>P. pestis</i> (48° hrs. at 32 C)
Casein (laboratory)	0.39	0.49
Casein (media)	0.30	0.36
Casein (H ₂ SO ₄ + HCl)	0.36	0.46
Peanut protein	0.29	0.64
Sesame protein	0.29	0.27
Safflower protein	0.19	0.58
Gawar protein	0.35	0.41
Gluten (BDH)	0.32	0.44

In the case of *P. pestis*, while safflower seed protein hydrolysate showed very good growth, sesame protein could give only 50% growth.

The hydrolysates were also analysed for calcium, phosphorus, magnesium, iron and copper (Table 29).

TABLE 29

Mineral content of casein and indigenous protein hydrolysates

Hydrolysate	Per 100 ml (N—270 mg)					
	Total ash g	Ca mg	Fe ug	Mg mg	Cu ug	P mg
Casein (laboratory)	1.65	6.25	9.0	1.18	23.0	17.5
Casein (media)	1.41	7.60	8.0	1.52	25.5	17.0
Casein (H ₂ SO ₄ +HCl)	1.44	7.10	8.0	1.38	24.5	16.0
Peanut protein	1.48	9.60	12.5	2.04	26.5	12.50
Sesame protein	1.92	13.2	11.5	2.34	55.0	2.90
Safflower protein	1.43	11.50	15.0	2.10	35.0	5.90
Gawar protein	1.22	9.80	9.0	2.50	41.0	11.00
Gluten (BDH)	1.53	13.65	13.0	2.60	49.5	9.00

Studies in protein metabolism of *T. pyriformis* W

T. pyriformis W is a proteolytic protozoan. It can, therefore, grow on intact proteins, liberating and utilizing the necessary amino acids. Earlier studies had indicated that the growth of the organism varied with different proteins. The ability of the organism to grow on a given protein may depend on its capacity to liberate the amino acids from the proteins. In addition to the amino acid composition of the proteins-nitrogenous substances—such as nucleic acids and the non-protein nitrogen may also have some effect on the growth of *T. pyriformis* W.

Therefore, systematic work was undertaken to study the nutritional requirements of this organism using (i) pure amino acids (ii) isolated proteins from various sources (iii) partially and completely hydrolysed-acid and enzyme digested proteins.

Earlier work was concerned with the (i) amino acid requirements of the organism and (ii) the growth of the organism on amino acid mixtures simulating the composition of certain proteins, such as whole egg (hen), cow's and human milk and the ideal protein pattern of Swaminathan.

Further work was carried out on the following lines—Growth of the organism on (a) seven commonly used food items such as whole egg, rice, wheat, jowar, bajra, red and bengal gram dhal (b) isolated proteins from the above foods, casein and leaf protein (supplied by CFTRI), (c) acid and enzymatic hydrolysates of the above isolated proteins, (d) supplementation of one or more deficient amino acids to the whole foods shown under (a), and (e) the above food items after extraction with hot TCA to eliminate the effect, if any, of non-protein nitrogenous substances including nucleic acids.

Some salient observations of the above studies are summarised below :—

(i) The regression coefficients of growth curves of the 7 foods studies was different from the individual proteins isolated from the foods. The growth response was linear with the nitrogen concentrations upto a level and thereafter the growth curves were flat. (ii) The acid hydrolysates of some of the food proteins had some advantage over the intact foods with reference to their effect on the growth of the organism ; however, the acid hydrolysates of cereal foods—such as rice, jowar, bajra and wheat depressed the growth of the organism to some extent. (iii) The enzymic hydrolysates (pepsin and trypsin for 72 hours) showed adverse effects on the growth of the organism. (iv) The proteins isolated from the cereals and pulses showed similar growth curves as those on intact foods. (v) The TCA extracted proteins caused no appreciable change in the growth pattern of the organism. (vi) The growth response of the protozoan to 4 different amino acid mixtures, simulating the composition of the ideal protein indicated that the organisms was sensitive towards slight changes in the amino acid composition of proteins. This being the highest, is indicative of a close relationship between the amino acid requirements of the human infant and *T. pyriformis* W. (vii) The significant increase in the growth response due to supplementation of the deficient amino acids such as methionine, lysine, threonine and tryptophane to the cereals and pulses indicates that the growth of the organism is directly related to the amino acid composition of the proteins.

Dietary fats and lipid metabolism

It is now very well recognised that the dietary fats, both in quality and quantity affect the tissue lipids. This effect can be generally recognised from the serum cholesterol levels. Much work has been done all over the world with reference to unsaturated dietary fats and the serum and tissue cholesterol and phospholipid levels. It is also recognised that while animal fats which contain high amounts of saturated fatty acids, tend to increase the serum cholesterol levels., ingestion of vegetable fats which contain a greater proportion of unsaturated fatty acids tend to lower the same.

Earlier work in the Department was carried out using safflower seed and coconut oils as the dietary fats in albino rats. Although of vegetable origin, coconut oil is highly saturated as those from animal sources namely butter, ghee and lard. Coconut oil elevates the serum cholesterol level as compared to safflower seed oil. The picture in the liver is the reverse indicating that the excess cholesterol is stored in the liver in the combined form with polyunsaturated fatty

The work was extended to other dietary fats, Lipids from serum and liver were studied in detail using thin-layer (TLC) and gas-liquid chromatography (GLC).

Animals fed with saturated fats like coconut oil, 'Pakav' and 'Dalda' have higher total saturated fatty acids than those maintained on groundnut, sesame and safflower seed oils. The concentration of polyunsaturated fatty acids in the serum are very much higher in the latter groups. The liver lipids contain a higher proportion of mono and polyunsaturated fatty acids as compared to serum lipids. On the whole, the 'Pakav' and 'Dalda' (hydrogenated vegetable oils) groups show a different fatty acid pattern than the vegetable oil groups including the coconut oil.

Although coconut oil is very highly saturated (about 90 per cent) as compared to 'Dalda' and 'Pakav' (35 %), its effect on the fatty acid spectra of serum and liver lipids seems to follow the same pattern as the other unhydrogenated vegetable oils-groundnut, sesame or safflower seed oils.

TABLE 30

Serum and liver fatty acid patterns in relation to dietary fats in the albino rat

		Coconut	'Pakav'	'Dalda'	Sesame	Ground-nut	Safflower seed oil
<i>Total saturated fatty acids</i>							
Dietary fat	..	91.7	36.0	34.6	14.2	18.4	11.0
Serum lipid	..	76.2	78.5	74.4	68.5	69.8	61.0
Liver lipid	..	47.3	59.6	51.8	46.9	44.6	44.7
<i>Monounsaturated fatty acids</i>							
Dietary fat	..	5.7	61.0	60.5	45.4	54.2	13.0
Serum lipid	..	16.5	18.1	20.1	15.1	15.4	12.3
Liver lipid	..	27.8	29.7	33.6	25.0	28.1	22.6
<i>Polyunsaturated fatty acids (PUFA)</i>							
Dietary fat	..	2.6	3.0	4.7	40.4	27.4	76.0
Serum lipid	..	7.2	3.3	5.3	16.4	14.8	26.5
Liver lipid	..	24.8	10.5	14.6	28.1	27.3	32.6

Influence of dietary carbohydrates on lipid metabolism in the rat

The influence of dietary carbohydrate on the lipid metabolism in man is of wide interest. Two factors are involved in this, the amount and the type of carbohydrates consumed. These two aspects assume clinical importance when it is considered that in certain parts of the world the diet of a large number of people consists mainly of carbohydrates while there are other groups whose carbohydrate consumption, although not high includes increasing quantities of refined carbohydrate as sucrose. It has been stated that the malnutrition in the former group is aggravated by the relative excess of carbohydrate while some of the diseases of the latter group are related to the increasing consumption of refined carbohydrates (mainly sucrose). The varying metabolic response to different types of carbohydrates is not quite understood. The little work that has been done in this sphere indicates that lipid metabolism can be considerably influenced by the type of dietary carbohydrate consumed. It is speculated that the refined carbohydrates play a serious part in the etiology of such diseases as atherosclerosis, ischemic heart disease and diabetes mellitus. The consumption of sucrose, in general, seems to increase with the prosperity of a community. Some work on experimental animals and human volunteers are reported in the literature. The present work in this laboratory has been taken up to study the effect of different carbohydrates on lipid metabolism using various dietary fats.

Earlier work in this laboratory has confirmed the observations that increased ingestion of the disaccharide sucrose can lead to elevated serum cholesterol levels in the rat. Sucrose can not only enhance the hypercholesterolemic effect of a saturated dietary fat like coconut oil but can also nullify the hypocholesterolemic effect of an unsaturated dietary fat like safflower seed oil. Marked alterations are observed in other tissue lipids and fatty acid components in the presence of increased amounts of sucrose in the diet.

The study has been extended further to glucose, lactose, corn and potato starches as carbohydrates, sunflower seed oil as the dietary fat and casein as the nitrogen source.

The experiments consisted of feeding groups of albino rats with diets containing casein (24 %) and an isocaloric mixture of the sugar and starch, sunflower seed oil at 10 per cent, along with salt and vitamin mixtures. The proportion of sugar and starch were varied at suitable levels.. At the end of 10 weeks, the animals were sacrificed and the tissues were processed for analysis. The results indicate the following : (i) higher proportions of lactose in the diet are not tolerated by the rats resulting in diarrhoea and subsequent mortality, (ii) as in the case of sucrose, lactose produces hypercholesterolemia which is lowered by the starches, (iii) the cholesterol:phospholipids ratio is not altered appreciably, (iv) the fat and cholesterol accumulation in the liver is more with lactose as well as sucrose) as compared to the starches.

Biochemical studies on *V. cholerae* and *V. eltor*

The normal cells under suitable environmental conditions synthesize different macromolecules, viz., proteins, nucleic acids, lipids, and carbohydrates, etc. and there is a definite pattern in the percentage composition of these macromolecules. Any change in the environmental conditions, such as temperature variation, condition of growth, composition of medium, presence of certain other substances such as antimetabolites or antibiotics, etc. may alter the pattern of the cellular composition. Hence studies on the effect of certain antibiotics and an anticholera drug on the biochemical reactions of *Vibrio cholerae* and *V. eltor* was taken up.

Earlier work in this laboratory had shown that the vibriostatic effect of the alkaloidal drug berberine is due to certain membrane damage, causing a leakage of the macromolecular pool of the cell. The net protein and nucleic acid syntheses are affected and the drug is found to be associated more with the lipid fractions. The *Vibrio cholerae* is more sensitive to the drug as compared to the *el tor* variety.

The present study has been extended using (a) furazolidone (b) ethyl alcohol and (c) chloromycetin.

(a) In 1944 Dodd and Stillman had reported that the introduction of a nitro group in the 5 position of the furan ring conferred antibacterial activity to 2-substituted furans. Furazolidone—(5-nitro-3-amino-2-oxazolidone) is now available commercially for clinical use against enterobacters. Paul in 1954 had first used this drug clinically against *Salmonella* infections. This has been confirmed by many others in recent years both in this country and abroad. It is reported to be more effective with less side effects than the other widely used antibiotics. The present work has been undertaken to study the effect of this drug on the two strains of cholera vibrios.

It is found that concentrations as low as 150-200 ug. % of this drug in a synthetic medium inhibit the growth of the two strains by increasing the lag period. *V. Cholerae* is found to be more sensitive to the drug than the *el tor* variety—1 ug./ml. concentration bringing about 50 per cent inhibition of the growth of *V. Cholerae* and only 30 per cent in the case of *el tor*. A detailed study of the cellular constituents revealed that the drug inhibited protein synthesis while increasing the RNA synthesis. This drug could be estimated by a convenient colorimetric procedure. It is found that the major portion of the drug is associated with lipids and RNA fractions of the cell. The absorption of *V. Cholerae* was found to be slightly more (48 per cent) than in the *el tor vibrio* (40 %). Tables 31 and 32 summarise the results.

TABLE 31

Effect of furazolidone on cellular constituents of V. cholerae cells

Cellular constituents				Percentage on dry weight basis	
				Control	Test (drug treated)
Nitrogen	..	..	..	11.2	12.0
Proteins	..	..	..	56.0	40.0
RNA	..	..	..	13.8	22.0
DNA	..	..	..	4.0	4.1
Sugars	..	..	..	11.9	14.0

TABLE 32

Percent of drug absorbed by various fractions of V. cholerae and el tor vibrios

Fractions				Percentage concentration of drug	
				<i>V. cholerae</i>	<i>el tor</i>
Acid soluble pool	..	..	..	8.9	9.6
Lipids	..	..	..	28.6	31.7
RNA	..	..	..	22.1	22.0
DNA	..	..	..	18.6	18.3
Carbohydrates-proteins	..	..	..	20.4	18.5

(b) the bactericidal action of ethyl alcohol has been known since the time of Koch. The probable explanation for the activity of alcohol is through its capacity to denature proteins. This fits in with the observation made by Daffley *et al* (1950) that the alcohol destroys bacterial dehydrogenases and the prolonged lag phase in the growth of aerobacter aerogenes can be reversed by adding certain amino acids.

Robertson *et al* (1948) made an observation that about 5 per cent alcohol inhibits the growth of most of the gram-positive and gram-negative non-spore forming bacteria.

It is of interest to note that from ancient time the tribal people of India treated cholera cases with alcoholic drinks. The present work was planned to study in detail the biochemical changes involved in this bacteriostatic action of alcohol. Table 33 summarises the results observed with *V. cholerae*.

TABLE 33

Effect of alcohol on cellular constituents of V. cholerae cells

Cell constituent	Percentage on dry weight basis				
	Control		Test		
Nitrogen	..	..	..	11.2	11.6
Protein	..	..	..	55.4	44.6
RNA	..	..	..	13.8	17.6
DNA	..	..	..	4.0	4.0
Sugar	..	..	..	11.9	13.4
Phospholipids	..	..	..	0.8	1.2

Ethyl alcohol at a concentration of 3.5 per cent in a synthetic medium is observed to inhibit the growth of cholera vibrios. The organisms are found to be without motility but with the flagella intact. Quantitative analysis shows that there is an inhibition in protein synthesis with enhanced RNA and lipid synthesis.

(c) Chloromycetin is an antibiotic very widely used for clinical treatment of all salmonella infections. In the present study, the effect of both ampicillin and chloromycetin on the growth and metabolism of *Vibrio cholerae* and *el tor* vibrio has been investigated in continuation of similar studies with berberine. Preliminary experiments indicated that while 25 ug./ml of ampicillin is required to produce 50 percent inhibition in both the strains, a very-low concentration, as low as 0.5 ug./ml. of chloromycetin is enough to produce the same effect in the two organisms.

The effect of these two drugs on the macromolecular composition have been studied in detail. Ampicillin-treated cells synthesize these constituents at a much lower rate than the normal cells, while in the chloromycetin-treated cells there is a greater accumulation of RNA and lower rate of synthesis of proteins. The pattern is the same for both the organisms.

To understand the effect of these drugs on the biosynthesis of the macromolecules a study of the incorporation of labelled glucose by these organisms has been undertaken.

Studies on the haptoglobin patters in certain clinical diseases

The haptoglobins (2-mucoproteins) are a family of serum proteins characterised by their ability to bind haemoglobin or certain derivatives of haemoglobin both *in vitro* and *in vivo* to form a stable complex. The haptoglobins in plasma, besides being useful genetic markers have been shown to be of diagnostic value in cases of haemolytic anaemia, liver disorders and various other diseases. The haptoglobin levels in liver cirrhosis can be taken as an index of hepatic cellular degeneration. Owen and his colleagues studied various liver disorders and found that the measurement of serum haptoglobin levels served as an index of hepatic disfunction viz. hepatitis and cirrhosis and also as an aid in the differential diagnosis of obstructive jaundice. No attempt has been made so far to correlate the haptoglobin levels with the genetic types. Hence a study on cases of hepatitis, cirrhosis of liver and other diseases was undertaken with reference to their genetic types.

The following laboratory investigations have been carried out in cases of hepatitis (both infective and amoebic), cirrhosis and other liver diseases. Serum glutamic oxalacetic transaminase (S.G.O.T.), serum glutamic pyruvic transaminase (S.G.P.T.), bilirubin, alkaline phosphatase and total proteins were studied. The haptoglobin levels are estimated quantitatively by acuglobin method (Connell, 1969) in the above cases, as well as in a few cases of malnutrition, pulmonary tuberculosis, haemolytic anaemia, diabetes, acute nephritis, etc. The phenotypes of the haptoglobin are determined by starch gel electrophoresis method. Markedly low values of haptoglobin have been obtained in liver disorders viz. hepatitis and cirrhosis of liver and very high values are noted in cases of nephritis.

DEPARTMENT OF PHARMACOLOGY

Studies in antileprosy drugs

The advent of Dapsone has been a positive contribution in the control and treatment of leprosy. However, the reports have been made of resistance developing to this drug. The ENL reactions are sometimes very severe and thirdly individual intolerance to this drug by some patients have been noted. These factors emphasise the need for alternative form of effective treatment. The Chemotherapy Department of this Institute has synthesised a wide range of new organic compounds for possible use against acid fast organisms. These have to be screened for their antileprotic activity.

Screening of drugs for their *in vitro* antileprotic activity

Screening of the compounds for antileprosy activity *in vitro* using ICRC bacillus in Dubos medium has been continued. Eighteen compounds received from Dr. S. M. Deshpande of Benares Hindu University were tested during this period and 4 compounds R 31/70, R 33/70, R 39/70 and R 35/70 showed significant activity. The compounds received from CDRL, Lucknow, are being tested.

A quicker method for testing antituberculosis drugs

Gaugas and Rees have shown that progress of tuberculosis can be rapid if agent like antilymphatic serum (ALS) is used in thymectomised mice. So, a quicker method for screening antitubercular drugs is suggested using the above techniques.

Albino mice about 6 weeks old were thymectomized by sucking out the gland under anaesthesia and used after 14 days. Antilymphatic serum was raised against mouse thymocytes in rabbits and assayed by using an agglutination test.

Mice, in groups of 10 were given ALS 0.2 ml. intramuscularly every alternate day. The infective dose of 1 mg. of H 37 Rv in 0.4 ml. was given intravenously, and the drugs were administered on the 4th day. All the drugs were fed orally, except streptomycin which was given intramuscularly. The mortality of the animals was checked till 80 per cent of the animals died.

From preliminary work it was found that more uniform and quicker results were achieved if the mortality of the animals was recorded at 80 per cent than the customary method of recording 100 per cent mortality. The drugs were administered only once that was on the 4th day, by which time the growth of the bacilli would be logarithmic. The results (Table 34) were taken to be significant if the survival period was increased from 7 in control to 9 or more in the drug-treated group. Conzelman and Jones (1956) found that the half life of cycloserine in mice was too short to have any effect.

This was confirmed.

TABLE 34

Drug				Dose/kg.	Days require for 80% mortality
Saline	..	..	..	0.4 ml.	7
Isoniazid	..	..	..	20 mg.	11
PAS	..	..	..	400 mg.	11
Streptomycin	..	..	..	25 mg.	12
Cycloserine	..	..	..	100 mg.	7
Ethinonamide	..	..	..	40 mg.	10
Pyrazinamide	..	..	..	20 mg.	9
Thiacetazone	..	..	..	40 mg.	11

Antidiabetic activity of indigenous drugs and synthetic compounds

Different extracts of eight indigenous plants were studied for their antidiabetic activity in rabbits. Out of which alcoholic extract of PY-IDR-1 *Kela* stem (*Musa paradisaca* Linn) showed 15 per cent fall in blood sugar at the dose level of 0.5 g/kg of crude fraction. The extracts of *Durva* (whole plant) *Papaya* seeds, *Ativisa*, *Dudurli* and *Kavit* did not exhibit any effect.

Twenty-two compounds belonging to oxa-series (from Chemotherapy Department) were tested for their antidiabetic activity in rats. The blood sugar lowering effect of 7 compounds was more than 20 per cent at the end of seven hours while only three compounds showed 15 per cent fall in blood sugar when tested in rabbits. Oxa-3, oxa-4 and oxa-5, were the most active amongst those tested during this period.

Experimental amoebiasis

In the present work antiamoebic activity of extract from different medicinal plants was studied. The activity against *Entamoeba histolytica*, the causative organism of amoebiasis was studied *in vitro* and *in vivo*. *E. histolytica* was grown in a monophasic medium and the compounds under test were added to it and the tubes were observed for the growth of amoebae after 48 hours, incubation at 37° C. Golden hamsters and albino rats were used for the study of *in vivo* activity of the test compounds. Hamsters were infected intra-hepatically and rats intracaecally. The compounds tested, the dose used and the results obtained are listed in the Table 35. Drugs were administered orally to animals per kg. of body weight.

TABLE 35

Plant extracts	Fraction	In vitro Concen-Result tration mg/ml	In vivo Dose Result mg/kg		
<i>Mesua ferrea</i>	.. Alcoholic extract (bark)	20	—	×	×
	Alcoholic extract (kernal)	20	—	250	—
	Alcoholic extract (fruit)	25	—	×	×
	Acetone extract (flower)	10	—	×	×
	Crystalline compound	20	—	100	—
	Chloroform extract ..	10	—	100	—
<i>Aconitum heterophyllum</i> ..	Hydrochloride of water insoluble fraction of alkaloid (root).	4	+	100	—
<i>Nyctanthes arborescens</i> ..	Acid hydrolysis of alcoholic extract (leaves)	4	+	100	—
	Alcoholic extract (flower)	20	—	500	—
	Alkaloid fraction (leaves).	20	—	×	×
<i>Adhatoda vasica</i> ..	Vasicinone ..	20	—	200	—
	Vasicinone hydrochloride ..	10	—	200	—
<i>Aloe vera</i> ..	Alcoholic extract ..	10	—	×	×

—inactive, + active, × not done.

Studies in testing drugs for possible use in the treatment of filariasis

At present, diethyl-carbamazine is the only available drug for the treatment of filariasis. There is a need for more effective treatment. With this end in view the work on testing of drugs against filariasis has been undertaken. At present there is no animal model available for testing drugs against human microfilaria. So the preliminary work of establishing a model is initiated. A colony of the vector-*Culex* mosquito has been built up. The next stage will be production of infective larvae in mosquito in order to transmit the infection to suitable laboratory animals.

Pharmacological evaluation of drugs Ativisa (*Aconitum hetrophyllum*)

Different fractions viz. crude alcoholic extract, alkaloid fraction (water soluble), alkaloid fraction (water insoluble) from root of the plant were tested pharmacologically.

In anaesthetised cat, the drug (alkaloid fraction, water soluble) lowered blood pressure considerably after a slight initial rise with a dose of 2.5 to 5 mg/kg of body weight and returned to original level after a considerable length of time. In the atropinised cat, 8 mg/kg produced a severe and prolonged hypotension. When adrenaline and the drug were given concomitantly, there was initial hypertension followed by prolonged severe hypotensive effective.

The alkaloid fraction (water soluble) in powdered and gummy form did not show any effect on guinea pig tracheal chain upto a concentration of 1 mg/ml. The same fraction produced significant relaxation and inhibition of rhythmic movement of ileum of guinea pig in a concentration of 0.8 mg/ml and in case of rabbit's ileum upto 0.5 mg/ml. After ergometrine maleate (0.005 mg/ml), the drug shows relaxation effect with partial inhibition of rhythmic movement with 0.5 mg/ml. Pregnant uterus (10 days old) of guinea pig produced initial powerful contraction followed by gradual relaxation maintaining the rhythmicity of muscle with contraction to a dose of 0.5 mg/ml. The virgin guinea pig's uterus shows gradual increase in tonicity of muscle and sustained for a long time with concentration of 0.2 mg/ml.

The alkaloid fraction (water insoluble) did not show any effect on tracheal chain of guinea pig to concentration of 0.5 mg/ml. Ileum of guinea pig has shown reduction of tonicity and depression of rhythmicity of muscle in a concentration of 0.25 mg/ml. Rabbit ileum produced relaxation and inhibition of rhythmicity with 0.5 mg/ml and after ergometrine maleate (0.005 mg/ml) the same effect was observed. The pregnant uterus (10 days old) of guinea pig produced significant persistent relaxation and complete inhibition of rhythmic movement with 0.5 mg/ml. Virgin guinea pig uterus showed depression of rhythmic movement with 0.25 mg/ml. But crude alcoholic extract produced increase in tonicity of the uterine muscle to a concentration of 0.5 mg/ml.

The alkaloid fraction (water soluble) was used in experimental cholera in infant rabbit model with doses of 10 mg/100 gm of body weight before infecting the animals and 10 mg every 6 hourly thereafter i.e. 400 mg/kg of body weight. Difference in the time of onset of diarrhoea between the test and control animals was 12 hours and the difference between the time of death of test and control animals was 15 hours. There was no significant effect of the drug.

The alkaloid fraction water soluble did not play a role as an antifertility agent in rabbits.

Vasicine and Vasicine HCL

Guinea pig tracheal chain did not respond upto the concentration of 0.8 mg/ml of vasicine and vasicine hydrochloride. Pregnant uterus of guinea pig (10 days old) produced initial significant contraction gradually and inhibition of rhythmicity but after reaching the peak gradual relaxation of uterus was seen upto the concentration of 0.2 mg/ml and the effect was further intensified with 0.4 mg/ml vasicine hydrochloride also produced contraction and inhibition of rhythmic movement upto the concentration of 0.2 mg/ml in case of pregnant guinea pig uterus.

Guinea pig ileum had produced powerful contraction initially followed by gradual relaxation upto the concentration of 0.5 mg/ml of vasicine. Rabbits ileum has shown significant increase in tonicity manifested by greater amplitude of contraction with the dose upto 0.25 mg/ml. But the concentration 0.5 mg/ml produced significant relaxation with inhibition of rhythmic movement. After ergometrine maleate (0.05 mg/ml) rabbits ileum has produced the same effect with the drug in the same dose (0.5 mg/ml) revealing that relaxation is not due to stimulation of sympathetic nerve endings. Vasicine and vasicine hydrochloride did not produce the preventive effect on the action of snake venom upto the dose of 250 mg/kg intraperitoneally in mice. Significant result was not observed with vasicine and vasicine hydrochloride in case of experimental cholera in infant rabbit upto the dose of 250 mg/kg day in four divided doses orally. The drugs did not inhibit the growth of *Entamoeba histolytica in vitro* upto the concentration of 10 mg/tube of media i.e. 1 mg/ml of media. In the fasting adult rabbit vasicine caused lowering of blood sugar to the extent of 29 per cent with 250 mg/kg in two divided doses for one day. -The fasting blood sugar level was 111 mg/100 ml of blood (average) and even at 17th hours the blood sugar level was 78 mg/100 ml (average), at 65th hour 95 mg/100 ml (average) and 114th hour the original fasting blood sugar level returned. This is a pilot study and further work is in progress.

DEPARTMENT OF VIROLOGY

Pathogenesis of rabies

Studies on the brown fat of rabies infected mice were continued. Anatomical changes produced in this tissue by rabies infection were found to be consistent and reproducible. These changes were not seen in mice given injections of rabies vaccine (containing an equivalent quantity of foreign brain substance), or in mice infected with the KFD virus (a group B arbovirus). The KFD infected mice showed no difference, compared to control, uninfected mice even on the day of paralysis, and when death occurred. The KFD virus, however, could be recovered from brown fat of infected mice on the day when paralysis was first noted. It appears, therefore, that the brown fat is a tissue in which an infecting viral agent can multiply, when the infection has set in. However, the anatomical changes seen with rabies infection, are not seen with the KFD virus which also produces paralysis and death in the mice.

An interesting feature of this study was the presence of virus at the site of inoculation from the 4th upto the 9th day after inoculation, when the animal was moribund. This may be due to persistence of virus in the local tissue. On the other hand, the virus may reappear at the site as a result of retrograde spread along the nerve trunks after multiplication in the central nervous system.

Diagnostic tests for rabies. Diagnosis of rabies infection by passive cutaneous anaphylaxis reaction

In an attempt to find out an easy method for the early diagnosis of rabies infection the passive cutaneous anaphylaxis technique of Ovary (1964) was taken up. As a continuation of the last year's work, the following antigens and antisera were tried in guinea pigs weighing 250-350 gms. Antisera were given intradermally (i. d.) in 0.1 ml. quantity in each side of the back of the guinea pig. After 3 hrs. 1 ml of 1 per cent freshly prepared Evans blue in normal saline was given by intracardiac (i. c.) or intravenous (i. v.) route. Following this, the antigen 1 ml was given as 10 per cent suspension by intravenous or intracardiac route. The reaction on the skin was noted after $\frac{1}{2}$ to 1 hr. Usually there was no bluing on the skin where normal sera were injected, but light to dark bluing of more than 10 mm in size was noticed on the areas injected with rabies sera, after the injection of rabies tissue antigen into the circulation. This increased bluing was interpreted as due to the accumulation of moredye due to the vaso dilatation caused by the antigen antibody reaction.

The sera used were : Horse hyperimmune rabies serum prepared by this Institute ; dog, guinea pig, sheep and rabbit antirabies sera, prepared against Semple's vaccine ; dog antirabies serum prepared against LEP Flury virus, and normal sera from horse, dog, rabbit and sheep.

Antigens tested were infected and normal (control) brains of mice, dog, sheep ; infected human brain and dog salivary gland ; and chick embryo suspensions, normal and infected with LEP or HEP Flury virus.

The results obtained so far may be summarized as follows :—

As low as 10^4 mouse LD_{50} of the virus was found to give positive reaction with PCA test.

The infected brain suspensions (CVS mouse brain at 10 per cent. and 5 per cent. concentration) gave the reaction.

Heat inactivation and dilution of rabbit antirabies sera did not have any effect on PCA test.

In one test, rabies infected guinea pigs were tested for PCA, by injecting 1 ml. of 1 per cent. Evans blue i. v. after the i. d. injection of 0.1 ml. antirabies serum and 0.1 ml. of normal serum at different sites on the skin ; significant (more than 3 mm.) bluing on the dermis was noted at the sites of antisera injection and not at the control sites.

Standardization and increased reproducibility are still to be achieved. Usually 1 or 2 guinea pigs out of 3 tested may show the reaction ; deaths of guinea pigs during intracardiac injection are not uncommon.

Rabies epidemiology (Carrier state in normal dogs)

The study of normal dogs for carrier state is continued. During the year, 30 dogs from the municipal dog-pound were sacrificed and their tissues examined as described in the previous report. Saliva samples from 150 dogs attending the Veterinary Hospital were also examined. The results from 710 saliva samples, and tissues of sacrificed dogs, examined upto date, have been negative for rabies. Thirty dog sera were examined by neutralization test for antibodies against rabies All were negative.

A dog was brought from I. I. T., Powai, Bombay, to Haffkine Institute for examination of saliva as this dog had bitten a child who developed hydrophobia and died. Saliva samples were collected daily for virus isolation. So far no saliva sample, of 60 tested, has yielded rabies virus.

Rabies in bandicoots

The study of bandicoots for rabies infection was continued. Ninety brains and 90 salivary glands were examined during the year. All were negative for rabies. Six salivary gland specimens showing an agent infective to mice are still under study.

Rabies from human patients

A study has been commenced to investigate the possible role of human rabies patients as transmitters of this disease. Saliva, urine and blood (for antibodies) from rabies cases admitted to city hospitals are collected and tested for presence of virus. Seven rabies patients have been studied so far. The saliva and urine of all 7, and tongue scrapings from two of these cases, were negative for rabies. The blood antibody levels are under test.

Interference phenomena

Interference between rabies fixed virus (RV) and Rous sarcoma virus (RSV).—A system has been established wherein rabies, a nononcogenic rhabdovirus, used as a suspension of infected rabbit brain, produced blockade of tumour induction by Bryan master standard strain of RSV, an avian sub-group. A leukovirus, in the subcutaneous wing web tissue of a high percentage of white Leghorn chicks. Controls inoculated with a suspension of normal rabbit brain (NRB) showed no blockade of tumour.

Experiments were performed to test whether the administration of hydrocortisone could reverse the blocking effect in the *plus* 48 hours experiment. The blocking effect was reversed *i.e.* in birds of RV and ultraviolet irradiated rabies virus (UVRV) treated groups, tumours appeared by day 7 post-RSV inoculation. Each bird received 4 mg. of hydrocortisone over two days.

Similarly, actinomycin D also reversed the blocking effect of RV and UVRV in *plus* 48 hours experiment. However, partial reversal of blockade was apparent in the 0 hour experiment. There was an increase in latent period in RV group ; birds developed tumour by day 12 as compared to 6.3 days of NRB group.

These experiments suggested that probably interferon or like substances may be responsible for the tumour blockade, at least at the *plus* 48 hours interval. Preliminary results indicated that serum from RV inoculated chicks when mixed with RSV produced blockade in chicks receiving the mixture.

The blocking effect of RV was strain specific *i.e.* RV failed to block Schmidt—Ruppin strain of RSV.

Surveillance of influenza

The surveillance studies were continued. The investigation of the epidemic in Dahanu was completed. The most severely affected were the Adivasi population, who were very poorly nourished, and many of whom were also suffering from other conditions like pulmonary tuberculosis. Out of the washings of 10 cases collected, one yielded a strain of A₂ Hong Kong influenza virus.

The number of throat washing collected in Bombay were 16. Out of these, one was positive for influenza. There was no epidemic outbreak during the year.

The study of paired sera received from 6 cases studied in the epidemic at Aurangabad was completed. The results showed a significant rise in titre against A₂ HK virus in 5 cases. In 4 of these 5, A₂ HK virus had been isolated from throat washings. One case showed no rise in antibody titre.

Epidemiology of measles

The measles vaccine trial was completed and the results are now under analysis by the Indian Council of Medical Research, New Delhi. The analysis so far shows that seroconversion obtained in Phase I of the trial, wherein blood samples were drawn before, and one month after the vaccine administration was very satisfactory, as measured by the neutralization test. Whereas 119 out of 120 (99·16 per cent.) children receiving the vaccine showed fourfold or higher rise in titre, none of the children receiving placebo showed a rise in titre. The haemagglutination inhibition test was nearly as sensitive for detection of response to vaccine. Complement fixing antibodies were developed in only 101 out of 120 vaccinees (84·16 per cent.). Follow up after one year for measles or measles like illness, showed an incidence of 2·5 per cent. in vaccinated and 9 per cent. in the placebo group. These figures are based on reports of parents or health visitors. No laboratory confirmation of the diagnosis of measles was possible. There was no epidemic of measles during the year.

Seroepidemiology

A measles test survey was carried out in co-operation with the Department of Preventive and Social Medicine, Aurangabad Medical College.

The survey was aimed at finding out the incidence and severity of measles in a rural population, and also to assess the reliability of the history of measles, obtained from parents. In Bombay the parent's history was found to be very reliable, as confirmed by serological tests. The reliability of such history in a rural, mainly illiterate and unsophisticated population, was therefore, of some interest. While all the population could not be tested serologically, it was decided that a sampling technique, bleeding every 10th child under survey, would give a fair indication.

The survey covered all the 564 families (comprising 3621 individuals) in two villages served by the Rural Health Unit at Paithan, 40 miles from Aurangabad.

The salient feature of the work may be summarized as follows :—

Approximately 44 per cent. of all children below 5 years of age have suffered from measles, the incidence rising gradually upto 80 per cent. of children between 4 and 5 years of age. The duration of illness in 80·2 per cent. was from 1 to 15 days. Complications and prolonged illnesses were relatively rare. Among 137 deaths (of children below 5 years) 10 were recorded as due to measles. Approximately 90 per cent. of children exposed to measles (family contact) got the disease, while 10 per cent. escaped.

The sample serological test covered 61 children consisting of those who suffered or had not suffered from measles and 10 children who were exposed, but did not develop the disease. The sera showed corroboration of the history (correct to 95·5 per cent. confidence limits) and indicated that sub-clinical infections did not occur in children in this community.

The incidence of measles appeared to be smaller in single child families, but otherwise was unaffected by family size. A higher rate of infection was found in the winter months. From these results, it appears that history of an attack of measles is a reliable index in judging the prevalence of the disease in a given population.

Rats as vectors of human disease

Studies on tissue of rats collected from houses in a rural and an urban area, for presence of viruses pathogenic to humans is continued. During the period under study, 4 virus strains were isolated from 40 samples studied. Two (out of 180 isolates) which are under study in MKTC were pathogenic to MKTC and 4 pathogenic to infant and adult mice. One strain previously isolated has been identified as belonging to the EMC virusgroup.

Epidemiology of rubella

Studies on the incidence of rubella antibodies in the different age groups of the population were continued.

Twenty four pairs of blood samples were collected at the Wadia Maternity Hospital from umbilical cord of the new born babies and their mothers.

Eighteen children with congenital malformation were studied and their blood samples (and, where possible, C.S.F. and throat swabs) along with the blood samples of their contacts were collected.

One hundred and thirteen blood samples were collected and tested (HA-I test). 94 of these showed significant antibodies. From the blood samples collected during the earlier year 26 in the age group 6-10 were positive out of 37 and 5 positive out of 6 in the age group 11-15.

poliomyelitis surveillance

Enterovirus Unit continued to collect epidemiological data on paralytic poliomyelitis in Greater Bombay. During January to December 1970, 545 cases of paralytic poliomyelitis were recorded. This incidence reflects only those cases which have been hospitalised at the principal hospitals in Greater Bombay.

The seasonal distribution of paralytic poliomyelitis during 1970 showed slight shift as compared to the epidemic years in the past. Considering June to September as the period during which majority of the cases are recorded, the incidence ratio between this period as compared to the rest of the year helps in differentiating an epidemic year from a non-epidemic year. During 1970, a total of 233 (51.7 per cent.) cases were recorded during June to September period. In contrast during 1968, the last epidemic year, the corresponding incidence was as high as 744 (67.8 per cent.). During the year 1968 (epidemic year), the ratio June to September rest of the year was around 2/1 while the corresponding figure for 1970 (non-epidemic year) was 1/1.

Age distribution of cases of paralytic poliomyelitis in Bombay has remained unchanged. The disease continues to be an infantile paralysis affecting mainly the children in the age group 7 months to 2 years. During 1970 as many as 78 per cent of the cases were recorded in children below 2 years. Children below 5 years accounted for 96 per cent of the total cases. Two cases were recorded in the higher age group (15-20). Both showed presence of virus in their stool specimens and they had residual paralysis at 60 days.

From the beginning of 1970 in addition to detecting paralytic cases in hospitals, the Unit has undertaken to follow up cases of paralytic poliomyelitis at the end of 60 days from the onset of paralysis. The aim of this investigation is to evolve a more accurate method for counting true cases of paralytic poliomyelitis and exclusion of cases of transient paresis or paralysis possibly due to other enteroviruses. This investigation also gives the true account of the morbidity and mortality on account of poliomyelitis. The "best available paralytic poliomyelitis cases count" includes those clinically and epidemiologically compatible cases known to have residual paralysis at 60 days plus those cases reported initially as paralytic poliomyelitis for which no 60 days report on residual paralysis is available. The details of this investigation are presented in the Table 36.

TABLE 36

Poliomyelitis surveillance—60 days follow up cases (1970)

Paralytic poliomyelitis cases with residual paralysis at 60 days	..	258 (49.1%)
Paralytic poliomyelitis cases without report on residual paralysis	..	150 (27.4%)
Fatal cases		42 (7.7%)
Paralytic poliomyelitis cases without residual paralysis	..	95 (17.3%)
Best available paralytic poliomyelitis case count	..	450

During the follow up visits for evidence of residual paralysis, information was also collected as to the extent of recovery from paralysis. The data collected are given in Table 7.

TABLE 37

Poliomyelitis surveillance—status of residual paralysis at 60 days (1970)

Severe residual paralysis (no recovery)		55 cases
Partial residual paralysis (partial recovery)		203 cases
Proved fatal		42 cases

Paralytic illness amongst vaccinated children

Introduction of Oral Polio Vaccine for immunisation of the susceptible population in India has brought to light the problem of paralytic illness occurring in individuals inspite of vaccination with Oral Polio Vaccine. During 1970, 82 cases were recorded with history of paralytic illness inspite of immunisation with oral polio vaccine in the past. Complete epidemiological and virological studies could be done in 70 cases out of these of which 54 yielded polio viruses. Inadequate immunisation was detected in 37 cases while 33 individuals had received all the three doses of Oral Polio Vaccine. Age distribution and seasonal distribution of these cases does not show any significant difference when compared with total number of cases recorded in 1970.

Study of Simian intestinal viruses

Majority of virological investigations are being carried out with the use of monkey kidney tissue culture and since it is known that monkeys frequently harbour viruses in their gut as well as in various tissues, it was decided to study the viral flora of the intestinal tract of the *Rhesus* monkey to begin with.

Intestinal contents of 100 rhesus monkeys were collected for virological studies. As many as 62 (62 per cent) monkeys were found to harbour a cytopathogenic agent in the intestinal tract.

The cytopathogenic agents could broadly be grouped into two types according to the morphological changes induced by them in tissue culture, viz. (a) those which produced cytopathogenic effect (CPE) like human adenoviruses, and (b) those which produced cytopathogenic effect (CPE) like enteroviruses. Accordingly 29 agents belonged to group (a) and 33 to group (b). All these have been studied for the haemagglutinating activity against chick, fowl, guinea pig, monkey and human 'O' red cells. Two strains showing adenovirus type of CPE could agglutinate human 'O' red cells at 4° C while all the cytopathogenic agents producing CPE like human enterovirus failed to agglutinate the red cells. Attempts are being made to prepare the antisera against these two strains in rabbits.

DEPARTMENT OF ZONOSIS

Plague epidemiological studies

Plague epidemiological studies were carried out in Aurangabad district and in the 'B' and 'C' ward areas of Greater Bombay.

The study will indicate the extent of the wild and peridomestic rodent fauna and the foci of infection serologically, if any, among these rodents. Attempts were also made to study the distribution of commensal rodents in different areas of the district. In all 1,630 rodents were examined from that district.

The wild rodent fauna, examined, primarily consisted of *R. rattus*, *R. blandfordi*, *B. bengalensis*, *L. buduga*, *L. nagarum*, *L. platythrix*, *G. ellioti*, *Tatera indica*, and *Funambulus* sp. *X. cheopis* was the predominant type amongst the fleas collected and examined from that district. *R. rattus* was the prevalent type amongst the commensal rodents. Rat density was high; the highest being 152 at Kazipura (Khuldabad). The average flea index, was 2.7.

In the 'B' and 'C' wards of Greater Bombay, *R. rattus* *R. norvegicus*, *B. bengalensis* constituted the predominant commensal rodent fauna.

In all 2,114 rats from 'C' ward, and 1,914 rats from 'B' ward area were examined. *X. cheopis* was predominant amongst the rat fleas in both these wards. From ward 'B' 1,400 fleas, and from ward 'C' 1,665 fleas were examined. *X. astia* constituted 4.5 per cent of the fleas collected from rats in wards 'B' and 'C'; whereas they constituted 5 per cent of the total flea collection.

Studies on susceptibility status of oriental rat flea to different insecticides

Studies on the assessment of susceptibility status of oriental rat fleas from Thana to different insecticides showed that *X. cheopis* has developed resistance to DDT in only some of the localities, whereas in other places fleas are still susceptible to this insecticide. In some parts of the district, fleas also showed tolerance to malathion and they were found to be susceptible to dieldrin in all the localities studied. Similar phenomenon was observed in Nasik district where fleas were seen to be susceptible to dieldrin and malathion and they showed resistance to DDT in some areas of the district.

Studies in ascariasis

In continuation with the epidemiological survey for ascariasis, stool-samples were collected from the students of primary sections of R. M. Bhat High School, Parel. The faecal samples were microscopically examined for the presence of intestinal parasites—both protozoa and helminths. Out of 135 faecal samples examined, 65 showed the presence of ova and cysts of pathogenic intestinal parasites. No trematode and cestode infections were observed, so also not a single case of ankylostomiasis was noted. Incidence of ascariasis was 40 per cent, trichuriasis 27 per cent, strongyloidiasis 6.8 per cent and giardiasis in 12.2 per cent. The prevalence of infection with *Enterobius vermicularis* (human pin-worm) was low, being found only in 1 case. All students screened belonged to age group of 5-8½ years.

Epidemiological studies in filariasis

In this venture, the Department of Zoonosis of Haffkine Institute and Malaria Organization at Thana and the Thana Municipality have participated. Every house covering a population of 1,61,000 was numbered. Every tenth household was surveyed by collecting blood for the detection of microfilariae between 22 hours to 02 hours. In this way the total number of slides that have been collected during the period from 22nd March 1971 to 8th April 1971, came to 14 491.

A colony of vector species *Culex fatigans* has been bred here for carrying out experimental studies. Till now (15th May 1971) 9,632 slides were examined for microfilariae out of which 382 were found to be positive. It is also proposed to examine the slides for eosinophilia.

Simultaneously, the entomological survey of the same area has been undertaken. The density of the vector species (of mosquitoes) *C. fatigans* has been determined. The mosquitoes were dissected with a view to find out the infection rate.

Along with these, a survey of environmental conditions particularly with reference to drainage system and collection of waste water has been carried out.

Blood has been collected for serological studies from 5 disease manifestation cases but no microfilariae in their blood could be detected. Blood from another 4 cases (with no disease manifestation) but having microfilariae in their blood has also been collected. The immunological studies of these blood samples been taken up.

It is proposed to take up further studies to determine the sensitivity of the population at risk by using Sawada's antigen, which has been recently received from W.H.O.

Efforts are being made to collect *Ornithonyssus bacoti* for rearing and conducting transmission experiments with *Leishmaniasis carinii*.

Proteus hydrophilus-infection in snakes

The mouth rot is a common disease resulting in swelling of the jaws in the snakes. This was found to be a common infection among the snakes purchased for the collection of venom. An organism *Proteus hydrophilus* was isolated from the creamy fluid after puncturing the swelling. The infection was successfully treated with sulpha methazine.

PART III
ROUTINE ACTIVITIES AND MISCELLANEOU
ACTIVITIES

ROUTINE ACTIVITIES AND MISCELLANEOUS ACTIVITIES

ANALYTICAL AND CONTROL DEPARTMENT.

As a major event, Analytical and Control Department was shifted from the premises of old K. F. D. Block to the Nutrition Department on the second floor. The Chemical Section which was housed in two rooms near the Pharmacology Department was also transferred and both the Sections are now brought at one single premises on the second floor of the main building. The long and broad corridor adjacent to the present Laboratory, is to be converted into full-fledged Bio-assay Section.

The Assistant Director of the Department Dr. R. B. M. Colah retired on superannuation and Shri K. G. Anantanarayanan took charge of the post of Assistant Director of the Department.

Dr. Saldanha, who had proceeded to U.S.A. on W.H.O. fellowship for advanced training in drug analysis and biological standardisation of drugs returned to the Institute recently. He also visited laboratories in Canada, England, Denmark and Sweden.

This Department is comparatively new but has evolved into a compact organisation in the Institute. It keeps rigid vigilance over all the products of the Institute. Quality control has been the watch-word of this Department.

The expansion of the sphere of activities of the Department was brought about by undertaking the new responsibility of analysing the raw material required to be used for the manufacture of pharmaceuticals and biologicals of the Institute.

Shri R. J. Turel, J.S.O., Shri D. P. Sule, S.T.A. and Kum. V. B. Mantri, joined the Department. Kum. V. D. Apte, Kum. A. R. Shinde and Kum. A. D. Púrandare joined the Department as Senior Technical Assistants in place of Kum. T. J. Bapat, Kum. S. S. Chakravarti and Kum. A. K. Shah respectively.

Shri Ramani, Senior Technical Assistant was transferred to Blood Bank Department on promotion and Kum. Bhandarkar joined in this place.

Technical training was imparted to students of M. L. T. course and other trainees.

Routine—

The number of different test and samples analysed by the Department during the year are tabulated below :—

TABLE 38

Product				Test	No. of samples tested
				Sterility	
Bacterial vaccine	..	..	..		11,101
Antirabic vaccine	..	..	..		8,522
Parenteral bulk solutions	..	..	..		7,835
Antitoxins and sera etc.	..	..	..		16,901
Miscellaneous ..	..	..	..		768
				Pyrogen	
Parenteral bulk solutions	..	..	..		1,204
				Chemical and physical exam.	
Parenteral bulk solns	..	..	..		1,212
Vitamin preparations	..	..	..		329
Sulpha drugs ..	..	..	..		193
Miscellaneous ..	..	..	..		75
				Toxicity	
Antitoxins, toxoids	..	..	..		676
Vaccines, etc					
				Absence of infective virus	
Viral vaccine ..	..	..	..		258
				Potency	
Antitoxin ..	..	..	..		70
Antivenine ..	..	..	..		39
Toxoids ..	..	..	..		29
Triple antigen ..	..	..	..		2
				Preservatives	
Vaccines antitoxins	..	..	..		554
				Free formaldehyde	
Bacterial, Vaccines toxoids	..	..	..		167
				Microbiological assays	
Pemon ..	..	..	..		89
Vitamin preparations	..	..	..		99
				Raw materials	
Vitamins ..	..	..	..		7
Sulpha drugs ..	..	..	..		7
Miscellaneous (fine chemicals, antiseptics pharmaceutical necessities, etc.).					96
				Total	50,233

DEPARTMENT OF BACTERIOLOGY

During the year under review the Department continued its studies on storage stability of typhoid vaccine at high altitudes for which the Ministry of Defence Research and Development Organisation had sanctioned aid until May, 1971.

The project to assess the efficacy of live and killed plague vaccines in langoons in collaboration with the George William Hooper Foundation, San Francisco, under the auspices of World Health Organization is approved by the Minister of Health, Government of India. Grant in Aid of \$ 4,000 has been received from World Health Organization for this project.

Shri Khleifat Hussein completed his studies for the degree of M.Sc. He was supported by a scholarship from World Health Organization. Mrs. L. C. Kashyap who joined the Media section as Junior Scientific Officer was transferred to the Department of Pharmacology. Kum. A. C. Gupte, joined the Media section in October 1970 as a Senior Technical Assistant.

During the year under review, the following quantities of different vaccine were produced.

TABLE 39

Product						Quantity (litres)
Cholera-El tor vaccine	..	..	..	..	..	8,735,399
T.A.B. vaccine	..	..	..	..	..	222,837
Plague vaccine	..	..	..	..	..	25,620

Media Section—

TABLE 40

Products						Quantity (litres)
<i>Parenteral solutions—</i>						
Dextrose and Sodium Chloride Injection, U.S.P.	..	..	..	..	..	71,229
Dextrose Injection, I. P. 5 per cent W/V.	..	..	..	..	..	74,507
Dextrose Injection, I. P. 25 per cent	..	..	..	..	..	769
Sodium Chloride Injection, I. P.	..	..	..	..	..	51,328
Water for Injection, I. P.	..	..	..	..	..	9,666
Sodium Lactate Injection, B. P.	..	..	..	..	..	7,052

Products					Quantity (litres)
<i>Culture Media—</i>					
Casein hydrolysate agar	..	..	..	..	20,519
Meat digest agar (Typhoid)	..	..	..	..	608
Agar medium for cholera research	..	..	..	..	36
Sterility Test media	..	..	..	..	17,233
Sabouraud's agar U.S.P... .. .	..	..	..	..	56
Peptone water	..	..	..	..	180
Nutrient Broth	..	..	..	..	191
Cooked meat medium (in tubes)	..	..	..	..	52
Nutrient agar	..	..	..	..	950
MacConkey's medium	..	..	..	..	23
MacConkey's agar	..	..	..	..	132
Löffler's medium	..	..	..	..	213
Lowenstein and Jensen's medium	..	..	..	..	157
Sabouraud's broth	..	..	..	..	31
Glucose broth	..	..	..	..	73
Taurocholate medium	..	..	..	..	25
Citrate saline	..	..	..	..	17
Lab. Lemco broth	..	..	..	..	7
Potato agar	..	..	..	..	4
Todal agar	..	..	..	..	2
D. E. C. medium	..	..	..	..	58
Glucose Phosphate	..	..	..	..	4
Selenite broth	..	..	..	..	9
Koser's citrate medium	..	..	..	..	2
Thioglycolate medium	..	..	..	..	41
Christensen's medium	..	..	..	..	2

Products						Quantity (litres)
Hoyle's agar ..	..	..	..	..	..	2
Glycerol agar ..	..	..	..	..	..	1
Lemco broth ..	..	..	..	..	..	7
Milk tubes ..	..	..	..	..	..	1
Brilliant green ..	..	..	..	..	..	8
Alkaline peptone water ..	..	..	..	..	..	53
Venkatraman medium ..	..	..	..	..	..	2
Ox-Bile ..	..	..	..	..	..	25
<i>Sugars for biochemical tests—</i>						
Glucose ..	..	..	..	..	..	22
Lactose ..	..	..	..	..	..	3
Sucrose ..	..	..	..	..	..	21
Mannite ..	..	..	..	..	..	20
Maltose ..	..	..	..	..	..	3
Dulcitol ..	..	..	..	..	..	13
Xylose ..	..	..	..	..	..	2
<i>Diagnostic Reagents—</i>						
Benedict's solution (qualitative) ..	..	..	..	..	..	967
Albert's stain No. 1 ..	..	..	..	..	..	25
Albert's stain No. 2 ..	..	..	..	..	..	13
J. S. B. stain No. 1 ..	..	..	..	..	..	27
J. S. B. stain No. 2 ..	..	..	..	..	..	20
Löffler's methylene blue ..	..	..	..	..	..	8
Carbol fuchsin ..	..	..	..	..	..	143
Gram's stain No. 1 ..	..	..	..	..	..	15
Gram's stain No. 2 ..	..	..	..	..	..	26
Hayem's fluid for R. B. C. ..	..	..	..	..	..	13
Methylene blue ..	..	..	..	..	..	104
Oxalic acid solution for W. B. C. ..	..	..	..	..	..	38
Safranine 0·5 per cent (aqueous) ..	..	..	..	..	..	17

DEPARTMENT OF BLOOD BANK

The post of Junior Scientific Officer has been filled up for plasma processing and fractionation. Dr. K. V. Kulkarni, Dr. A. J. Baxi and Shri V. M. Patankar attended the Annual Meeting of Indian Society of Haematology held in Mangalore and presented a paper on "Studies on Australia antigen and hepatitis."

Training

Training in the field of blood Group Serology and Blood Banking Technology was imparted to the medical and paramedical personnel deputed by various governments and medical institutions. The following were trained.

Shri H. Z. Patel, from New Civil Hospital, Ahmedabad, for 3 months. The remaining are for one month each ; Kum. R. M. Panchal, Kum. N. S. Patrawalla, Smt. B. A. Mistry, Smt. A. A. Apte, Shri S. P. Gosavi, Smt. A. V. Dhavle, Shri Y. M. Sambray, Kum. M. J. Turel, and Dr. P. M. Bhanej, private students each for one month.

Production

During the year the routine production activities of the Department, viz. collection of blood from blood donors both at the Institute and the mobile sessions, processing and lyophilisation of plasma, fractionation of human plasma and production of blood grouping reagents were carried out in a normal way with the greater drive made by the Public Relations Officer for the enrolment and of voluntary blood donors the collection of blood from this source showed a marked increase. The Public Relations Officer had established contracts with many establishments (educational institutions, factories, etc.) and mobile blood collection sessions were held at these institutions.

TABLE 41

No. of Institutions/Colleges/Factories, etc. where blood collection Sessions were held.	37
No. of voluntary donors enrolled	3,219
<i>Blood donors—</i>	
Voluntary	2,913
On payment	3,701
Prisoners	2,317
Hospitals	1,755
Total	10,686

Blood Plasma—

Name of product	Packing (ml.)	Produced	Supplied
Dried Plasma	.. 250	419·25 × 250 ml.	639·75 lit.
Human gamma globulin	.. 1	10,515 × 1 ml.	10,425 × 1 ml.
Human fibrinogen	.. 150	440 × 150 ml.	437 × 150 ml.
Human fibrinogen	.. 10	94 × 10 ml.	94 × 10 ml.
Fresh plasma	.. 250	7 × 250 ml.	7 × 250 ml.
Cryoprecipitate	.. 100	22 × 100 ml.	22 × 100 ml.
Packed cells	.. 150	164 × 150 ml.	164 × 150 ml.
Bovine albumin (in powder form) . .		200 gms.	200 gms.
Human Serum albumin (in powder form)		25 gms.	25 gms.

Tests carried out—

Rh tests		230
Coombs test		6
Cross-matching test		19

Blood grouping reagents—

Name of product	Produced	Supplied
Anti-A	 33,112 × 1 ml.	31,851 × 1 ml.
Anti-B	 33,198 × 1 ml.	32,436 × 1 ml.
Anti-A — B	 1,720 × 1 ml.	1,246 × 1 ml.
Coombs' reagent	 1,051 × 1 ml.	1,036
Bovine albumin	 461 × 1 ml.	456
Anti-D	 6,135 × 1 ml.	5,497
Anti-A _I (Lectin)	 67 × 1 ml.	63
Anti-H (Lectin)	 118 × 1 ml.	57
Anti-M (Serum)	 11 × 1 ml.	50
Anti-N (Serum)		16
Total No. of anti-venine ampoules decanted and given for lyophilization		67,861

DEPARTMENT OF CHEMOTHERAPY

The research problems taken up in hand progressed satisfactorily during the year under review. In the area of chemotherapy of tuberculosis, a series of thiosemicarbazones from various substituted acetophenones were prepared and when screened *in vitro* some of the compounds showed activity equal to and a few better than that of thiacetazone. Thiadiazole and oxadiazole derivatives synthesised and studied for possible hypoglycaemic activity have revealed a few to have significantly potent blood-sugar lowering activity both in normal and alloxan treated rats. Among the compounds synthesised and screened for antitumor activity some were found to possess good activity and relatively low order of toxicity. Piperazine and nitroimidazole derivatives were synthesised for antiamoebic and anti-parasitic activity.

Shri D. T. Choudhari, M.Sc., and Shri K. Y. Mahajan, M.Sc., joined the department as Government of India scholars and Shri B. G. Khadse as University Grants Commission fellow to carry out research work for Ph.D. degree.

Shri D. R. Shekawat, M.Sc., Unichem Research fellow, completed the work for Ph.D. degree.

Sarvashri M. Y. Mhasalkar, M.Sc., A. K. Bhat, M.Sc., and H. V. Patel, M.Sc. completed their research and their theses were accepted by Bombay University for the award of Ph.D. degree.

Also the thesis of Shri J. A. Fernandez was accepted for M.Sc. degree by the University of Bombay.

Four research papers were published and three more were accepted for publication during the year.

Routine

Production.—The production and supply of clinical products for the use of Government Hospitals, E. S. I. S. and public bodies were maintained at full level. New formulations and additional products were taken up for E.S.I.S. authorities. The list of items manufactured is given in the following Talbe 42.

TABLE 42

Product					Tablets manufactured (Number)
Vitamin A. D.	..	..	..	..	1,06,80,200
Vitamin B. C.	..	..	..	..	1,94,38,700
Vitamin B ₁ (10 mg)	..	..	..	..	12,03,300
Vitamin B ₂	..	..	..	..	2,42,760
Vitamin C	..	..	..	..	1,12,74,000
Nicotinic Acid	..	..	..	..	11,71,425
Vitamin B. C. No. 3	..	..	..	..	11,89,900
Multi-Vitamin	..	..	..	..	51,00,800
Vitamin K	..	..	..	..	1,60,800
Vitamin A. D. (coated)	..	..	..	..	2,11,848
Tolbutamide	..	..	..	..	4,03,280
Isoniazid (I.N.H.)	..	..	..	..	41,38,200
Polyvitamin	..	..	..	..	1,46,34,700
Iodochlorohydroxyquinoline	..	..	..	..	11,69,000
Vitamin B-Complex with C	..	..	..	..	1,07,96,760
Thiacetazone and I.N.H.	..	..	..	..	8,91,500
Di-iodohydroxyquinoline	..	..	..	..	33,14,100
Sulphaguanidine	..	..	..	..	78,67,500
Sulphadimidine	..	..	..	..	50,34,500
Sodium aminosalicylate	..	..	..	..	15,89,000
Chloropropamide (250 mg)	..	..	..	..	45,300
Chloropropamide (100 mg.)	..	..	..	..	72,700
Calcium with Vit. D ₂	..	..	..	..	25,62,310
Berberine/HCl	..	..	..	..	1,14,900
Sulphadiazine	..	..	..	..	1,21,48,000
Total				..	11,55,05,485
Pemon (anti-septic)	..	..	..	..	34,425 Litres

DEPARTMENT OF CLINICAL PATHOLOGY

This Department participated in the Inter-laboratory Evaluation Programme conducted by the Central V. D. Reference Laboratory, Madras Medical College, Madras. (Twenty-six laboratories participated from all over India).

W. H. O. combined course in Epidemiology organized by the National Institute of Communicable Diseases included: (i) a visit to the Haffkine Institute by the participants from various countries of the South East Asia and (ii) Discussions were held on various research problems on communicable diseases in progress in different departments of the Institute.

Mr. K. K. Das was awarded a degree of M.Sc. (Microbiology) for his thesis on "Studies on the Diagnosis of Enteric Fever". Mr. L. M. Rumao got the M. Sc. degree in Microbiology for his research work on "Streptococci". Mr. H. A. M. Khleifat was awarded degree of M. Sc. in Microbiology for his research work on "Studies on Dynamics of Disinfection."

The Government of India scholarship continues to be held by Shri K. N. Joishy, who has been working on bacterial genetics of mycobacterium.

The investigation on Leptospirosis was completed and the I. C. M. R. scheme has been terminated since 1st April 1971.

Mr. K. K. Das presented a paper viz., "Immobilisation test as an aid to the rapid diagnosis of *V. cholerae*" at the Indian Association of Pathologist Conference held in Calcutta in December 1970. Dr. Mrs. V. S. Vaidya has been appointed as Medical Officer under the Typhoid Scheme, Haffkine Institute.

Dr. S. Mukerji was deputed for the Radio Isotope Training Course conducted by the Medical Isotope Division of Bhabha Atomic Energy at Tata Memorial Hospital. The training comprised of the application of radio isotope techniques in medicine viz., I 131 uptake in thyroid disorder, auto-radiography, radioimmuno assay etc.

Seventeen trainees completed "short term" training course besides D.M.L.T. students.

Routine investigations carried out during the year 1970-71 are presented below (Table No. 43).

TABLE No. 43

					Total
<i>Histopathology :</i>					
Histopathological examination	..	..	..	..	3,257
<i>Haematology :</i>					
Haemoglobin estimation	..	..	..	..	375
Total R. B. C. count	..	..	..	..	376
Total W. B. C. count	..	..	..	..	415
Differential W. B. C. count	..	..	..	..	410
P. C. V. estimation	..	..	..	..	9
E. S. R. estimation	..	..	..	..	77
Prothrombin time	..	..	..	..	6
Platelet count	..	..	..	..	2
Bleeding time	..	..	..	..	5
Clotting time	..	..	..	..	5
Reticulocyte count	..	..	..	..	4
Blood smear for filariasis	..	..	..	..	
Blood smear for malaria	..	..	..	..	7
Bone marrow smear examination	..	..	..	..	1
<i>Clinical microscopy :</i>					
Urine routine examination	..	..	..	..	444
Stool routine examination	..	..	..	..	256
Semen examination	..	..	..	..	15
C. S. F. cytological examination	..	..	..	..	59
Other puncture fluid (cytological examination)			..	..	28
Pregnancy test (qualitative)	..	..	..	..	2,257
Pregnancy test (quantitative)	..	..	..	..	193

TABLE NO. 43—*contd.*

						Total
<i>Bacteriology :</i>						
Blood culture	..	..	..	..	..	23
Urine culture	..	..	..	..	..	378
Stool culture	..	..	..	..	..	3
C. S. F. culture	..	..	..	..	..	5
Body fluid culture	..	..	..	..	..	6
Throat swab culture	..	..	..	..	..	40
Antibiotic sensitivity	..	..	..	..	..	348
Autovaccine ..	..	..	..	..	..	48
Specimen for typhoid culture	..	..	..	..	..	857
Specimen for tuberculosis culture (sputum, urine)	..	..	..	..	..	82
Specimen for guinea pig inoculation for T. B.	..	..	..	..	..	11
Smear for acid fast bacilli	..	..	..	..	..	47
Swabs for diphtheria culture	..	..	..	..	..	307
Material for tetanus culture	..	..	..	..	..	264
Tissue for culture	..	..	..	..	..	
Tissue for autolysate preparation	..	..	..	..	..	7
Stool for cholera culture	..	..	..	..	..	7,588
Urine for leptospira examination	..	..	..	..	..	47
Smear for gonorrhoea culture	..	..	..	..	..	2
Miscellaneous culture	..	..	..	..	..	304
<i>Mycology :</i>						
Specimen for fungal culture	..	..	..	..	..	4
<i>Serology :</i>						
Widal test ..	..	..	..	..	..	857
Weil Felix test	..	..	..	..	..	857
Test for brucellosis	..	..	..	..	..	857
Viagglutination test	..	..	..	..	..	38
Paul Bunnel test	..	..	..	..	..	17
Test for leptospirosis	..	..	..	..	..	540

Products	Total
<i>Serology</i>	
Kahn test	283
V. D. R. L. test	339
Wassermann test	72
Casonus (skin) test	3
Frei's (skin) test	1
Schick test	152
<i>Diagnostic reagent section</i>	
Rideal Walker Co-efficient test	17

Production and supply :

Diagnostic Reagent Section was concentrating on improving the quality of all the products and has introduced new products viz. (a) E. coli Poly 'O' S. para-typhi A 'O', S. para-typhi B 'O' and S. para-typhi C 'O' antisera (b) Antigen S. Para-typhi A 'O' and S. para-typhi B 'O'.

TABLE NO. 44

Products	Production (ml.)	Supply (ml.)
Dead emulsions	4,32,915	4,18,515
High titre sera	6,425	5,823
Kahn antigen	15,250	12,500
W. R. antigen		1,570
Casoni's antigen	360	422
Cobra venom solution	486	433
R. V. venom solution	702	371
E. coli sera	1,609	996
Vi typhisera		83
Vi antigen	38	25
Biological stains	14,32,500	14,23,400
Total	1,889,285	1,864,138

DEPARTMENT OF IMMUNOLOGY

During the year the Department produced and supplied various antisera and toxoids as usual. At the Pimpri farm, the work of construction of 2 stables, 48 Class IV quarters and 12 Class III quarters has been completed. The construction of two new stables has started.

Shri A. D. Kulkarni and Shri S. M. Potdar, Junior Scientific Officers resigned from the service. Kum. S. R. Hardikar joined the Department as Senior Technical Assistant.

Shri S. V. Gadre was awarded a Research Fellowship of the Indian Council of Medical Research. Shri P. M. Naik joined as the I. C. M. R. research fellow in a project on "Studies in immune response in presence of steroid hormones and adjuvants".

The following students obtained their post-graduate degree of the University of Bombay :—

- M. Sc. (i) Shri R. G. Tamhane (Biochemistry).
- M. Sc. (ii) Shri S. M. Potdar (Microbiology).
- M. Sc. (iii) Shri A. D. Kulkarni (Biochemistry).
- Ph. D. (iv) Shri S. M. Desai (Microbiology).

The following research papers were presented from the Department —

- (i) Passive cutaneous anaphylactic reactivity of horse serum immunoglobulins and their fragments. S. A. Kulkarni and S. S. Rao, at the Indian College of Allergy and Applied Immunology (4th convention, New Delhi)
- (ii) Studies in Diphtheria phages S. V. Gadre and S. S. Rao at the Symposium on microbial spoilage of foods,

TABLE 45

Production

Products	Quantity (Litres)
Tetanus toxin—	
(a) Peptic digest medium	762
(b) Protein-free medium	1,464
Tetanus toxoid—	
(a) Peptic digest medium	55
(b) Protein-free medium	1,084
Diphtheria toxin—	
(a) Papain-digest medium	150
(b) Protein-free medium	200

Products	Quantity (Litres)
Diphtheria toxoid—	
(a) Papain-digest medium	55
(b) Protein-free medium	180
Adsorbed tetanus toxoid	30
Diphtheria-tetanus toxoid	55
Adsorbed diphtheria toxoid	40
Antivenin	140
Tetanus antitoxin	1,584 (M. U.)
Diphtheria antitoxin	140 (M. U.)

DEPARTMENT OF BIOCHEMISTRY

The Nutrition Department which was functioning as a separate office under the Surgeon General with the Government of Maharashtra since 1949 was amalgamated with the existing department of Biochemistry and placed under the administrative control of the Director from November 1962. The field unit and the laboratory section continued to carry out the multifarious activities pertaining to nutrition. With the introduction of the applied nutrition programme in the State from September 1965, the responsibilities and the field activities of the unit were increased very much. The National Nutrition Advisory Committee of the Ministry of Health, Government of India had been consistently recommending the formation and functioning of a compact Nutrition Cell under the Directorate of Public Health for proper co-ordination of the nutrition and other public health activities of the State. It was, therefore, decided to shift the Nutrition Department to the Public Health Institute at Nagpur under the administrative control of the Director of Public Health. This transfer was finally effected from December 1970. The temporary research scheme on dietary fats and lipid metabolism was, however, retained in the Biochemistry Department to avoid dislocation of the research programme.

With the shifting of the Nutrition Department from the second floor of the Institute, the existing laboratories had to be reorganised with a little more additional space in the first floor. These changes are expected to be complete by October 1971.

During the year under review, the Biochemistry Department continued the research, routine and training activities. The service facilities of the department are available to all the hospitals and private practitioners. This is particularly useful to the Employees State Insurance Service (E. S. I. S.) hospital where such facilities are still not available.

Dr. Mukund J. Modak, Ph. D., Senoir Diamond Jubilee Fellow left the department in August 1970 to work as a Post-Doctoral Research Fellow in the Department of Biochemistry, Nagoya University, Japan for a period of 6 months. He has now joined the Molecular Biology Division, College of Physicians and Surgeons, Columbia University, New York, U. S. A. as a Post-Doctoral Research Fellow. Kum. Ranjana Buit, M. Sc., is continuing the research work on "Biochemical studies on *Vibrio Cholerae* and *V. El. tor*" as a Diamond Jubilee Junior Research Fellow. Shri Syed Raziuddin has been appointed as a Junior Research Fellow from October 1970. The Research Scheme of Dietary fats and lipid metabolism has been extended for one more year from February 1970 and Shri V. D. Rane is continuing the work on "Influence of dietary carbohydrates on lipid metabolism in the rat".

One more post of S. S. O. II (Research) was sanctioned to which Dr. (Smt.) Shantha Modak has been promoted. Shri J. K. Kamath, Senior Technical Assistant in the former Nutrition Department has been promoted as J. S. O. in place of Dr. Shantha Modak. Shri V. P. Shinde, M. Sc., and Ranjana Buit, M. Sc., have registered for the Ph. D. (Biochemistry) degree of the Bombay University. Shri V. G. Rao and Shri U. J. Dumaswala have been awarded the M. Sc., degree of the Bombay University.

Routine Tests :

A total of 5,326 tests were carried out during the year as shown in the Table 46.

TABLE 46

Yearly statement of the Biochemical tests performed in the Department.

Name of the test						
<i>Blood and Serum</i>						<i>Number</i>
<i>Qualitative :</i>						
Aldehyde test ..	..	..	..	..	..	1
Icteric index ..	..	..	..	..	..	173
Vanden Bergh ..	..	..	..	..	..	62

Name of the test							Number
<i>Blood and Serum</i>							
<i>Quantitative</i>							
Amylase	..	..	..	..	..	..	27
Bilirubin	..	..	..	..	..	..	479
Calcium	..	..	..	..	..	..	52
Chlorides	..	..	..	..	..	..	102
Creatinine	..	..	..	..	..	..	36
Cholesterol	..	..	..	..	..	..	557
N. P. N.	..	..	..	..	..	..	9
Phosphatase (acid)	..	..	..	..	..	..	30
Phosphatase (alkaline)	..	..	..	..	..	..	324
Phosphorus	..	..	..	..	..	..	38
Proteins (total)	..	..	..	..	..	..	228
(a) Albumin	..	..	..	..	..	..	149
(b) globulins	..	..	..	..	..	..	149
Sugar	..	..	..	..	..	..	612
Thymol turbidity	..	..	..	..	..	..	118
Urea Nitrogen	..	..	..	..	..	..	125
Uric Acid	..	..	..	..	..	..	62
Plasma acetone	..	..	..	..	..	..	1
Total							.. 3334
<i>Body fluids :</i>							
C. S. F. chlorides	..	..	..	..	..	..	99
C. S. F. proteins	..	..	..	..	..	..	102
C. S. F. sugar	..	..	..	..	..	..	89
S. M. test	..	..	..	..	..	..	19
Pandy's test	..	..	..	..	..	..	1
Total							.. 310

Name of the test						Number
<i>Urine and stool :</i>						
Acetone bodies..	..	..	..	..	..	3
Albumin ..	..	..	..	..	..	3
Bence-Jones proteins ..	..	..	..	..	..	1
Bile salts and pigments ..	..	..	..	..	..	96
Bilirubin ..	..	..	..	..	..	2
Calcium ..	..	..	..	..	..	11
Chlorides ..	..	..	..	..	..	1
Diastase ..	..	..	..	..	..	1
Occult blood ..	..	..	..	..	..	1
Phosphorus ..	..	..	..	..	..	1
Sodium ..	..	..	..	..	..	1
Sugar ..	..	..	..	..	..	71
Urobilinogen ..	..	..	..	..	..	19
Total ..						211
<i>Special tests :</i>						
Amylase (pleural aspirate) ..	..	..	..	..	..	1
B. M. R. ..	..	..	..	..	..	5
Fecal Fat (stool) ..	..	..	..	..	..	3
Gamma globulin ..	..	..	..	..	..	1
Gastric function ..	..	..	..	..	..	13
Glucose tolerance test ..	..	..	..	..	..	70
Iron (serum) ..	..	..	..	..	..	1
17-Ketosteroides (urine) ..	..	..	..	..	..	45
Lange's test (C. S. F.) ..	..	..	..	..	..	46
Phospholipids (serum) ..	..	..	..	..	..	1
Potassium (serum) ..	..	..	..	..	..	89
Protein bound iodine ..	..	..	..	..	..	20
Proteins by paper electrophoresis ..	..	..	..	..	..	27
S. G. O. T. ..	..	..	..	..	..	506
S. G. P. T. ..	..	..	..	..	..	503
Sodium (serum) ..	..	..	..	..	..	122
Urinary stone ..	..	..	..	..	..	9
Total ..						1471
Blood and serum..	..	..	..	..	..	3334
Body fluids ..	..	..	..	..	..	310
Urine and stool ..	..	..	..	..	..	211
Special tests ..	..	..	..	..	..	1471
Grand Total ..						5326

DEPARTMENT OF PHARMACOLOGY

During the year under review the Department suffered a great deal due to the transfer of senior and experienced personnel. Shri K. G. Anantnarayanan M.Sc., Senior Scientific Officer, Class I, was promoted as Assistant Director in charge of Analytical and Quality Control Department Shri J. A. Fernandez left the Department for better prospects else where. Shri V. A. Aroskar, B.Sc., Senior Technical Assistant was promoted as Junior Scientific Officer (Pharmacology) but he subsequently resigned from the post. Smt. L. C. Kashyap, Junior Scientific Officer (Pharmacology) was transferred to the Department of Bacteriology.

Kum. Y. V. Dhairywan, B.Sc., Kum. N. D. Vaidya, B.Sc., and Shri S. D. Dholam, B.Sc., joined the Department.

Shri S. T. Nikam and Shri J. A. Fernandez were awarded the degree of M.Sc., by the University of Bombay.

There was unprecedented rise in demand for training in the Department. The following persons from the various pharmaceutical concerns were given opportunity of training. They were Sarvashri A. M. Munshi, Ashok Kumar Totla, C. S. Rao, S. S. N. Murti, S. Raghuvver, H. T. Jagasia, Durgh Pinjani, G. M. Mondre, R. V. Kulkarni, C. P. Joshi, G. L. Bhavsar, Smt. Sharda K. Gehani, Kum. Hemalata A. Wagh, Kum. Shobhana V. Thali, Kum. S. D. Vasavani, Kum. M. V. Naik, and Dr. A. Chowdhary.

Dr. M. B. Bhide, Assistant Director of the Department was deputed to attend the training of medical uses of radioisotopes conducted by Radiation Medicine Centre of Bhabha Atomic Research Centre, Bombay, for a period of 5 weeks.

Shri H. V. Nerurkar, Junior Technical Assistant was deputed for training in Diploma in trade Unionism and Industrial Relations conducted by the Bombay Labour Institute.

Routine activities

During the year under review, the Department continued the testing of drugs under the Drugs and Cosmetics Act, and testing of drugs on behalf of Prohibition and Excise under the Prohibition Act 1949. It also continued to extend the testing facilities to other Government agencies and private pharmaceutical manufacturers.

A collaborative assay of insulin for the establishment of National Reference Standard was undertaken and reported to the Central Drugs Laboratories, Calcutta.

It is found that chloramphenicol palmitate is present in different physical forms designated as polymorph A, B, etc. The oral suspension of chloramphenicol palmitate though passing in content of chloramphenicol by microbiological methods, may not be absorbed to the same extent as polymorph

form A, which is not absorbed properly in the gastrointestinal tract. So the Commissioner, Food and Drugs, Maharashtra has enforced that all the oral preparations of chloramphenicol palmitate should be tested for the limit of polymorph A according to the method prescribed by the F & D. A. (U. S.). In this State this is the only laboratory which is analysing the samples of chloramphenicol palmitate for polymorph 'A' content in samples. So far about 250 samples received from different manufacturers were tested and about 11 per cent failed to comply with the F & D. A. regulations. Nine samples out of 20 received from F & D. A. Maharashtra failed to comply with the test. Even some samples in which imported raw material for the preparation was used, failed to comply with the test. The Prohibition Department continued to enforce quality control measures of toddy through its testing these samples. A few samples of foreign liquor were also received for analysis. They were tested according to the Indian Standard Specifications.

The total number of samples tested for the period was 2,901. The percentage of non-standard drugs for the period was 7.73.

Government Analyst Section

Since August 1968 the Pharmaceutical Section of the Drug testing Laboratory has been transferred to the new laboratory established under the Commissioner of Drugs and Food Control Administration. It is expected that the remaining Sections will also be transferred in the near future. The percentage of non-standard samples under the Drugs and Cosmetic Act was found to be 12.9. Two hundred and thirteen samples of vitamin formulations including enzymes, were tested. Amongst these, 18 samples contained in addition to vitamins the well-known enzyme diastase and/or pepsin. The percentage of failing samples in this category was 39. Two out of 4 vitamin samples containing vitamin D₂ were found to be not of standard quality. Twenty two per cent of the parenteral preparations, containing vitamins of B-complex failed because particles of visible suspended matter were detected in them. Sixteen per cent of the samples analysed for vitamin B₁₂ content were found to be of non-standard quality. The percentage of non-standard quality in antibiotic preparations was very low. Out of 220 samples tested only 16 samples failed. The samples failed in potency or weight variation.

The Department continue to serve as Government Analyst for the State of Gujarat. The Drugs Controller, for the State had sent a few samples of coal-tar-distillant fluids sold under various names as essence of phenyle, *De-De oderant*, etc. under Drugs Act. They were tested for R. W. coefficient and for other specifications.

Samples received from commercial and government agencies

Commercial and government agencies continued to make good use of the special facilities offered to them. Samples received in this category were mostly for testing by biological methods. Two hundred and fifty samples were received for testing for polymorph A content.

Four hundred thirty five samples were tested for safety or undue toxicity tests. These samples were mostly antibiotics and a few samples of vitamin preparations.

Samples of synthetic oxytocins, antitoxins, antibiotics, adrenaline and insulin were some of the main products received under this category.

Testing of drugs received from private agencies for their efficacy against snake-bites has been continued. Testing of fractions of indigenous drugs supplied by a private firm was also undertaken for their hypoglycemic activity. An experimental antifatigue-drug was also tested.

Samples received from Police and Prohibition Departments

The Prohibition Department continued sending samples of toddy, ganja, bhang, opium, etc. for analysis. A few samples of toddy contained much more ethyl alcohol than it is supposed to contain. None of the toddy samples were found to be adulterated.

DEPARTMENT OF VIROLOGY

Dr. K. H. Dave, has been appointed Assistant Director, Department of Viral Vaccines. Shri M. M. Farro and Shri S. V. Pandit were appointed as Junior Scientific Officers, Department of Viral Vaccines.

Shri S. M. Desai, Junior Scientific Officer was invited to attend a Symposium on Viral Laboratory Services, held under the auspices of the World Health Organization in Tokyo in December, 1970.

Dr. S. M. Desai was awarded the Khanolkar Prize of the Indian Association of Pathologists for 1970, for his studies on interference-phenomena between the rabies and Rous sarcoma viruses in chicks.

Routine

Diagnostic studies.—Thirteen brain specimens were received for diagnosis of rabies of which 4 were human, 8 dog and 1 calf brain. Two brains were positive for rabies by Seller's strain, and 2 more were positive by mouse inoculation.

Enterovirus

During the period under view, 429 specimens from 159 cases were received for viral diagnostic work. These cases were as follows. Paralytic illness (47), aseptic meningitis (5), encephalitis (29), gastroenteritis (65) and others (13).

Subacute progressive leuco-encephalitis

Twelve samples of sera and one sample of cerebro spinal fluid were received from cases of subacute encephalitis for the study of antibodies to measles. The details are given in the Table 47.

TABLE 47

Case No.	Sex	Age	History of measles (Age)	Duration of neurological symptoms	Interval between onset of measles and onset of neurological symptoms	Antibody Titre*		
						HAI	CF	N
18	M	7 years	5 months	2 months	6½ years	160	16	256
19	M	4 years	2 months	6 months	1½ years	640	256	1024
20	M	5 years	1 year	6 months	3½ years	640	256	512
21	M	9 years	7 years	1 month	2 years	5120	512	4096
22	M	8 years	7 years	7 years	0 month	5120	512	4096
23	F	8 years	1 year	3 months	6¼ years	5120	512	4096
24	M	20 years	?	?	?	5120	512	4096
25	M	8 months	8 months	15 days	0 month	5120	512	4096
26	F	8 years	2 months	15 days	7¾ years	160	64	256
26a	F	8 years	2 months	15 days	7 years	20	4	32
27	M	9 years	7 years	2 months	2 years	160	64	128
28	M	11 years	4 years	7 years	7¾ years	80	32	128
29	M	4 years	6 months	2 years	1½ years	320	128	..

*Titres are reciprocals of serum dilutions.

a Cerebro spinal fluid

Production :

	<i>Product</i>			<i>Quantity</i>	
Rabies vaccine	..	..	..	..	3,17,1826 ml
Canine vaccine	..	..	..	..	22,268 ml.
Rabies antiserum	..	..	..	..	1,000 ml.
Polio vaccine	..	..	..	..	1,623,350 doses

DEPARTMENT OF ZOONOSIS

Plague epidemiological studies were continued in ' B ' and ' C ' Ward areas of Bombay city and Aurangabad district of Maharashtra.

Epidemiological studies were undertaken in Municipal areas of Thana city to determine the incidence of filariasis.

Two trainees from Bombay Municipal Corporation were trained in advanced medical entomology for three months.

Mrs. J. R. Bharucha continued to work as a Government of India Research Scholar. Dr. (Kum.) S. Shringi joined this Department as a C. S. I. R. Senior Research Fellow ; Dr. G. C. Chaturvedi, Senior Scientific Officer left this Department in May 1970.

Dr. W. N. Gatlwar, Senior Scientific Officer, Class I, of the Clinical Pathology Department, joined the department ; Mr. T. U. K. Menon and Kum. L. S. Iyer joined the department as Junior Scientific Officers. Mr. T. U. K. Menon was awarded M.Sc. degree in Applied Biology in 1971.

A large number of snakes and other items were received from outside for identification and advice. Besides attending to inquiries pertaining to rodents, snakes and insects, and identifying the specimens received, this Department also supplied insects, snakes and rodents to a number of educational and research Institutions. The Table below shows the number of different types of insects, snakes and rodents supplied :—

TABLE 48

	No. supplied					
<i>Cobra</i>	..	..	..	..	..	32
<i>Russell's viper</i>	..	..	..	..	..	14
<i>Krait</i>	..	..	..	..	..	93
<i>Echis carinatus</i>	..	..	..	..	..	6
Non poisonous snake	..	..	..	..	..	9
Scorpion	..	..	..	..	..	18
Snake fat	..	..	..	..	..	17 gms
Snake skin	..	..	..	..	..	5
Fleas (<i>X. cheopis</i>)	..	..	..	..	..	50
Fleas (<i>X. astia</i>)	..	..	..	..	..	50

Colonies of fleas *X. cheopis* and *X. astia*, flies (*M. domestica nebulosa*), cockroaches (*P. americana*) and rodents *Tatera indica*, *Rattus blandfordi* were continued to be maintained for insecticide and plague studies.

During the period, visitors from different institutions were shown the venom extraction technique by actual demonstration and were given detailed information on the identification of snakes and first aid treatment of snake bite cases. Demonstration of the extraction of snake venom from the snakes is one of the best attraction for Scientists, students and visitors alike. Numerous people visited the Institute for this purpose.

Shri Vad of this Department gave a talk on identification of snakes, first aid and treatment of snake-bite cases at Messers Lubrizon Ltd. He was also deputed to give a talk on the same subject at Messers Philips India, Ltd. Thana.

Production :

During the period under review, 258.10 gms. of venom was extracted and 145.21 gms. of venom was supplied for the manufacture of antivenin and research as shown in Table 49 and about Scorpion Venom in Table 50.

TABLE 49.

Venoms					Production	Supply
					(gms)	(gms)
Krait	..	..	..	..	128.19	93.88
Cobra	..	..	..	..	56.09	39.94
Viper	..	..	..	..	73.82	7.03
Echis	..	..	..	..	Nil	4.36 (supplied previous from stock)
Total					258.10	145.21

TABLE 50

Scorpion venom :

Species				Production	Supply	
Black scorpion (<i>Palamaneus gravimanus</i>)				..	1.59	0.20
Red scorpion (<i>Buthus tamulus</i>)				..	0.39	
Total				..	1.98	0.20

ANIMAL FARM

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

Students of the D.M.L.T. and Technician Training Course were trained in general management of laboratory animals. Trainees of the Laboratory Animal Technician Training Course conducted by the Laboratory Animal Information Service (I.C.M.R.) were provided facilities for practical work in connection with their course. There was a general strike for a fortnight by the class III and class IV Government Servants of the Maharashtra State. The working of the Animal Farm both at Bombay and at Pimpri (near Poona) where about 200 horses are kept, was greatly jeopardized. Fortunately the gazetted officers of the institute numbering about 80, rose to the occasion and carried out all the work of sweeping, cleaning and feeding of all categories of animals. As a result of the strike the breeding programme of the animals like mice, rats, guineapigs, rabbits etc. were upset. This will have a serious repercussion for future animals supply programme.

The below mentioned figures will show the number of different species of laboratory animals bred in the Animal Farm and supplied to the Department and outside during the year under review :

TABLE 51

				Production	Supplied to Departments	Supplied outside
White mice	..	..	..	1,90,242	1,61,155	6,355
Albino rats	..	..	..	11,428	5,313	4,126
Rabbits	..	..	..	3,372	2,076	919
Guinea pigs	..	..	..	4,240	3,277	1,044
Hamsters	..	..	..	1,078	713	101
Cotton rats	..	..	..	10		

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

LIBRARY SECTION

The work of reorganization of the library continued into this year. The books in the book-section were reclassified where necessary and were re-arranged according to 'universal decimal classification' system. The construction of the air conditioned seminar room was completed. This has satisfied the longfelt need for a room, where small groups could hold scientific discussions without disturbance.

There was lot of congestion in the Library due to lack of space and due to addition of new books and journals. The congestion was eased to some extent when space was made available in the former varandah on the east side of the Library. The varandah was converted into a big room and provided with adequate facilities to make the readers comfortable.

The current-journals-room has been provided with false ceiling and fluorescent tube lights. This room is going to be air-conditioned shortly.

Eighteen new journals (15 foreign and 3 Indian) have been added to library during this year.

Hours of work.—The practice of keeping the library open upto 7-00 p.m. was continued during this year to enable our research staff and outside readers to make use of the library facilities after office hours.

Library Services.—The library participated in the inter-library loan service with other scientific libraries. It collaborated in exchange programmes sponsored by the W. H. O., American Medical Library Association, British Medical Library Association and UNESCO.

Library Working.—The following figures indicate the working of the Library during the year.

Number of outside readers who visited the Library	..	3000
Number of books and periodicals issued to our staff	..	840
Number of books and periodicals issued under inter-library loan service.		270
Books and periodicals obtained for our staff from other libraries.		56

Additions to Library—

Number of books added during the year	..	..	177
Number of volumes bound	..	..	275
Number of current periodicals subscribed	..	..	160
Current periodicals received free of charge	..	..	115
Reports of scientific institutions	..	..	30

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MAINTENANCE ENGINEERING AND CARPENTRY SECTION

The year under review has been full of activity, increased work load and covering maintenance-work of the type which was previously not undertaken.

Four incubation-rooms for Polio section are prepared by this Department. The carpentry section has prepared frames for windows of the rooms and rooms are made air-tight. Every room has been provided with one 5 K. W.

heater with fan of G. E. make. The room is required to have exactly 37° C temperature. So special type of thermometer-type of temperature control (imported) which is very sensitive for a minor change in temperature is installed and for this temperature control special type of imported electronic relay is used. The complete installation and electrical wiring is carried out by the electrical section and has made incubation room ready for use.

In addition to this a large number of installations of refrigeration machinery equipment and repairs of innumerable items like ovens, centrifuges, compressors, vacuum pumps, various shaking machines, stirrers, pharmaceutical machinery, etc. are carried out.

The carpentry section also contributed substantially in the year's output. Specially required wooden items for research and production such as animal despatch cages, crates for 8, 12, 20, and 40 litres pyrex jars, preparation of laboratory racks, partitions etc. were made.

Also the annual hydraulic testing of all the equipment and machinery that work under pressure as per requirement under factory Act was carried out.

