



सत्यमेव जयते

GOVERNMENT OF MAHARASHTRA

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HAFFKINE INSTITUTE
FOR THE YEAR
1969-70

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CONTENTS

						PAGE
PART I						
GENERAL	..	..	..	..	..	1
PART II						
RESEARCH	..	..	..	..	..	9
A. Progressive Research in—						
Antifertility drugs	..	..	..	..	..	11
Cholera	..	..	..	..	..	12
Diphtheria	..	..	..	..	..	15
Enterovirus	..	..	..	..	..	17
Plague	..	..	..	..	..	17
B. Departmental Research—						
Department of Bacteriology	..	..	..	..	..	19
Department of Blood Bank	..	..	..	..	..	26
Department of Chemotherapy	..	..	..	..	..	32
Department of Clinical Pathology	..	..	..	..	..	35
Director's Laboratory	..	..	..	..	..	37
Department of Immunology	..	..	..	..	..	40
Department of Nutrition and Biochemistry	..	..	..	..	..	44
Department of Pharmacology	..	..	..	..	..	47
Department of Virology	..	..	..	..	..	49
Department of Zoonosis	..	..	..	..	..	72
PART III						
ROUTINE ACTIVITIES—						
Analytical and Control Department	..	..	..	..	..	77
Department of Bacteriology	..	..	..	..	..	79
Department of Blood Bank	..	..	..	..	..	82
Department of Chemotherapy	..	..	..	..	..	84
Department of Clinical Pathology	..	..	..	..	..	86
Department of Immunology	..	..	..	..	..	89
Department of Nutrition and Biochemistry	..	..	..	..	..	90
Department of Pharmacology	..	..	..	..	..	99
Department of Virology	..	..	..	..	..	102
Department of Zoonosis	..	..	..	..	..	104
Animal Farm	..	..	..	..	..	105
Library Section	..	..	..	..	..	105
Maintenance Engineering and Carpentry Section	..	..	..	..	..	112

						PAGE
MISCELLANEOUS ACTIVITIES—						
Critics Club	..	..	..	..	..	113
Distinguished Visitors	..	..	..	..	..	115
Academic courses and training of health personnel	..			..	..	121
Post graduate students	..	..	..	..	..	121
Study awards (Degrees)	..	..	..	..	..	123
Short-term trainees	..	..	..	..	..	124
Trainees admitted for Technicians' Training Course				..	..	125
Fellowships and scholarships		..	..	..	..	126
Guest workers	..	..	..	..	..	127
Grant-in-aid Research Schemes		..	..	..	..	127
Welfare Activities	..	..	..	..	..	129
Haffkine Institute Co-operative Credit Society Ltd.	..			..	..	131
Institute's Dispensary	..	..	..	..	..	132
Financial Summary	..	..	..	..	..	133

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STAFF OF THE INSTITUTE

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PART I
GENERAL

GENERAL

Haffkine Institute is one of the major organisations of advanced research in India and it is recognised as being the leader in many respects in this field. The Institute has now ten technical departments backed by administrative departments. There are services departments like the Library, Maintenance Engineering Section, Animal Farm, etc. There are about 300 scientists who work at this Institute.

The primary function of the Institute is to conduct researches in medical and allied fields. Scientists undertake investigation of problems, keeping in view the requirements of the State Government and the country at large. The nucleus of research has been built upon communicable diseases with reference to their causation, prevention and cure.

It is an important training ground for building up research and technical man-power of the State. It is a noted centre for post-graduate studies. It admits 67 students for undertaking post-graduate research work. It runs two training courses for science graduates, each of 9 months' duration.

The Institute renders public health laboratory services, as it is concerned with advanced investigations of diseases in the State. It tests drugs and other clinical specimens that need expert testing in the field of public health services.

Epidemiological studies on communicable diseases form an important part of the manifold activities of the Institute. At a short notice, the Institute sends expert teams to any part of the State for on-the-spot investigations of epidemics. The Institute manufactures biologicals like anti-sera, vaccines, antivenine, lyophilised plasma, gamma globulin, diagnostic reagents etc. Life-saving biologicals are available day and night at the Institute.

Haffkine Institute has expanded its activity greatly during recent years. Since the establishment of the Institute, the research, training, production and services activities have grown together and a time has come when it is required to be separated into two distinct units, namely, research, training and services units on the one hand and the production activities on the other. This may conveniently be done in two stages—firstly by the functional separation and secondly by physical separation. The second stage requires to be backed by construction of a multi-storeyed building within the premises of the Institute. It is gratifying to note that the Government has accepted the suggestion in principle and it is hoped that this will be implemented in course of time. During the last three decades, the Institute has expanded in its own way and in course of time, many units of a department have been spread out to different parts of the building. With the help of senior colleagues, it has been possible to reorganise some of the units of the Institute within the limitation of the space. For example, the canteen was formerly housed by the side of the library. It has been shifted to a much bigger place and is situated in the central position for the convenience of the members of the staff. The Analytical and Control

Department and the Nutrition and Biochemistry Department have exchanged places for the convenience of work. The Blood Bank will shortly be moving into a new spacious place. The library has been reorganized to give a better look and a better place has been created for the readers.

There was no room where classes, seminars, meetings, group discussions etc. could be held. All these were done either in the laboratory or in the library, much to the inconvenience of the members of the staff. This long-felt need has been removed. A new room has been created where, in future, all these activities could be carried out.

It is mentioned in the last year's report that the Government was pleased to sanction the construction of staff quarters for the lower income employees of the Institute. This construction has already begun this year and it is hoped that in course of a couple of years it will be possible to provide quarters for about 192 families.

The construction of the staff quarters at Pimpri (near Poona) has been going on and it is hoped, with their completion, a bulk of the staff will be accommodated within the premises of the Institute at Pimpri. The construction of the Class III quarters has begun this year. This will greatly facilitate the accommodation of the members of the staff, who at present have no quarters.

The construction of the Gymnasium, which began in 1969, has been completed. The members of the staff will now have a place where they will be able to play in-door games. The building will also house a circulating library which is being created for the benefit of the Institute staff.

A few years back, the Major General Sahib Singh Sokhey Oration was created by the Government to honour the illustrious past Director of the Institute, General Sokhey. The Second Major General Sahib Singh Sokhey Oration was delivered by Dr. P. N. Wahi, Director-General, Indian Council of Medical Research, New Delhi, on 31st January 1970. The subject of the lecture was 'Recent Trends in Cancer Research'. The lecture was very well attended.

Research

During the last 20 years, the growth of the medical and scientific research in our country has been larger than at any time before. The Institute has been keeping up the progress, bearing in mind the requirements of the State and the country. To those who have warned that the unlimited population growth would hamper the progress of the country, there is some reassurance that the Institute has entered the field of reproductive pharmacology to find out new remedies to combat fertility in females. In a series of drugs that were examined in experimental animals, fractions isolated from the seeds of *wild banana* (*Ensete superbum*, Cheesm) appear to be interesting. One of these water soluble compounds, yet to be isolated in pure form, when administered orally to the laboratory animals, prevented conception to a varying degree i.e. from cent per cent to 50 per cent, depending upon the species of the animal. The seeds of *wild banana* are being subjected to further investigation in other laboratories in the country.

Chemotherapy of cancer is one of the fields in which the Institute has been working for the last decade. Out of a large number of compounds synthesised, a few showed promising results. Anticancer compound N.S.C. 107639 is not only active at a much lower dose, but its Therapeutic Index has a definite edge over the corresponding compounds currently in use in man.

Reference was made in the last report that berberine, an alkaloid from the medicinal plant grown in India, has been proved to be an effective remedy against acute cholera and non-specific diarrhœa. It has gained further popularity. Several other clinicians have confirmed our work. It is being used in our State for the control of acute choleraic diarrhoeal cases.

The conversion of the Department of Entomology into the Department of Zoonosis is a major step that was taken in order to fit into the modern trend. The Institute is better poised now to tackle the diseases which occur both in men and in animals. Filariasis is a major problem of the Maharashtra State and other regions of the country. A co-operative study between the Institute and the Public Health Department of the State has been initiated. The team consists of bacteriologists, epidemiologists and public health workers who would engage on the epidemiological studies on filariasis in man and in mosquito—the vector responsible for the transmission of the disease.

Simultaneously, the scientists propose to study the epidemics of "gastro-enteritis" which come to light in certain areas of the State. The questions that should be answered are these cases due to cholera infection or due to other enteric organisms or due to any viral disease? Does the population of these places harbour cholera organisms in their intestines without any clinical symptoms and whether, under suitable circumstances, these organisms cause the so-called "gastro-enteritis"? These studies will help in tracing the origin of these epidemics of "gastro-enteritis" and to take effective preventive measures by the public health authorities.

The epidemiological studies have assisted in finding out reservoirs of plague in wild rodents, certain species of which were identified as resistant to the effects of plague infection. Such reservoirs of infection may break through at any time and lead to the infection of domestic rodents (rats) and then to a human epidemic. To take effective action against wild rodents is impossible in our country. Testing the susceptibility of the rats to plague infection has been an important feature of the plague epidemiological studies at this Institute. As part of these systematic studies, Nasik and Thana Districts were taken up in the year under review. Rats collected from Talasari (Thana District) showed susceptibility to plague infection. Rats of other parts of Thana District showed resistance to plague to the extent of about 60 to 80 per cent. Such data are invaluable in controlling the plague infection quickly.

A sub-epidemic outbreak of influenza took place in November 1969 and continued through January 1970 in India and other parts of the world. In Aurangabad town and district, a large number of cases with symptoms of influenza were reported in January 1970. A team of scientists from the Haffkine Institute, assisted by the scientists of the Medical College, Aurangabad after careful investigation, concluded that the incidence of the disease was no

doubt greater than the normal level but was not sufficiently high to constitute an epidemic. From the throat washings of 20 cases, seven positive cases were so far detected. A₂/Hong Kong/68 type of influenza virus was isolated. Influenza surveillance studies during the year showed the presence of this strain in many individuals suffering from cold and cough in Bombay.

The Institute has embarked upon a study of rubella. Several cases suspected to be rubella were reported. Because of the teratogenic sequelae of the disease, advice was sought on the termination of pregnancy in women. No data were available on the prevalence of the disease nor its severity. Serological survey was considered essential and, therefore, undertaken. Out of the 234 sera tested, 194 showed positive results. It was further observed that a majority of children above the age of 6 years and adults possessed antibodies against rubella.

Poliomyelitis is a disease of the children. Survey carried out by this Institute had shown that most of the incidence occur below the age of 8 years in Bombay area. It is necessary to ascertain the occurrence of the disease in rural areas of Maharashtra, for which no data were available. In collaboration with the Director of Public Health and the Department of Social and Preventive Medicine, B. J. Medical College, 4 villages in Sirur Taluka (Poona District) were selected for study with the object of determining (i) the enterovirus pattern in the child population, (ii) the existence of natural immunization in the child, and (iii) the efficacy of the oral polio vaccine.

The incidence of poliomyelitis amongst vaccinated children is a disturbing feature in any part of the world where polio vaccines were in use. Sixty-seven such cases were studied virologically in Greater Bombay. Forty-seven cases (70 per cent) showed the presence of virus during the acute stage of illness. Reasons for such incidence of the disease in vaccinated children are being investigated. All isolates so far made are wild strains of polio virus.

It is now known that leprosy is not a highly contagious disease but, on the contrary, a weakly infective malady. In India, there are over 2 million cases. Treatment at an early stage is curative. If the reservoir of infection can be reduced by treatment, this disease will eventually disappear. Diaminodiphenyl sulphone (D.D.S.) is a drug of choice at present. There is scope for finding out a better remedy. The Institute has specialised in synthesising anti-tubercular drugs. Some of these drugs could also be tested against *Leprae bacilli*. They might be effective.

Testing of antileprotic drugs may be performed in two ways. Inhibition of the growth of *bacilli* in the presence of the drug can be measured turbidimetrically. This method could be used to screen a large number of compounds quickly. The second method is the use of mouse foot pad technique of Shepard. It is a time-consuming process. One of the Officers of the Institute has been trained to make a beginning of the mouse foot pad method.

The Institute introduced human gamma globulin for the first time in the country some years back. Its use has been getting popular day by day. Gamma globulin was studied for its antibody level against some of the common

infective diseases. The antibodies that were detected were against poliomyelitis, diphtheria, tetanus, streptolysin 'O', small pox, measles and typhoid. Amongst these, polio and streptolysin 'O' antibodies showed good concentration (ten times more concentrated than original plasma). Hospital trials of human gamma globulin in cases of infective hepatitis, serum jaundice (post-transfusion cases) and measles, demonstrated good prophylactic and preventive value.

The use of adjuvants, which stimulates antibody production, is of importance in the commercial production of antisera. So also, immuno suppressors are of value in inhibiting the antibody production during organ transplantation and in autoimmune diseases. The Institute's scientists have entered into this field of investigation, which may lead to some fruitful discoveries.

The work has been going on, for the past several years, to grow *S. typhi* by submerged culture method. Different synthetic and semisynthetic media were tried to improve the growth, but none succeeded. A modified complete casein hydrolysate medium (C.C.H.) was tried, both under stationary and shaken conditions. The yield of organisms from one litre of a C. C. H. medium in liquid or solid form, when compared with that of routinely used medium obtained from acid digest of meat agar, showed 3 to 4 times yield in quantity in terms of the conventional stationary method.

The vaccine prepared from the new medium was non-toxic and the antigenic pattern remained the same as in conventional vaccine. Thus, the submerged culture method for the growth of the typhoid vaccine appears to be better than the method that is now being used for the production of typhoid vaccine.

The effect on the potency of typhoid vaccine due to repeated freezing and thawing was being investigated. The results demonstrated that there was no deterioration in the potency of T. A. B. vaccine, either heat-killed and phenol-preserved or the formalin-killed but preserved with phenyl mercuric nitrate (p.m.n.) for a period of six months. It was interesting to observe that the heat-killed and phenol-preserved vaccine showed granular appearance after the third thaw. This made the vaccines unsuitable for human administration.

Bacteriophages are associated with *C. diphtheriae*. Phage typing of the infection will demonstrate the source of the epidemic and the extent of the infection in healthy carriers. About 340 strains of diphtheria were isolated in different cases. Eighty per cent of the strains belonged to Type 19 and 6 per cent showed phage pattern, while the remaining were untypable. The prevalent organisms, therefore, belonged to the Type 19.

A processed food containing leaf proteins was tested for its human acceptability. Responsible staff members of the Institute served a human guinea pigs. Analysis of results revealed that the dark green colour of leaf proteins was largely responsible for the unacceptability of the leaf-protein recipes. Secondly most of the leaf protein recipes did not seem to be so palatable as the plain counterpart.

Institute News

Dr. N. K. Dutta, Director, was appointed member of the WHO Expert Advisory Panel on Bacterial Diseases (Cholera) for another 5 years. He has also been appointed member of the Governing Body of the Indian Council of Medical Research, New Delhi.

Dr. Dutta delivered two orations, one at Trivandrum and the other at Madras on 'Drugs against Cholera and Severe Diarrhoea' and 'Modern Concept on the Prevention of Cholera as evidenced by experimental work' under the auspices of Indian Academy of Medical Sciences, New Delhi.

Dr. Dutta was the guest lecturer at the Indo-Soviet symposium on the 'Chemistry of Natural Products' including Pharmacology, held at New Delhi from 2nd February 1970 to 6th February, 1970, sponsored by the National Institute of Sciences of India, New Delhi.

He was also invited to be the Chairman of the Section of Pathophysiology in the National Seminar on 'Immunity and Immunoprophylaxis in Cholera' held at the Cholera Research Centre, Calcutta in March 1970, under the auspices of the Indian Council of Medical Research.

Dr. M. V. Sant, Assistant Director, Department of Zoonosis, Shri Y. S. Nimbkar, Assistant Director I/C Department of Bacteriology and other scientists, Dr. M. J. Modak, Department of Biochemistry, Kum. Perviz H. Marker, Department of Pharmacology, Shri P. K. Chatterjee and Smt. Sha-kuntala Kumar Bhindey also participated in the seminar on invitation.

Dr. S. S. Rao, Assistant Director I/C Department of Immunology, has been appointed a member of the Expert committee on Genetics and Immunology of the Indian Council of Medical Research, New Delhi.

Dr. K. H. Dave, Officer of Enterovirus Unit, Indian Council of Medical Research, has been appointed member of the Virus and Arthropod-borne Diseases Expert Committee of the Indian Council of Medical Research, New Delhi.

Shri Y. S. Nimbkar, Assistant Director I/c Department of Bacteriology, is appointed as a member of the Advisory Committee of the Small-Scale Industries' Research Institute, Poona.

Dr. Sant and Dr. S. S. Rao took active part in the organization of the International Conference on 'Global Impacts of Applied Microbiology' held in Bombay in December 1969. They were assisted by several members of the staff.

The Institute organized several exhibitions at different places and one of them was held in Nagpur.

Under the Indo-Bulgarian Cultural Exchange Programme, Mr. Georgi D. Dimitrov from the Institute of Chemical Technology, Sofia, Bulgaria, worked at this Institute for a year. He left in November, 1969 after completing his studies on snake venoms. Dr. J. J. P. Ratnayake, Bacteriologist, Anti-

V. D. Campaign of the Department of Health Services of Ceylon, worked at this Institute as guest worker. Dr. Laxman Pondgyal from Nepal had spent a short period at the Institute under WHO Fellowship.

Dr. F. L. Saldanha, Department of Analytical and Control, proceeded abroad on a year's study in U. S. A. under WHO Fellowship. The subject of study is 'Drug Analysis and Biological Standardization'.

Shri S. M. Desai, Junior Scientific Officer, Department of Virology, attended the WHO Seminar on the 'Laboratory Diagnosis of Small-pox', held in Bandung, Indonesia. His training was sponsored by WHO.

WHO scholarship was awarded to Md. Khleifat Hussein of Jordan, who has been working here since 1968. His scholarship was extended to September 1970.

The Institute continued to attract new grants from different sources. The George Williams Hooper Foundation, California, sanctioned a grant of \$ 4,000 (U. S.), through W. H. O., Geneva, for a co-operative study between the Haffkine Institute and the Foundation on "the protective effect of killed and live plague vaccines".

The International Atomic Energy Agency sanctioned a grant of \$ 1,250 (U. S.) for a joint project between the Haffkine Institute and Dr. Oscar Felsenfeld, Chief, Division of Communicable Diseases, Professor of the Tulane School of Public Health and Tropical Medicine, U. S. A., on the "Use of antigens labelled with radio-isotopes in serological epidemiology".

Dr. N. K. Dutta received a grant of \$ 4,000 (U. S.) for his study on cholera from the World Health Organization.

Dr. S. S. Rao, Assistant Director, Department of Immunology, received a grant for a research scheme 'Cellular aspects of antibody production and immune paralysis', from the Department of Atomic Energy, Government of India.

A fellowship from the University Grants Commission was awarded to Shri H. V. Patel, Department of Chemotherapy.

Many Research schemes sanctioned earlier by the Indian Council of Medical Research, Council of Scientific and Industrial Research, Government of India, Government of Maharashtra Defence Department, etc., were continued. A new scheme on the study of leptospirosis was sanctioned under Dr. M. V. Sant by the Indian Council of Medical Research.

As pointed out before, the Institute offers facilities to the members of the staff to continue their studies in various subjects and simultaneously to take Master's Degree or Doctorate Degree of the Bombay University under recognized guides. At present, there are 16 University Teachers at the Institute. The subjects covered by them are microbiology, biochemistry, organic chemistry, applied biology, zoology, pathology, bacteriology and pharmacology.

In this year, 10 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 8 students admitted to the Diploma in Medical Laboratory Technology Course (Bombay University), six qualified for it. Six science graduate students successfully completed the Technicians' Training Course and were awarded certificates.

Many distinguished scientists and dignitaries, numbering about 125, visited the Institute. Several eminent scientists were invited to address the Critics Club of the Institute.

The Director and the senior members of the staff were greatly benefited by the advice and guidance given by the members 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 Director is very conscious of the fact that the successful implementation of any policy or change in any system depends on the integrity, conscientiousness drive and skill of the individual members of the staff. All these qualities were in evidence among the members and he takes this opportunity to thank them for the continued support from the administrative, scientific and non-scientific workers of the Institute.

PART II
RESEARCH

A. PROGRESSIVE RESEARCH

Antifertility Drugs

Seeds of Banakadali (wild banana) *Ensete Superbum* (Cheesm) is being used in the treatment of small-pox and measles by the physicians practising the indigenous system of therapy. The seeds were investigated for their antiviral properties. It was found that there are several chemical fractions which are effective against variola and vaccinia grown on the chorio-allantoic membrane. These fractions were also effective in mice infected in the variola or vaccinia viruses.

It is further shown that one of the fractions (VIDR-2GD) possesses antifertility action.

VIDR-2GD was dissolved in water and administered orally to animals during pre and post implantation periods. In mice, rat, guinea pigs and hamsters the drug treatment between the period which lay between fertilisation of the ovum and implantation of zygote rendered the animals non-pregnant. In case of rabbits and rats, complete inhibition of pregnancy could not be achieved. The dose requirement is critical, as high doses were ineffective in both the species of animals.

Acute toxicity.—Dose upto 5 gm/kg of the body weight of VIDR-2GD given orally did not produce any reactions or mortality in white mice during the 7 days observation period. Injections of 1,000 mg/kg intravenously and 2,500 mg/kg intraperitoneally, did not produce mortality or any reactions. But intraperitoneal injection of 3,000 mg/kg caused 75 per cent and intravenous injection of 2,000 mg/kg produced cent per cent mortality in these animals.

Subacute toxicity in albino rats.—Daily doses of 190 mg/kg of body weight (subcutaneously) and 400 mg/kg (concentration 500 mg/ml in distilled water) (orally) were given, for 60 days to rats. The group with subcutaneous injection did not show any abnormal symptoms. The weight was reduced slightly after 30 days, but there was slight increase at the terminal period. The haemogram showed slight leucopenia and decrease in haemoglobin. The bone marrow was normoblastic. Histological examination of the tissue revealed cloudy changes in the kidney, fibrinous material covering the intestinal mucosa, oedema of the submucosa of the stomach, slight oedema in the large intestine and lung. Rest of the tissue did not show any abnormality.

Subacute toxicity in dogs.—Four dogs, one for control and three for experiment were taken. Two dogs were given 500 mg/kg/day (42 mg/kg/day) orally in divided doses and the remaining dog took 250 mg/kg/day in the same way and all of them were observed for 60 days.

Clinically, the experimental dogs which were given 500 mg/kg/day did not show any abnormality at the beginning. After 4 weeks the weight was reduced by 1.5 to 2 kg. and then it slowly recovered by the end of the 8th week. Initially, the haemoglobin was 13G (average) per cent. At the end of 5th week it came down to 11G (average) per cent and 10.5G (average) per cent at the terminal period. During the corresponding time, the R.B.C. levels were 4.33, 3.86 and 4.69 million/c.m.m.(average) of blood respectively. Total leucocyte count initially was 12,350 at 4th week, 14,000 and 6,700 per c.m.m.(average) of blood at the termination of the treatment. The differential count did not show any discernible changes. Bone marrow and urine analysis did not reveal any abnormality. Blood sugar was not altered. The glucose tolerance curve was prolonged towards the end of the second month. The transaminases, sgot and sgpt levels initially were 35 units and 20 units/100 ml of serum, but it was increased to 70 and 50 units/100 ml of sera at the end period. The microscopic examination of the liver showed some congestion and nutmeg appearance.

The dog which received 250 mg/kg/day of the drug orally behaved normally and there were no toxic signs or symptoms.

CHOLERA

Preparation of live parenteral vaccine

The field trials of cholera vaccines in our country as well as in other countries of the world have shown limited protective power and, therefore, study in the preparation of live parenteral vaccines using attenuated vibrio strains is of importance. It is a continuation of the work, which has been started a couple of years back. Further grounds have been covered.

Vaccines containing 4,000 million organisms per ml. from two apathogenic strains, viz. *V. cholera* C₁₄ S₅ and *V. cholera* E1 tor Water 6 were prepared and subjected to protective tests using two experimental animal models along with a formol killed vaccine. The two methods used for evaluation of the potency of vaccines were (i) active mouse protection test, (ii) the infant rabbit passive protection test. The active mouse protection test showed that live vaccines were significantly superior to the killed vaccines while the infant rabbit test using two doses for immunization of adult rabbits did not show a clear-cut superiority of live vaccines over the killed ones.

In addition to the above, the passive protection test in infant rabbits with sera prepared by hyper-immunization of adult rabbits using the live vaccines and their syncase toxins were carried out. Hyperimmune sera prepared against live cultures of *V. cholera* C₁₄ S₅ and *V. cholera* E1 tor showed very high degree of protection as compared to the strain *V. cholera* Ogawa 5321. The antisera derived from the toxin of *V. cholera* E1 tor Water 6 also showed a higher degree of protection as compared to those produced from *V. cholera* C₁₄ S₅, *V. cholera* Ogawa 5321. The toxicity of these vaccines on guinea pigs and mice was also tested. The vaccines at this high level of vibrio content were found to be toxic to guinea pigs in a dose of 5. The guinea pigs could tolerate lower doses. The vaccine was well-tolerated by mice in a dose of 0.5ml.

Cholera vaccine and concentration of vibrio

It has been observed in field trials of cholera vaccines that the conventional vaccines containing 8×10^9 million vibrios per ml. do not offer adequate protection. It was thought that vaccines containing higher concentration of vibrios might give greater antigenicity.

With this end in view, two sets of vaccines containing 24, 20, 16 and 12 billion organisms per ml. were prepared. In one of these series, the vibrios were killed by 0.15 per cent formalin, while in the other, vaccine was prepared by initially killing the vibrios with 1 per cent phenol and then preserving it with 0.5 per cent.

These two sets of vaccines were tested for freedom from abnormal toxicity in guinea pigs and mice. Five ml of each vaccine was injected into guinea pigs subcutaneously, as well as intraperitoneally. The two vaccines in a dose of 0.5 ml. each was injected subcutaneously into mice. All the animals were observed for a period of seven days.

It was seen that all the guinea pigs inoculated with vaccines containing 24, 20 and 16 billion organisms per ml. in both the types of vaccines died within 48 hours. The formalised vaccine containing 12 billion organisms per ml. showed no reaction in guinea pigs or mice. In the case of phenolised vaccine of 12 billion organisms per ml. some toxicity was observed in the guinea pigs. Mice also showed shivering after the inoculation. There seems to be some residual toxicity in the phenolised vaccine. The formalised vaccine containing 12 billion organisms is now being prepared for use.

Pathophysiology of cholera

(i) *Absorption of toxin*.—The belief is widely held that cholera is a local disease of the intestine and that any toxin that may reach the circulation is of minor importance. Investigations were carried out in order to find out whether the toxin is absorbed in the intestine and brings about signs and symptoms of the disease. For this purpose, *V. cholerae* organisms were introduced into the intestine of 10 day old rabbits *via* laparotomy and it was thought a new approach to the problem of absorption could be made, if it were found possible to prepare isolated "Thirty" — type segments of intestine in such animals. The segments, with one end blind and the other opening to the skin, would have blood, nerve and lymph supply intact, but would have no communication between the segmental lumen and that of the remainder of the intestine, the continuity of which would be restored by anastomosis. When the animals were fully recovered from the operation, vibrios could be introduced into the segment through its opening on to the abdominal wall. If this caused diarrhoea or accumulation of fluid in the intestine, the implication would be that a choleragenic agent had been absorbed from the isolated segment and distributed by the blood to the rest of the gut.

Isolated jejunal segments, with one end blind and the other opening on to the abdominal wall, were prepared under sterile conditions in infant rabbits anaesthetized with ether. There was no communication between the lumen of

the segment and that of the rest of the intestine, the continuity of which was restored by an end-to-side anastomosis.

When the animals had recovered, *V. cholerae* (Inaba 569) was introduced into the isolated segment. Most of the infected rabbits had diarrhoea and died within 48 hrs. No vibrios could be grown from the contents of the large intestine. Operated controls survived and gained weight.

Evidence was obtained that toxin prepared from *V. cholerae* could be absorbed from the isolated segment. The most probable explanation for these results is thought to be that a choleraenic(s) agent was absorbed in the isolated segment in which vibrios were growing, and that this agent reached the remainder of the intestine *via* the blood.

(ii) *Stomach in the pathogenesis of cholera*.—The electrolyte and fluid loss in experimental cholera originated in duodenum, small intestine, liver and endocrine pancreas. Here it is examined whether cholera toxin exerts any similar action on stomach.

Rabbits were fasted for 48 hours. They were opened under anesthesia and gastric loops were prepared by passing ligatures at cardiac and pyloric ends. In the loops 20 mg of syncase toxin was injected. The animals were allowed to recover and after 24 hours the animals were reopened. The gastric loops were found to be full with fluid containing a good amount of H^+ , Na^+ and Cl^- ions and a small amount of K^+ ions. This fluid accumulation in the loops could be partially antagonised by the action of antigestrin. This suggests that the part of the fluid accumulation was due to the mediation of gastrin.

Mechanism of action of berberine

The action of berberine on the cholera organisms is shown to be bacteriostatic in nature but this effect is only during the initial stages of growth of the organisms. During this initial static period, there is a leakage of intracellular micromolecules probably due to the membrane damage. The cell free preparations made from berberine treated vibrios (crude toxin) fail to produce choleraic symptoms in the infant rabbit model of Dutta, suggesting that the toxin is either neutralised or inactivated. The drug causes a static period during which there is an inhibition of the synthesis of all the macromolecules, particularly of the nucleic acids. Once the growth is resumed the synthesis of all these macromolecules begins. However, the protein and lipid syntheses are stimulated very much (to the extent of 2—2 1/2 times more than control). Since cholera toxins (endo as well as exo) are known to contain both of these components, the formation of toxin in the presence of berberine is questioned. A further analysis of fatty acid composition (liquid components) *V. cholerae* in the presence of berberine revealed that this alkaloid causes the disturbance in the relative proportion of various fatty acids presumably due to the biosynthetic disturbances. High rate of turnover of proteins also suggests the possible formation of heterogeneous protein which is degraded. Thus berberine most likely did not allow the formation of active and intact toxins.

DIPHTHERIA

Studies in diphtheria toxin production in acid hydrolysed casein medium

The nutritional requirements of *C. diphtheriae* for toxin production were determined using acid hydrolysed casein as the basal medium. The acid hydrolysed casein is a highly reproducible medium. The aim was to get better yields of toxin.

The carbohydrate requirement of *C. diphtheriae* (Weissensee strain) was first to be investigated. Glucose, sucrose, glycerol, acetate, lactate and citrate were tested for their ability to replace part or whole of maltose. It was found that the best source of carbohydrate for toxin production was maltose followed by glycerol, acetate and sucrose in that order. About a fourth part of maltose could be replaced by acetate or by glycerol with no decrease in toxin production.

The vitamin requirements were then investigated for toxin production. Nicotinic acid was essential for growth. Thiamine and B-alanine were stimulatory. The other vitamins of the B group were not necessary. The Lf of toxin produced in modified casein hydrolysate medium with supplements, was about 100-120 Lf/ml in 5 days.

Casamino acids could not be replaced even partially with urea or ammonium sulphate.

A simple synthetic medium for the study of diphtheria toxin production

The advantage of using a pure, simple and completely synthetic medium is that it can be used for the study of metabolism of the organism. It may also help to elucidate the role of iron and other factors in toxin production.

A purely synthetic medium, capable of giving 60 to 70 Lf/ml of diphtheria toxin in 5 days was evolved. The strain of *C. diphtheriae* used was 'Weissensee G5'. The medium consisted of 5 amino acids (glutamic acid, cystine, valine, isoleucine and methionine), 4 vitamins (nicotinic acid, B-alanine, thiamine and pyridoxine), salts, acetate and maltose. This medium was formulated using the "all minus one" technique. Using this technique, it was possible to get clear-cut results on the nutrients which were 'essential' and without which the Weissensee strain would not grow and those which stimulated the production of toxin.

This medium will now be used to study the metabolism of the organism and to elucidate the role of iron in the secretion of toxin.

Cellular composition of *C. diphtheriae*

The analysis of the cells of *C. diphtheriae* may throw light on a number of problems such as, virulence, ability to form pseudomembrane, resistance to drugs, etc. Earlier studies on the cellular constituents of the 7-day old culture had given varying analytical data from batch to batch. Therefore, it was felt necessary to study systematically 1 to 7-day old cultures.

The RNA, DNA, proteins and fatty acid patterns were studied at intervals from 1-7 days. The results indicated that, while the DNA content went down from 5 to 3.3 per cent during the seven-day period, the RNA content decreased with the incubation period rather fast as shown in the Table 1:

TABLE 1
mg per cent on dry weight basis

		1	2	3	4	5	6	7
Proteins	..	.. 42	47	37.7	42.4	33.9	43.7	27.0
RNA	..	.. 15	13.2	9.8	9.8	7.3	5.3	4.7
DNA	..	.. 5	4.8	4.6	3.2	3.1	2.8	3.3

The growth of the organism could not be assessed by the usual turbidimetric method, as the organism grew only on the surface layer of the medium. However, the growth could be evaluated by estimating the RNA and proteins formed at various intervals during the growth phase of the organism. The synthesis of toxin was mainly dependant on the formation of RNA in the cells eventually leading to the production of toxin. It was observed that the maximum RNA content was present in the first few days. As the protein was released as toxin into the culture medium, the RNA content was seen to decrease in older cultures.

Fatty acid composition of *C. diphtheriae*

Precise knowledge of the detailed composition of fatty acids in the organism may help to elucidate some of the biochemical properties of the organism. The study was undertaken to ascertain the nature and role of the lipids of *C. diphtheriae*.

Total lipid content of 1-7 day old cultures were estimated. The lipids were separated into neutral lipids and phospholipids by silicic acid column chromatography. Fatty acid pattern of the total lipid, phospholipid and neutral lipid by gas-chromatography was carried out. The neutral lipids and phospholipids were separated into different fractions by thin layer chromatography.

The detailed study of the fatty acid spectrum of *C. diphtheriae*—WG-5 (the standard strain used for toxin production) at intervals revealed that the total lipids of the organism contained 11 fatty acids, the palmitic (C16) and palmitoic (C16:1) being predominant. Oleic acid (C18:1) was next in abundance. The proportion of low molecular weight fatty acids was much less as compared to the higher ones.

The neutral lipid and phospholipid fractions also exhibited a similar trend. In phospholipid fractions, palmitic and palmitoic acids were the only constituents. There was no significant amount of the lower fatty acids.

Phage typing of *C. diphtheriae*

Since, phages are associated with toxigenicity of *C. diphtheriae*, the work of phage typing was undertaken, so that the source of an epidemic outbreak and the extent of infection in healthy carriers can be determined.

About 340 strains of *C. diphtheriae* were isolated from suspected patients. They were tested biochemically and also for toxigenicity. *C. diphtheriae* *mitis* were 60 per cent, *gravis* 35 per cent and *intermedius* 4 per cent and the remaining 1 per cent were other diphtherioids. Eighty-five per cent of the strains were found to be toxinogenic. All the strains were subjected to phage typing. About 80 per cent of the strains belonged to type No. 19 and 6 per cent showed phage pattern, while the remaining strains were untypable. Thus, No. 19 phage type *C. diphtheriae* was found to be predominantly prevalent.

ENTEROVIRUS

Poliomyelitis

Previous studies in the Greater Bombay areas have shown that paralytic poliomyelitis is mainly a disease of the children below 8 years. What is the state of affairs in rural areas of Maharashtra is not known. A survey is an essential step to assess the incidence of the disease in different parts of the State. This is the first step taken in this direction.

In collaboration with the Director of Public Health and Department of Social and Preventive Medicine, B. J. Medical College, Poona, 4 rural areas in Sirur taluka (Poona District) were selected with the following objectives :

- to study the enterovirus pattern in the child population

- to study the extent of natural immunization against poliovirus in the local community and

- to study the efficacy of live oral polio vaccine in the rural community.

The work began in February 1970. Out of the four rural areas selected for the above project, materials for enterovirus survey and study of natural immunization against poliovirus have been collected from two areas. The field team is working at present in the third area. Till todate 355 stool samples and 64 blood samples have been collected. On completion of these preliminary studies, oral polio vaccine will be fed to the child population of these areas and virus excretion and seroconversion studies would be carried out.

PLAGUE

During the year under review, the work of determination of susceptibility of rats to plague infection was continued. Rats from three areas, viz. Thana District, some areas of Greater Bombay, Nasik District were collected and challenged with approximately 5,000 living organisms of the virulent *P. pestis* strain, 195/P. It was seen that in Thana District, the rat population showed some variation in resistance. At Talasari, the rats showed a very high susceptibility of 66 per cent while in Kalyan town, the rats were found to be highly

resistant, the susceptibility being only 12·5 per cent. In Palghar again, the susceptibility was 52 per cent. The susceptibility did not seem to be related to the urban or rural nature of the areas. However, in all towns such as Bhivandi, Kalyan and Dahanu, the resistance of rats varied from 67 per cent to 87 per cent. Some rural areas like Dhasi also showed high resistance (72 per cent) in the rat population.

In Bombay city, rats from two areas, viz. Sewri and Ward A were included in this study. At both the places, the *R. rattus* found to be highly resistant, the resistance being 70·75 per cent. On the other hand, the *B. bengalensis* collected from both the above mentioned areas were susceptible to the extent of 82 per cent which was indeed was very high.

In Nasik District again, varying degree of susceptibility was observed in the rodent population of different places. The *R. rattus* from Surgana, Kuban and Abhona were highly susceptible, the susceptibility varying from 57 to 71 per cent, while on the other hand those of Nasik, Dindori, Niphad, Malegaon etc. were resistant. Their susceptibility varied from 20 to 40 per cent. In between these two extreme ranges, rats varying in susceptibility from 40 to 60 per cent were also found in areas like Kundewadi, Chandor and Ravalgaon. This only showed that a varying pattern of resistance of rats to plague infection, irrespective of the nature of area, exists in this district.

From out of the three areas mentioned above, the sera of rats collected from different places of Thana District were tested by haemagglutination test to determine the presence of specific antibody to plague by using purified fraction 1 antigen. All the sera were negative, indicating that there was no latent plague infection in this district and the resistance to infection with *P. pestis* observed in these rats was probably of genetic nature.

In addition to the above, samples of human and rat sera collected from Marathwada area, where on an outbreak of plague like syndrome was reported. All these samples were negative to plague. Rat tissues and flea pools collected in this area were also inoculated into mice in order to isolate *P. pestis*. Presence of *P. pestis* was not seen in any of the samples.

B. DEPARTMENTAL RESEARCH

DEPARTMENT OF BACTERIOLOGY

Studies on the cultivation of *S. typhi* by submerged culture methods were further investigated. Different synthetic and semi-synthetic media were tried with a view to improve growth, but none found to be superior to the complete casein hydrolysate medium previously used. Use of a silicone compound as an antifoam agent was found to be satisfactory. Vaccines prepared by submerged culture methods were tested for toxicity. The survival rate of *P. pestis* during lyophilization was found to improve by using mixtures of sodium glutamate and lactose instead of lactose alone. Formalized T. A. B. vaccine did not show any deterioration in potency after storing it in frozen condition for one year or on repeated freezing and thawing upto six thaws. A live parenteral cholera vaccine containing $4,000 \times 10^6$ organisms per ml. prepared by using attenuated strains, was found to be toxic to animals on parenteral administration. The course of disinfection of *S. typhi* and *S. paratyphi* B. with disinfectants like formalin and phenyl mercuric nitrate was found to be different.

Studies on the growth of *S. typhi* in shaken and submerged culture

The work on submerged and continuous culture method was continued during the year under review. For devising a suitable medium, which would give abundant growth of *S. typhi* under shaken and fermentor conditions and production of all the three antigens, namely O, H and Vi in proper quantities, various synthetic and semi-synthetic media were tried. These were (i) a synthetic medium reported by Troitsky and Hecker (1959) of Gamalaya Institute of Epidemiology and Microbiology, Moscow ; (2) plain casein hydrolysate and (3) complete casein hydrolysate (C. C. H. medium, i. e. casein hydrolysate fortified with yeast extract, sodium- β -glycerophosphate and other supplements).

Under stationary and shaken conditions, the growth of *S. typhi* was the best in C. C. H. medium and resulted in the production of all the three antigens. Plain casein hydrolysate showed the same antigenic pattern, but the growth was less than in C. C. H. medium. Synthetic medium gave a very poor growth and production of H antigen was negligible, though the production of O and Vi antigens was as good as in the other two media. In spite of fortifying the synthetic medium with yeast extract and yeast dialysate, it continued to show a poor growth and absence of H antigen in both shaken and stationary cultures. Another synthetic medium, containing inorganic nitrogen source, sodium lactate and buffer salts, was tried but this also gave poor growth and failed to produce H antigen under both the conditions.

With a view to improve the growth and antigen production, the organism (*S. typhi* was grown in a modified C. C. H. Medium wherein casamino acids (Difco),) along with buffer salts, replaced casein hydrolysate. It was observed

that the formation of H antigen was not so good as that in C. C. H. medium, though the growth was the same. One more modification of C. C. H. medium was done wherein casein hydrolysate was replaced by 2% tryptone, an enzymatic hydrolysate of casein. In this method, the growth and antigen production by *S. typhi* equalled to that in the C.C.H. medium.

Effect of changing the amount of yeast extract in the C.C.H. medium showed that the growth was considerably reduced in the absence of yeast extract, while 1% yeast extract, either in powder or paste form, yielded the maximum growth. Growth in shaken cultures was about five times that in the stationary cultures.

Effect of addition of glucose to the C.C.H. medium resulted in the loss of H antigen formation, whereas buffer salts, when added to the medium, yielded poor growth in both the cultures. Thus, among all the media tried so far, not a single medium showed its superiority over the C. C. H. medium with respect to growth and antigen production and, so, it was decided to continue the use of C. C. H. medium for the future work.

The yield of organisms from one litre of C. C. H. medium in liquid and solid form, was compared with that of routinely used medium obtained from acid-digest of meat agar. It was observed that one litre of C. C. H. liquid medium yielded about 15 litres of typhoid vaccine containing $1,000 \times 10^6$ organisms per ml as against 8 litres obtained from C. C. H. agar and 5 to 6 litres from acid-digest of meat agar medium. Thus the yield of vaccine from liquid C. C. H. medium was found to be the highest.

Apart from studying the growth and antigen production by *S. typhi* in various media, typhoid vaccines were prepared in complete casein hydrolysate medium and tryptone modification as well as on solid C. C. H. medium. Toxicity tests of these vaccines were performed in duplicate in laboratory animals, viz. white mice and guinea pigs. The vaccine was injected both intraperitoneal and subcutaneously in both the animals. A dose of 0.5 ml was used for mice inoculated by both the routes, while a dose of 5.0 ml was used in the case of guinea pigs. It was found that all the vaccines proved to be non-toxic in the experimental animals.

After completing the small-scale studies on growth and antigen production in various media, an attempt was made to grow the organism on a large-scale in C. C. H. medium. This was done by growing the organism in 500 ml of the medium in 3 litre jar and 4 litre of the medium in 12 litre jar, under shaken and stationary conditions. It was found that growth and antigen production in both the cases was the same as in small-scale experiments, but there was abundant froth formation in the jar where 4 litre of the medium was used.

For preventing froth formation, ground-nut oil and silicone (Union Carbide Chemicals Product) were separately incorporated into the medium. It was found that the amount of ground-nut oil required to stop the formation of froth was 0.1%, whereas that of silicone it was 10 p.p.m. of the medium. Growth and antigen production were not affected due to the addition of anti-foam agents in the medium. Silicone was preferred as anti-foam agent because

of the small amount required to stop the formation of froth in comparison to that of ground-nut oil. Ground-nut oil also formed an emulsion, making the medium turbid which interfered in the measurement of growth by opacity methods.

Encouraged by the results obtained in large-scale experiments, an attempt was made to study the methods for cultivation of *S. typhi* in a laboratory scale fermentor.

In the preliminary experiment, 5 litres of C. C. H. medium was taken in a 15 litres fermentor jar and inoculated with 100 ml of a 4 hour old culture of *S. typhi*. Volume of air supplied to the culture in the jar was 5 litres per minute and agitation was done with a stirrer rotating at a speed of 490 r. p. m. per minute. Under these conditions, it was observed that the antigenic pattern was the same and the amount of the growth was 3 to 4 times than that in stationary cultures.

Detailed studies for working out optimum conditions as regards aeration and agitation in the fermentor affecting the growth of *S. typhi* are yet to be undertaken. Vaccines prepared by different methods outlined above will be subjected to immunogenicity tests in experimental animals.

Studies on lyophilisation of *P. pestis*

The investigations undertaken to determine the optimum conditions for lyophilisation of *P. pestis*, with a view to develop methods for obtaining high survival rate after lyophilisation for use in preparation of a live vaccine, were continued. The studies on use of different suspending media before lyophilisation were studied. In addition to the suspending media, such as gum acacia, nutrient broth, 1% sucrose and different concentrations of lactose as reported earlier, 1% and 3% sodium glutamate, different mixtures of sodium glutamate and lactose and a mixture of sodium glutamate, sucrose and human serum albumin, were tried. The effect of using different concentrations of organisms for lyophilisation was also simultaneously investigated. The results obtained are given in the following Table-2.

TABLE 2

Suspending medium	No. of org. before lyophilisation	Percentage survival on the day shown			
		1	4	12	24
1% Lactose	.. 3590 × 10 ⁶	0.003	0.001	0.0012	0.0021
Do.	.. 101 × 10 ⁶	0.00060	0.0008	0.003	0.005
1% Sodium glutamate	.. 9449 × 10 ⁶	0.06	0.05	0.07	0.06
Do.	.. 4322 × 10 ⁶	0.09	0.05	0.02	0.04
3% Sodium glutamate	.. 6630 × 10 ⁶	0.25	0.30	0.28	0.30
Do.	.. 1582 × 10 ⁶	0.19	0.16	0.16	0.50

TABLE 2—*contd.*

Suspending medium	No. of org. before lyophilisation	Percentage Survival on the day shown			
		1	4	12	24
3% Sodium glutamate + 6% Lactose	12400 × 10 ⁶	2.67	2.01	1.008	
Do. ..	4650 × 10 ⁶	1.84	1.07	1.02	
Do. ..	1155 × 10 ⁶	7.96	3.07	6.06	
0.2 Molar phosphate buffer + 2.5% human serum albumin + 7.5% sucrose + 0.7% sodium glutamate	21700 × 10 ⁶	0.05	0.08		
Do. ..	7612 × 10 ⁶	0.13	0.10	0.03	
3% Sod. glutamate + 12% lactose	9562 × 10 ⁶	1.15	1.02		
Do. ..	39500 × 10 ⁶	0.09	0.54		
Do. ..	13750 × 10 ⁶	1.52	2.87		
3% Sod. glutamate + 3% Lactose	33500 × 10 ⁶	0.15	0.53		
Do. ..	11450 × 10 ⁶		0.94		
Do. ..	2912 × 10 ⁶ ..	2.36	1.07		
6% Sod. glutamate lactose	12% 34600 × 10 ⁶	0.36	1.15		
Do. ..	6125 × 10 ⁶ ..	6.45	2.56		
Do. ..	1840 × 10 ⁶ ..	4.45	3.69		

It will be seen from the Table 2 that the number of organisms surviving the process of lyophilization was considerably less as compared to the initial number taken for lyophilisation. Different suspending media show different rates of survival. With 1% lactose, the rate of survival varied from 0.008 to 0.005 per cent. The effect of initial concentration of organisms was not apparent in the concentrations used. Use of 1 per cent sodium glutamate showed a survival rate of about 0.05 per cent. However, increase of sodium glutamate concentration to 3 per cent gave a survival rate of 0.2 per cent to 0.3 per cent, which was much higher than in 1 per cent lactose alone. Mixtures of 3 per cent sodium glutamate with 3 to 12 per cent lactose gave higher rates of survival varying from 0.5 to 6 per cent. A mixture of 3 per cent sodium glutamate with 6 per cent lactose gave the highest survival rate of 3 to 6 per cent when the initial concentration of organisms was about 1000X10⁶ organisms per ml. The medium containing phosphate buffer, human serum albumin, sucrose and sodium glutamate did not give high rates of survival under our conditions of lyophilisation as reported by others. Experiments using other combinations of glutamate and lactose, which had given the best results in our hands so far, are in progress. The moisture content of the cultures lyophilised, using different suspending fluids, was determined. The moisture content of freeze dried cultures varied from 1.8 to 3.5 per cent excepting that in case

of 12 per cent lactose which was found to be as high as 6 per cent. The effect of moisture content on survival of organisms during storage is under investigation.

Studies in dynamics of disinfection

Studies in dynamics of disinfection by common bactericidal agents, such as formalin, phenyl mercuric nitrate (p.m.n.) etc., reported earlier, were continued using *S. typhi* and *S. paratyphi B* as test organisms.

The effect of phenyl mercuric nitrate on *S. typhi* (Strain Ty2) at concentrations of 20 mg, 10 mg, 5 mg, 2.5 mg, and 1.25 mg. per 100 ml of culture was investigated at three different pH levels, viz. 5.5, 7.5 and 9 at room temperature. The concentration of 20 mg/100 ml of p.m.n. completely inactivated the culture in 19 minutes at pH 5.5, while at pH 9 it was inactivated in 7 minutes. This showed that pH had marked effect on the inactivation process by p.m.n., the bactericidal activity increasing with rise in pH. The lower concentrations took more time for complete sterilization. The concentration exponent of p.m.n. was about 1.

Similar studies were also carried out using the same concentrations of p.m.n. with *S. paratyphi B*, at room temperature at 3 pH levels as well as at 4°C but only at pH 7.5. In contrast to *S. typhi*, p.m.n. showed a decreasing bactericidal activity with increasing pH. The time required for complete sterilization at a concentration of 20 mg/100 ml of p.m.n. was 17.5 minutes at pH 5.5, while at pH 9, it took 195 minutes for complete inactivation. The time required for complete sterilization also, increased with lowering of concentration of p.m.n. at all pH levels. Another important finding observed in the case of *S. paratyphi B* was that rate of inactivation was not logarithmic. An initial lag in the inactivation process was observed at alkaline pH levels, viz. 7.5 and 9, while no such lag was observed at acid pH, viz. 5.5. Concentration coefficient of *S. paratyphi B* was also about 1. The inactivation process at 4°C at the concentrations used, viz. 20 mg/100 ml to 5 mg/100 ml was considerably slow at pH 7.5. Fifty-seven and half hours were required for complete sterilization of culture at 4°C as against 37.5 minutes required at room temperature.

Similar studies were also carried out in the case of *S. paratyphi B* using formalin as a bactericidal agent. At pH 7.5, 0.2 per cent formalin required 40 hours for complete inactivation of the culture as against 2 hours taken at room temperature, viz. 29°C. In addition to the above studies, experiments on absorption of formalin by the two organisms mentioned above were also carried out at pH 5.5, 7.5 and 9 using different bacterial concentrations as well as different concentrations of formalin. It was observed that the amount of formalin adsorbed was directly proportional to the bacterial concentration, while it was independent of the amount of formalin added.

The amount of formalin absorbed, increased with the increase of pH in the case of *S. typhi*, while in the case of *S. paratyphi B* at pH 5.5 practically no absorption of formalin was observed though the organisms were killed by formalin. This suggests a different mechanism of action at this pH level.

At alkaline pH levels, *viz.* 7.5 and 9, absorption of formalin by the cells was observed. It increased with pH and duration of contact. These results, clearly bring out the difference in action of formalin on the two organisms.

Storage stability of typhoid vaccine at high altitudes

Bacterial vaccines are usually stored between 2°C and 10°C and not allowed to freeze. However, at high altitudes, where atmospheric temperature is below 0°C, the vaccines freeze under normal conditions of storage. It is generally believed that bacterial vaccines, if allowed to freeze or if subjected to repeated freezing and thawing, deteriorate in their protective efficacy and also show changes in their physical characters. The defence establishments stationed at places of high altitudes are faced with the problem of storing the vaccines without freezing. This investigation was, therefore, undertaken to study, in detail, the effect of freezing and thawing on vaccines.

During the year under review, the following investigations were carried out. Initially, a formalized T. A. B. vaccine preserved with phenyl mercuric nitrate was stored in a deepfreeze at—35°C for different periods. The immunogenicity of the samples of vaccines stored for different periods was compared with that of the same batch of vaccine stored at 4°C in a refrigerator. Similar experiments were also carried out on a batch of phenol-killed and phenol-preserved vaccine.

In another set of experiments, a batch of formalin-killed and phenyl mercuric nitrate (p. m. n.) preserved vaccine and a batch of heat-killed and phenol-preserved vaccine were stored at—35°C. They were thawed and refrozen six times at weekly intervals. After every thaw, both the vaccines were subjected to potency test by mouse protection test along with the sample of the same batch stored at 4°C. The results of these experiments are summarized in the Tables 3, 4 and 5.

It will be seen that formalised vaccine stored at—35°C for one year did not show any fall in potency by the mouse protection test as compared to the same batch stored without freezing at 4°C. The phenolised vaccine also did not show any deterioration in potency for three months for which period the tests were completed. The agglutinin response in adult rabbits to the formalized vaccine stored at—35°C up to six months remained unaltered. In fact, the results showed that the Vi antigen of the vaccine stored at—35°C was better preserved, as it produced significantly higher response as compared to that given by the same batch of a vaccine stored, at 4°C.

The results presented in Table show that there was no deterioration in potency of T. A. B. vaccine, either formalized and p. m. n. preserved or heat-killed and phenol-preserved, on repeated freezing and thawing for six times. However, in the case of heat-killed and phenol-preserved vaccines, it was observed that the vaccine showed some granulation after the third thaw. Potency tests were carried out on the same vaccine with granules suspended. It would be interesting to study the effect of the granules on the potency of the vaccine.

TABLE 3

Effect on potency of T. A. B. vaccine on storage at 35°C and at 4°C

Period of storage		ED 50 of formalized T. A. B. vaccine stored at -35°C and at 4°C with their relative potency			ED 50 of phenolised T. A. B. vaccine stored at -35°C and at 4°C with their relative potency		
		-35°C	4°C	Relative potency	-35°C	4°C	Relative potency
2 days	..				0.0103	0.0076	0.74
2 weeks	..	0.0104	0.0145	1.39	0.0129	0.0146	1.13
8 weeks	..	0.0173	0.0188	1.09	0.0019	0.0037	1.90
6. months	..	0.0123	0.164	1.33			
9 months	..	0.0118	0.0148	1.25			
12 months	..	0.0147	0.0148	1.01			

Potency stored at 4° C was taken as 1 for determination of relative potency

TABLE 4

Agglutinin response in rabbits to formalised vaccine after storage at -35° C and at 4° C

Vaccine used	Temp. of storage	Geometric mean titres against different antigens									
		Vi		O		H		Para A		Para B	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
13 B	.. -35°C	20	201	16	1613	31	1613	10	1016	10	508
13 B	.. 4°C	10	40	10	640	10	806	10	320	10	640

TABLE 5

Effect of repeated freezing and thawing on the potency of the vaccine

Relative potency of the formalised and p. m. n. preserved vaccine after each thawing						Relative potency of the heat-killed and phenol-preserved vaccine after each thawing					
Serial Number of thaw						Serial Number of thaw					
1	2	3	4	5	6	1	2	3	4	5	6
0.88	1.17	1.22	0.85	1.25	1.25	1.18	1.09	0.95	1.46	1.10	1.70

Potency of the same batch stored at 4° C was taken as 1 for determination of relative potency.

Potency of the same batch stored at 4°C was taken as 1 for determination of relative potency.

DEPARTMENT OF BLOOD BANK

Studies on genetical characters in some endogamous groups in Indian populations

This work was undertaken to study the distribution of genetical characters in 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. As compared to the data available in other countries, the data available in this country is rather meagre.

A study of blood and serum groups was undertaken on some Muslim endogamous groups of Bombay together with other genetic markers viz., colour blindness, ability to taste phenyl thiocarbamide, abnormal haemoglobulins and G-6-PD deficiency. The blood groups studied were the ABO, Rh (C, c, D, E, e), MNSSs, Duffy, Kidd, Diego and Kell system (Cellano, Penny, Sutler). The serum groups studied were the haptoglobins and transferrins.

This study, comprising of 5 endogamous groups of Muslims, viz., Khojas, Bohoras, Moplas, Misgar and Random Muslim group, was completed. Some interesting results were seen. G-6-PD deficiency ranged from 0 to 2 per cent. One male Khoja had haemoglobin A + D. One male Khoja had a variant of the N antigen. There was a higher percentage of A₂ blood in the Misgar group, three males of the 153 Misgars, 1 male of Khoja. Of the 200 mixed random muslims tested, three were found to be colour blind in the areas of green and red.

Bhanushali group.—This group (600 people) was investigated in detail for haemoglobinopathies, G-6-PD deficiency, haptoglobins and transferrins. There was a high incidence of the enzyme deficiency (12 per cent.) Hp frequency was higher as compared to the population groups in Maharashtra and Gujarat.

Reference work

Seventy cases of neonatal jaundice were referred for the red cell G-6-PD deficiency. Four male babies were found to be deficient.

Twentyfive cases of haemolytic anaemic were referred for enzyme studies. Four were found to be deficient in G-6-PD.

Eight cases of multiple myeloma were referred for the globulin typing. Two of these were found to have monoclonal IgM (Waldenström's macroglobulinemia). Remaining five showed the IgG specificity and one showed the paraprotein in X₂ region.

Development of rare blood grouping reagents

Rare blood grouping reagents like Anti-Rh and Coomb's reagents which were being imported so far, have now been developed by this Department and

are being supplied regularly. Rarer anti-Rh sub-grouping sera like Anti-e, Anti-E, Anti-c and Anti-C are still not being prepared here. Attempts to produce these sera here were undertaken. Four donors of R_1R_1 type were being immunized with R_2R_2 blood with the intention of preparing Anti-E serum. Each donor was given 8 injections up till now, but none had so far produced the antibody. As E was a poor antigen, longer courses of immunization would be needed.

Bacterial contamination of 'banked' blood

This work was undertaken to minimise the wastage of blood collected by the blood banks. During the last two national emergencies when considerable quantities of blood were collected by mobile units by outside agencies, they were found to be heavily contaminated. The blood collected at institutional centres was satisfactory, whereas that collected in mobile vans, especially long distances away, was affected. The major contaminants were *Staphylococci* and *E.coli*. Antibiotic sensitivity test of the organisms isolated was carried out and it was found that chloromycetin was the most effective antibiotic. Chloromycetin was, therefore, added to the A.C.D. solution in a strength of 1 mg per ml before autoclaving. It was found that 30 per cent. of antibiotic was lost during autoclaving. Blood was collected in this solution in outstation centres. Sterility tests were carried out on all the blood bottles which were found to be sterile. Plasma was separated and the amount of antibiotic in the plasma was estimated before and after lyophilization. It was observed that the lyophilization did not affect the chloramphenicol content in the plasma.

It is found that plasma contained less than 10 mg percent of chloramphenicol and 24 mg of chloramphenicol was adsorbed by the blood cells, as it was less soluble in water.

The addition of antibiotic in the preserving anticoagulant solution considerably brought down the percentage of contamination. However, its use as a routine was not advocated due to chances of sensitization and anaphylactic reactions.

Haemolysis of blood

Haemolysis observed in blood bottles was studied to find out its causes in respect of (a) temperature of storage, (b) transport of blood, (c) number of days of storage of blood, etc. The haemoglobin percentage in fresh plasma and those collected at different centres was determined colorimetrically. The haemoglobin content of the plasma of blood collected at Haffkine Institute Blood Bank was 2 mg per cent, while that in other centres in Bombay city was 11.75 mg. per cent and that in centres outside Bombay city was 17.03 per cent.

Different methods were used for collection, such as slow cooling, centrifuging of blood bottles, separation of plasma before diffusion of haemoglobin, keeping exact percentage of dextrose in A.C.D. It was found that quick transportation, slow cooling and immediate separation of blood minimised haemolysis.

These studies helped to reduce chance contamination and haemolysis in the routine blood collection and plasma processing.

Studies on normal human immunoglobulins

Human gamma globulins are being produced for clinical use for the first time in our country. It was, therefore, advisable to study and characterise the product in all parameters. World Health Organization Expert Group on Immunoglobulins have opined that "It is possible that human immunoglobulins from certain geographical areas might prove to be rich in particular antibodies for which a demand exists elsewhere. Information, is therefore, required concerning the amount of various antibodies to be found in immunoglobulin preparations from different parts of the world".

The gamma globulin prepared at this Institute was tested for the presence of IgG and IgM (qualitatively), by gel diffusion technique using the specific antisera obtained through the courtesy of Dr. H. W. Krijnen, Central Blood Transfusion Laboratory, Amsterdam. Some batches showed presence of traces of IgA and IgM; the major portion was IgG.

Ten batches of the gamma globulin were referred to World Health Organization, International Reference Centre for Immunoglobulins at Lausanne, Switzerland. Their report confirmed the finding that the immunoglobulins in these preparations were probably somewhat denatured as compared with immunoglobulins in normal serum but the results obtained were similar to those which were reported for commercial batches of gamma globulins.

Gamma globulins were studied for their antibody level against some of the common infective agents. So far, they were screened for the presence of anti-polio, anti-diphtheria, anti-tetanus, anti-streptolysin 'O', small-pox, measles and typhoid antibodies. They show good concentration of polio antibodies and antistreptolysin (ten times concentrated than original plasma pools).

These gamma globulins were tried clinically. The reports so far received showed good prophylactic value against cases of infective hepatitis, serum jaundice (post-transfusion cases) and measles.

Further studies on immunoglobulins

Preparative starch block electrophoresis method had been standardized to isolate immunologically pure immunoglobulins. Plasma from a case of multiple myeloma was collected in a large quantity. Immunologically, pure IgG was isolated from this plasma by the above method for the preparation of specific antisera.

The preparation of Anti-IgG serum in rabbits has been started using locally isolated IgG. Similarly, Anti-IgA and Anti IgM will be prepared.

Experiments are being carried out for the isolation of IgA and IgM by using preparative starch block electrophoresis, column chromatography and gel filtration, using DEAE cellulose and Sephadex-200.

Specific immunoglobulins. (anti-tetanus)

Immunoglobulins separated from plasma of donors specifically immunised against particular infections have established themselves as safe remedies in

the treatment of those dreaded infections. Their main advantage over the anti-sera derived from horse serum is the freedom from reactions, both anaphylactic and serum sickness type and the low dosage needed to treat cases as also prolonged maintenance of circulating antibodies in the patients. For this purpose, batches of prisoners at Nasik and Yerwada Prisons were selected and they were being immunized by giving them absorbed tetanus toxoid injections. These donors, when fully immunized, will be bled and from their plasma specific tetanus immunoglobulins will be isolated.

Anti-Rh (Anti-D) immunoglobulins

Recent development in the field of prevention of Rh isoimmunisation of women is the administration of Rh immunoglobulin IgG (Anti-D) to the vulnerable women before isoimmunization takes place. These immunoglobulins are prepared from high-titre Anti-D sera obtained from naturally immunized donors and deliberately hyperimmunised donors. In our country, high titre sera from naturally immunised donors are not available in large quantities. Hence, we had to use sera from a hyperimmunised donors. Here also, pure Anti-D sera was not available, so mixed sera like C+D had to be utilised.

A batch of Rh immunoglobulins (Anti-D) was processed from Anti-Rh sera obtained from deliberately hyperimmunised blood donors. This sample showed fairly pure IgG.

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.

Cryoprecipitates from fresh human plasma, were processed from the freshly collected plasma. The method described by D. L. Brown was followed, but siliconised bottles are used in place of plastic bags as the latter were still not available in the country. The precipitates were clinically used in a few cases at the K.E.M. Hospital, Bombay. In these cases, there was very good response (Anti-haemophilic globulin level rose from 15% to 85% of normal).

In order to preserve cryoprecipitates over longer periods, they were lyophilised. The results showed that lyophilised cryoprecipitates were as effective as frozen ones.

Tissue banking

Tissue banking technology is being developed to make available to the surgeons different human tissues for replacement surgery in a freeze-dried form.

Surgeons were interested in preserving ossicles (middle ear bones) removed from cadavers and preserved in antibiotic fluid and taken up for freeze-drying. They were freeze-dried under varying conditions and lengths of times, to find the optimum conditions for drying these bones. Each charge was subject

to different conditions of freeze-drying. The samples were studied histologically for the structural variation in bones. So far, 5 batches have been processed on these lines. A satisfactory method of preservation is still to be worked out.

Blood group substances from different sources

Blood group substances are needed in Blood Bank operations. They were being imported all these years. The following substances were being processed here from muconium and blood donors were immunised.

" A " group substance	..	..	..	442 donors
" B " group substance	..	..	..	360 donors
" AB " group substance	...	..	..	40 donors

Muconium samples were collected, pooled and the blood group substances were processed and used for immunisation of donors.

The responses were quite satisfactory and high-titre grouping sera were obtained.

TABLE 6

Mean values of antibody levels in 50 batches of pooled plasma and 50 batches of gamma globulin

Sample	Tetanus	Diphtheria	Typh	Antistreptolysin	INFULUENZA		Measles	Small-pox	POLIOVIRUS		
					A ₂ India 47/57	A ₂ Taiwan/ 64			Type I	Type II	Type III
Pooled Plasma	Insignificant	Do.	Do.	125	16	64	128	64	316	316	126
Gamma globulin	0.01 IU/	0.1 IU ml	50	1,250	64	256	640	256	3,162	3,162	562
Gamma globulin prepared in Russia and Amsterdam	10.8 IU/ml	5.33 IU/ml	50	1,250		128	640	512	1,600	1,600	560

DEPARTMENT OF CHEMOTHERAPY

Synthesis and *in vitro* antitubercular and antifungal activity of styryl aryl ketones of 5-bromo and 5-Nitro-salicylaldehydes and their thiosemicarbazones

It has been reported in the literature that α , β -unsaturated ketonic compounds exhibited bacteriostatic and fungistatic action, and also that this action might be due to the presence of $\text{CH}=\text{C}-\text{CO}$ -system. Further, it has been shown that this action was associated with the tendency of substituents to withdraw electrons from the double bond. In the present work, it was thought worthwhile to study the compounds containing $\text{CH}=\text{C}-\text{CO}$ -in place of azomethine ($-\text{N}=\text{CH}-$) which was present in the thiosemicarbazone and hydrozone series studied by us earlier. Accordingly, styryl aryl ketones of 5-bromo-, and 5-nitrosalicylaldehydes as well as their thiosemicarbazones were prepared to evaluate their antifungal and antitubercular activity. In all 28 compounds have been synthesised and tested *in vitro* for their antifungal and antitubercular activity. Many of these compounds have shown antifungal activity in the range of 1-5 $\mu\text{g}/\text{ml}$ and antitubercular activity in the range of 5-20 $\mu\text{g}/\text{ml}$.

Synthesis and *in vitro* antitubercular and antifungal activity of N¹-Salicyloyl (or 5-Bromosalicyloyl) N⁴-substituted piperazines

Derivatives of salicylanilide have been finding increasing mention in the literature and some of the substituted salicylanilides are currently in use for the treatment of skin disorders due to bacterial or fungal infection. Literature survey also shows that piperazine derivatives have manifested a wide range of antibacterial and antifungal activity. In the present work, it was planned to modify the structure of salicylanilide and 5-bromosalicylanilide by replacing the amine moiety with substituted piperazines. Accordingly, 16 compounds were synthesised and tested for *in vitro* antitubercular and antifungal activity. None of the compounds could exhibit any significant activity against the test organism.

Synthesis and *in vitro* antitubercular and antifungal activity of N¹-(5-bromo-salicylyl ethyl or methyl) N⁴-substituted piperazines

Mannich bases have shown interesting biological results in the field of medicinal chemistry and many compounds besides their CNS depressant activity and antihistaminic activity, have also exhibited antimicrobial properties. Some of the ketonic Mannich bases, having piperazine moiety, prepared in our laboratory for the evaluation of CNS depressant activity, have also shown good antitubercular and antifungal action. This observation prompted us to synthesize Mannich bases from 5-bromo-2-hydroxyacetophenone and different N⁴-substituted piperazine. Most of the compounds showed antitubercular action in the range of 20-100 $\mu\text{g}/\text{ml}$ and antifungal activity in 4-100 $\mu\text{g}/\text{ml}$ range.

Synthesis and study of N¹, N⁴-disubstituted piperazine derivatives

Over 50 new compounds like N¹-[β-(p-chlorophenyl-benzylamino) ethyl-N⁴-(substituted) piperazines and N-[β-(1, 2-diphenyl ethylamino)ethyl]-N⁴ (substituted) piperazines were synthesized and studied *in vitro* for anti-tubercular activity. Eight compounds were found to be active at 5 μg/ml and nine at 20 μg/ml.

Synthesis of N¹[β-Phenyl-β(p-substituted benzoyl) ethyl-N⁴-(substituted) piperazines.

The mannich bases containing substituted piperazines were synthesised and screened for *in vitro* anti-tubercular and anti-fungal action. Out of the 40 compounds tested, six were found to be active at 5 μg/ml and seven at 10 μg/ml against H₃₇Rv strain of *M. tuberculosis*. Five compounds from this series were also active against *T. rubrum* and *T. mentagrophytes* at a concentration of 2 μg/ml, 5 at 5 μg/ml and 18 compounds were active at 10 μg/ml.

Schiff bases from benzaldehyde nitrogen mustards and amino phenyl thiazoles

One of the approaches to better chemotherapeutic agents against cancer is to have compounds with "latent activity", so that they are not toxic *per se* but get activated at the tumor sites by the action of specific enzymes with which the tumor tissues are rich.

The work in the department has been directed towards this end by the preparation of Schiff bases of nitrogen mustards, which would liberate the free alkylating agent at the site of action.

Over 100 Schiff bases from different benzaldehyde nitrogen mustards and amines such as 4-(p-aminophenyl)-2, 5-disubstituted thiazoles and 4-[4'-amino-2'-chlorophenyl]-2-substituted thiazoles have been synthesised and representative compounds screened by the C.C.N.S.C., Bethesda, U.S.A., for anti-tumor activity. Many of the compounds displayed significant activity against L 1210 lymphoid leukemia, Walker 256 (intramuscular) and Dunning leukemia (solid). [2,5-Dimethyl-4-[p-[4-[N, N-bis (2-chloroethyl) amino]-3-methoxybenzylidene] amino]-2-chlorophenyl] thiazole, 2-benzyl-4-[p-[4-[N, N-bis(2-chloroethyl) amino]-2-methyl-benzylidene] amino]-2-chlorophenyl] thiazole, 2-benzyl-4-[p-[4-[N,N-bis (2-chloroethyl) amino]-2-methylbenzylidene] amino] phenyl] thiazole and 2-(p-methoxy-benzyl)-4-[p-[4-(N, N-bis (2-chloroethyl) amino] benzylidene] amino] phenyl] thiazole were the most active compounds among those screened in the present series.

Schiff bases from benzaldehyde nitrogen mustards and amino phenyl thiazoles

Several Schiff bases from different benzaldehyde nitrogen mustards and 2-phenyl-4-[(3'-amino-4'-methoxy)phenyl] thiazole have been synthesised and studied for anti-tumor activity. One of the compounds designated as R-1492 showed very good activity with high margin of safety against experimental animal tumor, viz. lymphoid leukemia. The following Table No. 7 gives activity data of R-1492 against L 1210 (Lymphoid leukemia).

TABLE 7

Lymphoid leukemia

Compound	Lowest active dose (mg/kg)	Survivors	LD ₅₀ mg /kg
R-1492	2.2	6/6	42

5-Aryl-4-alkyl-3-mercapto-1,2,4(H) triazoles as hypoglycaemic agents

Earlier work on this class of compounds was concerned with the possible diuretic activity. However, random screening of some of the compounds from this series revealed them to possess interesting hypoglycaemic activity. It was, therefore, thought worthwhile to extend this series further in search for better hypoglycaemic agents. Over eighty new 3, 4, 5-trisubstituted 1, 2, 4 (H) triazoles have been synthesised and studied.

Majority of the compounds have shown significant hypoglycaemic action. Two among them, 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 were the most active and exhibited a long duration of action showing blood sugar lowering activity both in normal and alloxan diabetic rats. They had also a very low order of toxicity in mice and were devoid of any anti-bacterial action.

Synthesis and hypoglycaemic activity of 5(N-alkyl-ureidosulphamonylphenyl)-4-alkyl-4H-1, 2, 4-triazole-3-thiols.

Since 5-p-sulphomylphenyl-4-ethyl-4H-1, 2, 4-triazole-3-thiol possessed pronounced and prolonged hypoglycaemic action in experimental animals, a series of 5(N-alkyl-ureidosulphomylphenyl)-4-alkyl-4H-1, 2, 4-triazole-3-thiol were prepared and studied. The introduction of ureido moiety did not enhance the activity.

Synthesis and study of 3-aryl (or pyridyl)-5-alkyl (or aryl) amino-1, 3, 4-thiadiazoles as possible hypoglycaemic agents.

During our study of hypoglycaemic compounds, we found that thiosemicarbazides, corresponding to two very active triazoles, were also hypoglycaemically active. This led us to synthesise the corresponding and some more thiadiazole derivatives for screening for blood sugar lowering property. Thirty-seven representative 2-aryl-5-alkyl (or aryl) amino-1, 3, 4-thiadiazoles have been tested and four of them were observed to be active even at the end of 24 hours. These compounds are being studied in greater details.

DEPARTMENT OF CLINICAL PATHOLOGY

Immunological slide test for pregnancy-Latex agglutination inhibition test (Gravindex) compared with rat ovarian hyperaemia test.

The Gravindex test was found to be very accurate in the detection of normal pregnancy and was more sensitive than the rat test.

Quantitative estimation of chorionic gonadotropin in urine

The hormone was assayed in the urine of thirty-seven normal and eighteen abnormal pregnancies. The quantitative estimation was found useful in the prognosis of cases of threatened abortion. It is also possible to predict the course that the pregnancy will take. Thus one can employ this test for the diagnosis of certain types of abnormal pregnancies and institute the indicated therapy in time.

Toxigeny and phage typing of *C. diphtheriae*

About 80 strains of *C. diphtheriae* were isolated from suspected patients. They were tested biochemically for the differentiation of gravis, mitis and intermedius. Mitis were 64 per cent, gravis 34 per cent and intermedius were 2 per cent. The strains were then subjected to toxigenicity test, both by Eleck's plate method and rabbit intracutaneous test. Ninety-three per cent of the strains were found to be toxigenic by both the methods. The strains were subjected to phage typing. About 84 per cent strains belonged to type No. 19 and 4 per cent showed a phage pattern, while the remaining 12 per cent strains were untypable. Thus No. 19 phage was found to be prevalent.

Carrier studies and studies on immunization status of contacts (under 10 years of age) of diphtheria cases

These studies were undertaken with the object of determining the level of immunity against diphtheria in contacts of diphtheria cases.

Throat swab examinations of 400 school children and contacts of patients were done to find out carriers amongst them, which revealed 3 per cent carriers. Schick test was performed on 100 non-immunized children under 10 years of age, of whom only 6 per cent were Schick positive.

Studies in leptospirosis

The study on leptospirosis has been continued during the year. Two hundred sera from human beings and animals were screened for leptospirosis. These sera were subjected to the microscopic agglutination test and the sensitized erythrocytelysis test. There was good correlation between the two tests. The percentage of infection among dogs (31 per cent) and aborted cases of sheep, cattle and goat (28 per cent) were found to be high.

Studies on beta-haemolytic streptococci of Group A

The study of beta-haemolytic streptococci group A was continued. Three hundred throat swabs and 101 skin swabs were collected from cases of scabies. Sixty-nine of the throat swabs were of beta-haemolytic streptococci, 15 out of which belonged to the group A streptococci. Fifty-five of the scabies cases were of beta-haemolytic streptococci, out of which 42 belonged to the group A streptococci.

Studies on mycophages and drug resistance in mycobacteria

In continuation of the work reported earlier, the effect of dimethylsulfoxide (DMSO) and bacitracin on the drug resistant pattern of mycobacteria was studied. It was observed that the incorporation of bacitracin along with DMSO further decreased the resistance of mycobacteria to drugs. This showed that the resistance might be due to the lesser penetration of the drug through the cell wall.

Seven more mycophages, supplied by Dr. Froman, were tested on mycobacteria. About 40 strains of mycobacteria were phage-typed. The lysogenic strains obtained were studied in detail for their biochemical and biological characteristics. Deoxyribonucleic acid (DNA) preparation with reference to drug resistance marker is in progress. Effect of kanamycin sulphate and mitomycin C on the phage induction from the lysogenic strains was studied. Experiments were also put up for studying the effect of rifampicin on mycobacteria.

Bacteriological study of acute gastro-enteritis in infants and children

This investigation was undertaken to find out the various bacteriological agents incriminated in acute gastro-enteritis cases in infants and children. Rectal swabs or stool samples were obtained from Sion Hospital and from Bai Jerbai Wadia Children's Hospital. A total of 172 cases were investigated during the year 1969.

E.coli was the predominant organism isolated, either present alone and/or in combination with other organisms. The anti-biogram pattern of all the isolated strains in the order of sensitivity sequence was as follows: (i) Neomycin, (ii) Paromomycin, (iii) Furaxone, (iv) Chloromycetin, (v) Streptomycin and (vi) Sulphadimidine.

E.coli strains were further serotyped against 15 pathogenic *E.coli* 'O' type sera. Types O126, O119, O111, O86 and O127 were the commonest serotypes encountered.

Bacteria isolated from sweetmeats in Bombay

One hundred samples of sweetmeats were analysed. The most frequent micro-organisms isolated were *Staphylococcus aureus*, *Streptococcus faecalis* and *E.coli*, followed by *Kl.pneumoniae*, *Aerobacter aerogenes*, *Ps. aeruginosa*, *trep. viridans*, *Staph. albus*, *Staph. citreus*, spore bearers and yeast too were noted amongst the contaminants. The results revealed lack of hygienic conditions in the sweetmeats sold in Grade I and Grade II establishments in Bombay.

DIRECTOR'S LABORATORY

Experimental Amoebiasis

Since the introduction of emetine as an antiamoebic agent in the therapy of amoebiasis, many synthetic compounds and antibiotics have been tried to cure amoebic infection. For the experimental study of amoebiasis, a successful cultivation of *Entamoeba histolytica*, the causative organism of the disease *in vitro* was necessary.

In the present work, a study was made of the antiamoebic value of different chemotherapeutic agents mainly extracts from different medicinal plants. The *in vitro* testing of the compounds was done using a monophasic medium. Golden hamsters and albino rats were the two species of animals used for the study of *in vivo* activity of the compounds. Hamsters were infected intrahepatically and rats intracaecally. Different fractions and compounds isolated from the following medicinal plants were studied :

1. *Mesua ferrea* (Nagkesar)
2. *Nyctanthes arbortristis* (Parijita)
3. *Asparagus racemosus* (Satavari)
4. *Aconitum heterophyllum* (Ativisha)
5. *Piper longum*

Of all the plants tested, only three fractions, namely glycoside fraction of *Asparagus racemosus*, an alkaloid from *Piper longum* and alcoholic extract from *Nyctanthes arbortristis* showed activity *in vitro*. Also, of the 19 synthetic compounds tested *in vitro*, none was found to be active.

Indigenous drugs in experimental cholera

In infant rabbit model for experimental cholera, the following drugs were studied to see the anti-cholera effect :

- (1) *Cynodon dactylon* (whole shrub)
 - (a) Crude alcoholic extract (water soluble fraction)
 - (b) Alkaloidal fraction
 - (c) Glycosidal fraction
 - (d) Gluco-alkaloidal fraction
- (2) *Asparagus racemosus* (root)
 - (a) Crude alcoholic extract
 - (b) Glycosidal fraction
- (3) *Nyctanthes arbortristis* (leaf)
 - (a) Crude alcoholic extract
 - (b) Alkaloidal fraction

- (4) *Mesua ferra* (flower, seed, kernel, bark)
 - (1) Chloroform extract
 - (2) Methanol extract of flower
 - (3) Petroleum-ether extract of bark
 - (4) Alcoholic extract of bark
 - (5) Ether extract of flower
 - (6) Water soluble fraction of alcoholic extract of seed
 - (7) Water soluble fraction of alcoholic extract of kernel of seed
 - (8) Mesuol (Sol. in acetone and alcohol)
 - (9) Acetone extract of flower
 - (10) Crystalline compound
 - (11) Water soluble fraction of alcoholic extract of outer part of fruit
- (5) *Ensete superbum* (*Banakadali* seed)
 - (1) VIDR-2GD fraction
- (6) *Adhatoda vasica* (*Vasaka*)
 - (1) Vasicine
 - (2) Vasicine hydrochloride
 - (3) Vasicinone
 - (4) Vasicinone hydrochloride
- (7) *Aconitum heterophyllum* (*Ativisha*)
 - (1) Crude alcoholic extract
 - (2) Alkaloidal fraction (water soluble)
 - (3) Alkaloidal fraction (water insoluble)

Out of these drugs, none showed any anticholera activity to the significant extent. VIDR-2GD delayed onset of diarrhoea by few hours and prolonged survival period.

VIDR-2T

Pharmacological studies on VIDR-2T, fraction isolated from wild *Banakadali* seed.

This fraction possesses a good antifertility action, Its toxicological and pharmacological studies are in progress.

Five mg of VIDR-2T did not have any effect on the frog's isolated heart. In anaesthetised cat, 5 mg (2 mg/kg) of the drug produced significant transient hypotension, but the same dose could not check the action of 0.1 mg adrenaline when given concurrently. After adrenaline, the drug sensitivity upto 5 mg was reduced. After atropine sulphate (1 mg/kg), 5 mg drug produced significant transient hypertension which indicated that it caused hypotension by stimulating the parasympathetic nerve ending and hypertension might be due to direct constrictive action on blood vessels showing vasomotor reversal like effect.

Ileum part of intestine of rat showed increase in amplitude of contraction with 10 mcg/ml. Adrenaline caused relaxation after 10 and 20 mcg/ml of the drug. After atropination, 30-60 mcg/ml did not show any effect. Ileum of guinea-pig also showed increase in amplitude and motility with 45 mcg/ml and it retarded the peristaltic movement with 40 mcg/ml. Guinea-pig uterus did not produce any effect with 40 mcg/ml. The drug showed antihistaminic effect on bronchial musculature of guinea pig when 6 mg VIDR-2T and 5 mcg of histamine were administered concurrently. But when the drug was given alone at the same dose, bronchial musculature did not show any significant changes.

***Aconitum heterophyllum* (Ativisha)**

An alkaloid fraction isolated from *Aconitum heterophyllum* (Ativisha), which is soluble in water, produced significant hypertensive effect with a dose of 3 mg/kg when given intravenously in anaesthetised cat but 1.5 mg/kg caused elevation which was not significant.

DEPARTMENT OF IMMUNOLOGY

Stability of purified antitoxic sera

It is known that enzyme-refined and purified antitoxic serum deteriorates in its antitoxic potency even when stored between 2°C to 5°C. The deterioration is much faster at higher temperatures. The exact reason for this deterioration is not known. A number of factors were, therefore, examined for their effect in either stabilizing or accelerating the deterioration of antitoxic sera.

The effects of heat, various antiseptics, amino acids, salts etc. were tested. Preliminary results showed that these factors had no effect in accelerating the loss of potency during storage at 2-5°C and at 37°C as compared with controls. An interesting result was that pepsin-treated antisera could stand heating at 60°C for 10 hours without showing any decline in potency as assayed by neutralisation tests using animals. The antiseptics used, e. g. 0.25 per cent phenol, 0.01 per cent merthiolate and 0.1 per cent PCMC did not have any effect on the rate of deterioration of antibody activity. Preliminary results showed that proteolytic enzymes added to antisera caused deterioration of antibody activity. These experiments will be repeated for confirmation.

Insolubilisation of pepsin

The use of insolubilised pepsin for the digestion of proteins has a number of advantages. It does not contaminate the system as it could be removed by centrifugation or filtration. The reaction can be stopped at any stage by simply separating it. It could be used over again if still active. There will be no antigens contributed by pepsin which may cause sensitization, fever or other untoward effects. It is also likely that any deterioration in antibody activity of stored antisera, if due to residual pepsin, would be eliminated.

In preliminary experiments, a number of methods and reagents were tested for polymerisation of pepsin with a view to insolubilise it. The results were all unsuccessful. Ethyl-chloroformate, which has been used at Pasteur Institute, Paris, to insolubilise many antigens, did not show a precipitate with pepsin. After a large number of failures with this reagent, finally this reagent did show a precipitate when pepsin was copolymerised in the presence of other proteins such as gelatin or in presence of amino acids such as lysine or glutamic acid. Even here, the results were variable. The product obtained in some experiments showed activity, while others did not. It was found that factors such as initial pH, amount of reagents added, time and temperature of reaction etc. exerted their effects on the enzyme activity of the product.

A sample of insolubilised pepsin was used in wet condition to see whether it acted on horse anti-toxic plasma. The results showed that it was active and was able to degrade antibody proteins in horse sera. Further work is in progress to improve the conditions for the insolubilisation of pepsin to get

consistently active products. When stored in cold in a wet condition, the activity of the insoluble pepsin was stable for 6 months. Study of the effect of longer storage is underway.

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

The purpose of the work was to fractionate Russell's viper venom using mild physical methods and to isolate and purify the biologically important factors such as coagulase, L-amino acid oxidase, skin necrotising factor, protease etc. The purified factors could then be taken up for study of their chemical nature and mode of action. It is likely that some of them will have value as laboratory reagents or in therapy. Our previous work had shown that Russell's viper venom could be separated using Sephadex gel filtration technique. However, this procedure gave very dilute solutions. In order to avoid this difficulty, the use of starch block electrophoresis was tried.

In the present work, the fractionation of the Russell's viper venom was carried out using starch block preparative electrophoresis method. In this way, 10 gms. of venom was separated into 5 fractions. These fractions were tested individually for their activity. It was found that the proteolytic activity was distributed in fractions II, III and IV and it seemed that the venom had two or more proteolytic enzymes. L-amino acid oxidase and coagulase were present mainly in fraction III. Phospholipase A was present in fraction IV. Fraction IV was then taken up for further purification by using gel filtration technique on Sephadex G-75. By this method, it was possible to separate the phospholipase A from the proteolytic enzyme. The work of separation and examination of other fractions for their biological activity is in progress.

Studies in tetanus toxin production

The production of tetanus toxin in protein-free medium based on tryptic digest of casein (Tryptone-T-Oxoid) seems to be governed by a number of empirical factors such as: (i) the batch of tryptone-T used, (ii) its purification by calcium phosphate precipitation, (iii) colour changes in medium during growth, (iv) presence of iron as iron powder or as $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, (v) concentration of cystine in relation to glucose and (vi) time and temperature of sterilization cycle. The potency of toxin produced in this medium was, therefore, variable. The aim of the work was to avoid the uncertainties of the Massachusetts medium by substituting the tryptic digest of casein with acid hydrolysate of casein (Casamino acids-Difco), which is more constant in composition.

In our previous experiments, it was found that the Harvard strain of *Clostridium tetani* could grow in deferrated Casamino acid medium supplemented with yeast dialysate, cystine, tryptophan and salts. The yeast dialysate could be replaced with thiamine, paraaminobenzoic acid, biotin, vitamin B12, pyridoxine, riboflavine, nicotinic acid and pantothenic acid. The optimum amount of these vitamins for maximum toxin production was then estimated. There was a consistent stimulation of toxin production when 0.01 per cent of KCl was added to the medium. The results were used to improve the Massa-

chusetts medium. The toxin titre has now improved from about 15-20 Lf/ml to about 30-40 Lf/ml.

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

The use of adjuvants, which enhance antibody production, is of great importance in the commercial production of antisera. Similarly, immunosuppressors are of great value in inhibiting antibody production during organ transplantation and in auto-immune diseases. Diphtheria toxoid is a poor antigen and it was, therefore, used for testing adjuvants and immunosuppressors.

Previous work had shown that Freund's complete adjuvant was a powerful adjuvant, while Millicorten (Ciba) acted as an immunosuppressor. Different adjuvants and immunosuppressors were tested in groups of about 10 rabbits each of which received three injections of 50 Lf of diphtheria toxoid at intervals of 2 weeks. The drugs were administered either along with the diphtheria toxoid or separately. The control group received diphtheria toxoid alone by intramuscular injection. The highest antibody level in the sera of the control rabbits was about 9.7 units/ml, which was reached 7 days after the 3rd injection (i.e. 37 days after the first injection). It was found that there was no significant difference when the toxoid was given intraperitoneally. Oil emulsion adjuvant by intramuscular injection with paraffin oil, lanolin and aqueous toxoid in the ratio of 2 : 1 : 3 showed an average antibody titre at its highest of 19.6 units/ml.

The use of rabbit anti-lymphocyte sera prepared in cockerels, in a dosage of 0.2 ml subcutaneously every day for 5 days, followed by 3 injections per week reduced the highest antibody titre to 4.6 units/ml.

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

The use of diphtheria toxoid in rabbits for screening adjuvants and immunosuppressors has disadvantages because of the large size of the animal and the cumbersome procedure for estimating the titre of diphtheria antitoxin in blood using skin reaction in rabbits. The use of sheep red blood cells in mice has none of these disadvantages.

Groups of mice were given a single dose of 0.2 ml of 10 per cent SRBC (about 5×10^8 cells) intraperitoneally. The mice were bled periodically and the haemolysin titre and the haemagglutination titre were determined in perspex plates using 1 per cent SRBC suspension in two-fold dilution. The maximum haemolysin titre was reached on the 6th day and a haemagglutination titre of 1:2048 was reached on the 15th day after the injection and then the titres gradually came down.

The passive cutaneous anaphylactic (PCA) reactivity of horse serum immunoglobulins and their fragments.

Hyperimmune equine antisera are extensively used for the treatment of diphtheria, tetanus, gas gangrene and bites of venomous snakes. Recently, horse anti-lymphocyte serum is used for immunosuppression in organ transplantation. Serum therapy, however, involves serious reactions like serum sickness and serum anaphylaxis. The incidence of such reactions has declined

considerably with the use of pepsin-refined antisera, but not abolished. With a view to studying the anaphylactic reactivity of these antisera, experiments were carried out in guinea pigs using passive cutaneous anaphylactic (PCA) test for Ovary. The choice of this test was motivated by its close resemblance to Prausnitz-Kustner (P-K) reaction in humans.

Guinea pigs were sensitized intradermally with rabbit anti-normal horse serum (anti-NHS) and rabbit anti-pepsin refined horse antiserum (anti-PRS). It was observed that, on challenging intravenously with horse antidiphtheria plasma (ADP), PCA reactions occurred with anti-NHS and anti-PRS serum. The challenge with pepsin refined antidiphtheria serum (PRS), however, could elicit PCA reactions only with anti-PRS serum and not with anti-NHS serum. A good correlation was found to exist between the PCA reactivity and the precipitating ability of the antiserum. It was found that only the antigens against which the precipitating antibody content in the rabbit antiserum was fairly high and the precipitation occurred within 1 hour at 45° C, could elicit a detectable PCA reaction within 30 minutes. It was concluded that pepsin-refined horse antiserum is strongly antigenic in rabbits when injected with Freund's complete adjuvant and the antibody formed can sensitize the guinea pig skin for PCA reaction. It is speculated that the incidence of serum sickness in humans is due to the decrease in the quantity of therapeutic dose administered as the units/ml of antiserum have increased considerably due to peptic digestion and purification.

Antigenic relationship between horse serum IgG and IgG (T) immunoglobulins

Several striking differences are recognised between the two horse serum immunoglobulins IgG and IgG(T). IgG(T) has a faster electrophoretic mobility and a higher carbohydrate content than IgG. It also has an additional disulfide bond linking the two heavy chains as a 5·6 S fragment is formed, whereas 3·58 fragments are formed when IgG is digested with papain. From a comparative study of C-terminal amino acid sequence of heavy chains, it has been recently suggested that the horse IgG(T) may represent a different class of immunoglobulins than horse IgG though others had previously suggested that IgG(T) is a sub-class of IgG. Thus, it was of interest to find out the antigenic relationship between IgG and IgG(T). For this purpose, IgG and IgG(T) isolated from hyperimmune horse antidiphtheria plasma were analysed by gel-diffusion test and by immuno-electrophoretic technique against rabbit anti-normal horse serum and rabbit anti-immunoglobulin serum. The two immunoglobulins were then digested with papain which is known to degrade the rabbit and human IgG immunoglobulin molecule into functionally active fragments, Fab, Fc and Fc'. Fab comprising the N-terminal portion of the molecule contains the antigen reactive site of antibody, while the Fc fragment is the C-terminal portion and possesses some other important biological properties. From the analysis of the intact IgG and IgG(T), it was concluded that the two immunoglobulins contained some common and some distinct antigenic determinants. The analysis of isolated fragments revealed that the two immunoglobulins differed in the C-terminal portion. In view of all the differences between IgG and IgG(T), it is speculated that IgG(T) is a distinct immunoglobulin class.

DEPARTMENT OF NUTRITION AND BIOCHEMISTRY

Nutrition Section

Studies in protein metabolism of T-pyiformis W.—*T-pyiformis W* is a proteolytic protozoon. 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, besides the amino acid composition of the proteins. Therefore, the work has now been extended to study growth responses of the organism to intact protein, acid hydrolysate of protein, the enzymic (pepsin and trypsin) digest of casein, whole egg protein and wheat gluten at different levels of nitrogen content. The results so far obtained, showed that the growth of organism on intact protein was slightly more than that on the acid hydrolysate as well as enzymic digests of the proteins. It seemed, therefore, that the growth of the organism depended mainly on the amino acid composition of the proteins, as it was capable of liberating the amino acids more easily from the food proteins.

Dietary fats and lipid metabolism.—It is now very well recognised that dietary fats, both in quality and quantity, affect tissue lipids. This effect can be easily 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 has also been 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 albino rats was carried out using safflower seed and coconut oils as the dietary fats. 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 reverse indicating that the excess cholesterol is stored in the liver in a combined form with poly-unsaturated fatty acids.

Since it was necessary to get a picture of the fatty acid pattern of lipids from all the tissues, experiments have been extended using (a) ground-nut oil, (b) hydrogenated ground-nut oil, (c) coconut oil and (d) safflower seed oil in the diet of the albino rat. The lipid fractions from serum liver and heart are being studied in detail by thin layer and gas chromatography for fatty acid patterns.

The relative effect of dietary fats and carbohydrates on serum and tissue cholesterol and phospholipid levels in the albino rat.—The role of the dietary carbohydrates with reference to the dietary fat is not properly understood.

This study was concerned with the effect of two carbohydrates sucrose and corn starch at 4 different levels in the presence of the two dietary fats on the serum and tissue cholesterol and phospholipid levels. Keeping the dietary fats (coconut oil and safflower seed oil) at 20 per cent level, the carbohydrates were fed as follows:

Sucrose 0%
Starch 54%

Sucrose 10%
Starch 44%

Sucrose 44%
Starch 10%

Sucrose 54%
Starch 10%

for 8 weeks. It was observed that the beneficial effect of the unsaturated fat in lowering the serum cholesterol level was nullified by the excess of the sugar in the diet. In the case of the liver, there was an increase of 40-50 per cent cholesterol, as the sugar level in the diet was raised irrespective of the type of dietary fat.

Biochemistry Section

Biochemical studies on vibrio and El tor cholera: Effect of ampicillin, berberine and chloromycetin on macromolecular biosynthesis.—Normal cell 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 and 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.

Berberine and chloromycetin are known to be active against cholera organisms. The antibiotic ampicillin (2-6-Aminophenyl acetamido penicillic acid) has been found to be effective against both gram-positive and some gram-negative organisms.

It was planned to undertake a systematic study of the cellular make-up and the macromolecular biosynthesis of *Vibrio cholera* and *El tor* vibrios, both normal and cells treated with ampicillin, berberine and chloromycetin. Preliminary studies on the cellular composition indicate that these antibiotics and berberine interfere in the biosynthesis of the macromolecules and alter the pattern of percentage composition of the cells.

Berberine is found to increase the protein and lipid content of the cells. In the case of ampicillin, it decreased the lipid content, while there was no effect on the other cellular constituents. Chloromycetin decreased the protein content while the percentages of RNA and total sugars were increased to an appreciable extent.

Detailed studies on the biosynthesis of individual macromolecules are being carried out, using radioactive precursors like labelled amino acids, sugars and purines.

Effect of some drugs on the biosynthesis of macromolecules—In our studies on the mechanism of vibriostatic action of berberine, it was demonstrated that the lassical vibrio differs from its biotype in several biosynthetic mechanisms. In

order to throw more light on these differences, a systematic study, involving action of a few drugs on the two organisms has been undertaken. The study consist of the examination of cellular make-up and their biosynthesis under drug-stressed condition and their differential response to the drug. Three drugs have been chosen for the study.

Ethanol.—This was found to inhibit the growth of *V. cholerae* at higher concentrations than 4 per cent. The lower concentrations did not cause complete growth inhibition, but loss of motility was noticed. This peculiar action is being investigated by analysis of structural components of the cells grown in the presence of alcohol. There was no loss of flagellum.

Furazolidine.—This drug has recently been recommended against enterobacteriace group. A detailed investigation on the action of this drug would help in understanding acetate handling by the two cholera strains and their capacity to produce the energy fuel.

Tetracycline.—It is a drug which is already being used against cholera and it will be possible to know whether the two organisms, though treated as similar clinical entity, respond differently.

A simple and rapid polyacrylamide gel electrophoretic method for the study of genetically controlled serum proteins.—Polyacrylamide gel disc electrophoretic technique is becoming increasingly popular for the qualitative and quantitative separation of proteins. This technique was standardised and adopted to screen a number of multiple myeloma cases. All the samples of multiple myeloma sera show typical bands of proteins in the region of β -globulins which was not easily discernible with the conventional 'starchgel' electrophoresis.

DEPARTMENT OF PHARMACOLOGY

Screening of drugs for their antileprotic activity

The present treatment of leprosy consists of the use of sulphones. The treatment is not very satisfactory because of (i) slow rate of emergence of resistant organisms and (ii) frequent *erythema nodosum leprosum* reactions. This presents the need for development of new drugs. The progress in this direction was held up till now because there was no model available for testing drugs against *M. leprae*, as they could not be grown either *in vitro* or *in vivo*. Now Shepard's method of cultivation of the bacilli in mouse foot pads has been standardised and can be utilised for testing drugs. But the method is too costly and time-consuming to be used routinely. It cannot cope with the synthesis of new compounds. So, as a preliminary screening procedure, ICRC (acid-fast) bacilli were grown in artificial media *in vitro*.

Inhibition of the growth of the bacilli caused by the presence of the drug was measured turbidimetrically. Twenty-two compounds supplied by Dr. S. M. Deshpande of Benares Hindu University were tested. Compounds bearing Nos. 1, 11, 20 and 22 were active in the same concentration as Dapsone.

Shepard's method : The work on standardisation of the technique is in progress. An inbred colony of CBA mice is being raised. The technique of performing thymectomies and raising of antilymphatic serum in rabbits by using thymus cells from mice was standardised.

Antidiabetic activity of indigenous drugs and synthetic compounds

In the literature on Indian medicine, a number of plants are mentioned as useful in *diabetes melitus*. Some work has been done in our country in testing a few plants in different laboratories. So far, the results reported are not encouraging. Moreover, a systematic screening of plant material may sometimes be rewarding. With this aim in view, screening of plant extracts for this type of activity has been undertaken.

During the past two decades, a large number of synthetic compounds (like sulphonylureas, biguanides, etc.) have been studied for their hypoglycaemic activity, but none has proved to be an ideal drug. Even well-known oral drugs like tolbutamide, chlorpropamide, penformin etc., which are being used extensively for diabetes, have some side reactions and, in some cases, they are not able to control diabetes properly. It may be said that these drugs do not satisfy the conditions required to be considered as an ideal drug. The present aim in undertaking this study is to obtain a good hypoglycaemic compound which should have better activity and least toxicity.

Indigenous plants

Different parts of *Nagkeshar* were extracted with various organic solvent, and the extracts tested in experimental animals. None of the extracts, however

showed any significant hypoglycaemic activity. The alkaloids obtained from the leaves of *Parijita* were found to be inactive.

Synthetic compounds

Work on the synthetic compounds supplied by the Chemotherapy Department has been continued. Some new types of compounds, viz : thiodiazole, have also been investigated. Twelve compounds of S-triazole series, 23 compounds of S-triazine series and 25 compounds of thiadiazole series were tested. Although six compounds of s-triazole series were found to possess significant hypoglycaemic activity. Toxicity studies indicated that those were not superior to tolbutamide. Three compounds of s-triazine series were found to be only moderately active. Four compounds of thiadiazole series were found to possess significant hypoglycaemic activity even when administered at a low dose in normal experimental animals.

DEPARTMENT OF VIROLOGY

RABIES

Tissue culture studies on rabies virus; assessment in tissue culture by demonstration of interference phenomena

The Paris strain fixed virus, passed in HKTC gave a titre of 10^3 mouse LD₅₀ per 0.03 ml. Passages were continued but progress was delayed by difficulties in obtaining Hamster Kidney Cultures.

RABIES PATHOGENESIS

Study of the brown fat as a target organ or virus reservoir in rabies infection

The brown fat in hibernating bats was shown by Sulkin to harbour rabies virus, often for long periods before the bat showed any signs of the disease. To discover whether a similar process occurred in mice, animals were infected by the peripheral route and sacrificed at regular intervals and the brown fat studied for evidence of rabies virus. No virus could be detected in this organ before virus multiplication occurred in the brain and signs of the disease became evident. However, progressive anatomical changes in the brown fat of infected mice were noted starting 48 hours after infection, which were absent in control mice injected with rabies vaccine which contained an approximately equal amount of brain substance, and in normal, uninoculated controls. These changes were consistent and reproducible. To determine the specificity of these changes a similar experiment was performed with an unrelated virus producing paralytic illness in mice. The K. F. D. virus, a group B arbovirus, was used. Changes in the brown fat produced by this infection appeared to grossly resemble those produced by rabies infection. Histological changes are under study.

Tests for detection of rabies virus

The demonstration of rabies virus requires (a) the presence of specific antigen, demonstrable by the fluorescent antibody technique, or (b) the successful infection of mice or other small animals in the histological test. Both these procedures are laborious and time consuming and require considerable experience. Simpler methods are, therefore, being sought for demonstration of the virus.

Haemagglutination test

Several virus preparations, viz. C. V. S. mouse brain suspension, Paris strain sheep brain suspension, Flury virus HEP and LEP (Chick embryo suspension), and street virus mouse brain suspension were used. Agglutination of cells of fowl, guinea pig, human O group, frog, and goose was attempted under different conditions of pH and temperature. Fowl, goose and frog cells, at pH 6.2 and 6.4, after 2-3 hours incubation at 4°C, were found to produce

positive haemagglutination with the various virus preparations used. Fowl cells, being most easily available to us, were used in subsequent tests. Optimum concentrations of virus and cell suspensions and other factors were worked out. HA titres obtained were generally low, 1 : 8 to 1 : 16, except with the Flury virus, which gave titres up to 1 : 64. Agglutination in the cold, at pH 6.2 and pH 6.4, using a 0.5 per cent red cell suspension gave the best results.

Neuraminidase activity of virus was tested by using agglutinated and eluted red cells, in parallel with fresh ones, for agglutination by influenza virus. Both groups of cells agglutinated to the same degree showing that no cell receptors had been destroyed by previous agglutination and elution by rabies virus, i.e. neuraminidase activity was absent.

Haemagglutination inhibition (HI) test

Horse and dog antisera against rabies were used for the H. I. test. Clear-cut results were obtained in both. Horse serum (120 I. U. per ml.) showed an H. I. titre of 1 : 512, while dog antiserum inhibited agglutination in a titre of 1 : 246.

P. C. A. test

Passive cutaneous anaphylaxis has been used as a diagnostic tool for several antigens or antibodies. An attempt was made to use this test for diagnosis of rabies. To standardise the test, known antigens from various sources were tested against antisera prepared in the dog, rabbit and guinea pig.

All white guinea pigs of 250-300 gm weight were taken and the hair of the back and flanks removed with a depilatory powder or clippers. Zero point one ml of antirabies serum was injected intradermally at the test site. On the opposite side, 0.1 ml of normal serum from the same species was injected as a control. Three hours later, 1 ml of 1 per cent Evans blue dye was injected by the intracardiac route and the animals observed for non-specific reaction or shock for 10 minutes. One ml of the selected antigen was then injected by the intracardiac route. After 15 minutes to 1 hour, the skin at the site of intradermal inoculation was observed. A positive reaction showed marked blue colouration over an area of about 10 mm. or more. The negative test showed no bluing, or at times a diffuse pale blue colouration with no defined zone. As there were individual variations in the production of PCA in guinea pigs, a minimum of three animals was used for each sample of antigen, and if one guinea pig showed a positive test, it was taken as positive.

About 30 tests have been performed so far, using various antigens, viz. CVS mouse brain, Flury HEP chick embryo suspension, human brain street virus, dog brain street virus, etc. Anti-sera were derived from horse, dog and rabbit. Antigen controls, i.e. normal mouse brain and serum controls of normal sera were also used. The results have been hopeful, but further

standardization of the test procedure has been found necessary to increase the reliability and specificity of the test.

RABIES EPIDEMIOLOGY

Study of normal dogs for evidence of carrier state

This study was undertaken to determine the presence or absence of a carrier state in the normal dog population. Normal dogs were kept for a week during which period, saliva samples were collected daily and were inoculated intracerebrally into mice. To rule out the possibility that the dog was in the presymptomatic stage of infection, it was kept under observation for another week. The dog was then sacrificed, and its blood (for antibody), brain, salivary gland, and dorsal brown fat were harvested for virus isolation. Ten per cent suspensions were prepared of these tissues and each tissue suspension was inoculated into 6 adult mice and 6 baby mice by the intracerebral route. The mice were observed, for paralysis or death, upto 21 days after inoculation. Sick or paralysed mice were sacrificed and the brains harvested for further passage and identification. If no ill effects were observed upto 12-14 days, two animals were sacrificed and the brain harvested and pooled for blind passage, and the same procedure of observation etc. was adopted. If there was no sickness on the second passage, the material was considered negative for rabies.

In addition to dogs taken from the Municipal dog pound, saliva samples were also taken from domestic dogs brought to the veterinary hospital for various ailments and processed as above.

Fifty dogs were kept under observation and were examined as above, and 500 saliva samples were collected from 100 domestic dogs. From a total of 850 saliva samples processed, none showed the presence of rabies virus. From 50 samples each of brain, salivary gland and dorsal brown fat, passed in adult and infant mice, no rabies virus was isolated.

From 50 blood samples of street dogs, put up for neutralization test, two sera showed neutralizing activity against approximately 100 LD₅₀ of rabies fixed virus in mice, the titres being 1:14 and 1:16. All other sera were negative. Since no history was available, it was not possible to exclude vaccination in case of the dogs whose sera showed neutralizing antibodies against rabies.

Epidemiological studies on animals other than dogs

Owing to its very wide host range, rabies presents a very confused epidemiologic picture, and it is hardly possible to exclude any warm-blooded animal as a possible host. However, there appear to be species differences in the frequency with which virus is excreted in the saliva, so that bites of certain species may be less likely to cause infection than those of others. One perpetual source of confusion is rat bite, and a study has been started to assess the possible role of rats in transmission of rabies.

rabies from rats

Rat bites are common in a city like Bombay, and it is not physically possible to treat every case of rat bite with a full course of antirabic vaccine. Further, there is no report known of natural rabies infection following rat bite. However, in view of the frequency with which rat bites are seen, it was thought necessary to try and obtain specific information on the pattern of rabies in rats, the incidence of rabies infection, if any, in wild caught rats, and, for any rabies infected rats found, the frequency of salivary excretion of the rabies virus.

Rats and bandicoots trapped by the municipality in various parts of the city, are brought daily, for plague surveillance studies, to the Institute. From among these rats, a few were picked out at random, and sacrificed. The brains and salivary glands were removed under sterile precautions. Ten per cent suspensions in 2 per cent horse serum saline were made of these tissues, by grinding in a sterile mortar and pestle, and these suspensions were inoculated by the intracerebral route into 6 mice each. The mice were observed for 21 days for signs of sickness or paralysis. Sick or paralysed mice were sacrificed, and the brains collected for further passage and identification of the disease agent. When no sickness was seen, on the 12-14th day, 2 mice of the 6 inoculated animals were sacrificed and the brains harvested, pooled and passed again by the same method into 6 mice. This process was continued upto one more passage. Where no symptoms of illness appeared up to the third passage in mice, the material was considered negative and discarded. Similar work was started in September 1969, on bandicoots, which are more fierce and more liable to attack or bite humans. Up-to-date, 50 brains and 50 salivary glands from apparently normal bandicoots have been examined for virus, six salivary gland specimens have yielded agents producing sickness in mice. These are yet to be identified. No virus was isolated from brain specimens.

Vaccine Studies

This study was done in order to determine whether any correlation exists between sheep brain virus titre, and potency of vaccine prepared from it. Sheep brains were routinely infected with the Paris strain of fixed virus for preparation of antirabic vaccine. When the sheep became paralyzed, they were sacrificed, and the brains were harvested for vaccine preparation. Before carbolization of the sheep brain suspension, aliquots of the 10 per cent brain suspension were removed for titration in mice. Mean titres of the vaccine

pools prepared from these brains were calculated, and the vaccine pools subsequently tested for potency by the NIH test. Sixty-two sheep brain suspensions were tested for the titre of the rabies virus, by mouse inoculation. The titres obtained ranged from $10^{5.5}$ to 10^8 LD₅₀ per 0.03 ml. Potency tests on the vaccine pools prepared from these brains are yet to be done.

STUDIES ON INTERFERENCE PHENOMENA

Effect of pre-treatment by various interferon inducers on mice challenged with CVS rabies virus

Various agents known to induce production of interferon were injected into mice, as shown in the Table, and followed up after varying periods by challenge with C. V. S. mouse brain suspension, by the intracerebral or intraperitoneal route. The experiments performed and the results obtained are given in the Table. It will be seen that pretreatment with statolon, cycloheximide and *Banakadali* extract showed no protective effect against the CVS virus, though some protection was seen when statolon was used with an enhancer of interferon, *i. e.* Vit A in liquid paraffin. The best protection was achieved by the use of U. V. irradiated rabies virus as the interferon inducer (50 per cent protection) and, to a lesser extent, by the La Sotta vaccine strain of NDV (Table No. 8).

TABLE 8

Protective effect of various interferon inducers against rabies virus in mice

Interferon inducers	Route of injection and dose		Enhancer	Route of injection and dose		Challenge virus	Route of injection and dose	Time of challenge	Exp. mortality	Control mortality
(1)	(2)		(3)	(4)		(5)	(6)	(7)	(8)	(9)
Statolon	..	I. P. 25m /mouse.	Nil	..	Nil	.. CVS 10 ⁵	0.03 ml I. C.	72 hrs	6/6 ..	6/6
Do.	..	Do.	..	..	..	.. CVS 10 ⁶	Do.	Do.	4/6 ..	5/6.
Do.	..	Do.	..	..	..	.. CVS 10 ⁷	Do.	Do.	4/6 ..	5/6.
Do.	..	Do.	..	Vit A. in liq. paraffin 24 hrs prior and 2nd dose on the day of statolon injection.		I. P. 5 mg/mouse.	CVS 10 ⁵	Do.	Do.	6/6 .. 6/6
Do.	..	Do.	..	Vit A in liq. paraffin.		I.P. 10 mg/mouse. 24 hrs prior to statolon.	CVS 10 ⁶	Do	Do.	6/6 .. 6/6
Do.	..	Do.	..	Do.	..	Do.	.. CVS 10 ⁷	Do.	Do.	4/6 .. 6/6
Do.	..	I. P. 5 mg/mouse.	DEAE dextran 24 hrs prior to statolon.		I. P. 2.5 mg/ mouse.	CVS 10 ⁵	Do.	Do.	5/6 ..	6/6
Do.	..	Do.	..	Do.	..	Do.	.. CVS 10 ⁶	Do.	Do.	6/6 .. 6/6
Do.	..	Do.	..	Do.	..	Do.	.. CVS 10 ⁷	Do.	Do.	4/6 .. 6/6

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VIDR-2 GD (<i>banakadali</i>)	1st dose 1 mg/ mouse I. P.								
	2nd dose 2a5 mg/ mouse 48 hrs post-virus chal- lenge.			CVS 10 ⁻⁵	Do.	Do.	6/6	6/6	
Do.	.. Do.			CVS 10 ⁻⁶	Do.	Do.	5/6	5/6	
Do.	.. Do.			CVS 10 ⁻⁷	Do.	Do.	4/6	4/6	
U. V. inactivated 10 ³ suspension CVS mice brain suspension.	I. C. 0.03 ml ..			CVS-10 ⁻⁵		18 hrs	3/6	5/6	
Do.	Do.			Do.	Do.	24 hrs ..	5/6 ..	5/6	
Statolon	.. I. P. 10 mg/mouse.			CVS 10 ⁻	0.05 ml I.M.	Do.	24/24	8/8	
Do.	.. Do.			Do.	Do.	48 hours	23/24	8/8	
Do.	.. Do.			Do.	Do.	72 hours	24/24	8/8	
Statolon	.. I. P. 25 mg/ per mouse.			Do.	Do.	48 hours	24/24	8/8	
Cyclohemimide	.. I. P. 1 mg/ mouse			Do.	Do.	Do.	22/24	8/8	
Do.	.. Do.			Do.	Do.	24 hours	22/24	8/8	
La Sotta vaccine	0.25 ml of diluted vaccine, i.e. one chick dose I.P.			Do.	Do.	48 hours	17/24	8/8	
Do.	.. Do.			Do.	Do.	72 hours	15/24	8/8	

Interference between rabies fixed virus (RV) and Rous Sarcoma Virus (RSV)

Interference by various arboviruses with tumour formation due to RSV in chicks has been demonstrated in the past. A preliminary study using rabies fixed virus (RV) as an interfering or blocking agent to prevent development of tumours by inoculation of RSV showed promising results, *i.e.* tumour blockade in approximately 50 percent of the birds treated in this way. The dose and time relation of this phenomenon were further studied. RV (infected rabbit brain suspension having titre of 10^6 mouse LD₅₀ on intracerebral inoculation of 0.03 ml) was found to block tumours, without producing overt rabies in the chick, at a dilution of 10^{-4} . At a higher dose level, 10^{-3} dilution, many of the chicks succumbed to rabies. Using RV in the dilution mentioned as a blocking agent, and tumour homogenate (w/v) having titre of $10^{6.9}$ to $10^{7.0}$ P. F. U./0.1 ml, the degree of blockade when RSV was given at different time intervals and in different dosages, was determined. Two sets of controls were used for the blocking agent, *i. e.* ultraviolet irradiated RV (UVRV) which had lost its infectivity to mice, and normal rabbit brain suspension (NRB) were injected for pretreatment instead of RV, the subsequent process remaining the same. One set of controls was given RSV alone to see the extent and time of tumour formation at the dose level used, and another given R V alone.

The results obtained showed that at minus 24 hours, *i. e.* when RSV was inoculated 24 hours earlier than the blocking agent, no blockade of tumour formation was seen. At 0 hours, *i. e.* simultaneous inoculation of both agents, 24.4 percent blockade of tumour formation was seen in R V treated birds as compared to those receiving RSV alone at the same dosage. At 24 hours (*i. e.* RSV 24 hours after RV), the level of blockade fell to almost 16 percent and again came up to about 44 percent blockade at 48 hours and 72 hours. The blockade effect with varying dose of RSV at 0 hour and at 48 hours also showed different slopes, suggesting that the mechanism of blockade was different at 0 hours from that which became effective at 48 hours. In general the NRB controls showed no blockade, whereas the UVRV controls showed a variable response usually lying in between the RV and the NRB treated birds.

Further experiments on the mechanism of blockade showed the following interesting results : Table Nos. 9 and 10.

TABLE 9
Percentage blockade at different RV-RSV time interval

			Time (hours) of inoculation of RSV after blocking agent.						
			-24	0	+24	+48	+72	+96	+120
RV	..	..	0	24	17	44	40	29	5
UVRV	..	..	0	20	0	11	10	10	0
NRB	..	..	0	0	0	0	0	0	0

TABLE 10

RSV on contralateral side

		Time (hours) of inoculation of RSV after blocking agent						
		-24	0	+24	+48	+72	+96	+120
RV	..	.. N.D.	N.D.	N.D.	33	N.D.	29	N.D.
UVRV	..	.. N.D.	N.D.	N.D.	17	N.D.	12	N.D.
NRB	..	.. N.D.	N.D.	N.D.	0	N.D.	0	N.D.

Composite table representing results of several individual experiments.
N.D.=Not done.

Blockade of tumour formation could be produced in the contralateral wing at 48 hours suggesting a blood-borne mechanism of blockade, like interferon. The blocking effect was short, i. e. no blockade was seen when RSV was injected 5 days after RV. Blockade was complete when it occurred and there was no multiplication of RSV in the blocked birds. This was demonstrated by absence of antibodies to RSV in the blood of these birds, and also by lack of resistance to RSV on subsequent re-challenge, i.e. after 30-40 days, the birds on rechallenge with RSV developed tumours similar to RSV controls. The blockade by RSV was produced in the apparent absence of multiplication of the RV i. e. the blocked birds showed no antibodies to RV and no resistance to subsequent re-challenge by RV.

All these findings present several further lines of study, which will be followed up in future. At the present time, the effect of cortisone and of actinomycin-D on the blockade produced by RV are under study, to try and elucidate the role of interferon in the mechanism of blockade. The results described are summarised in the above Tables.

Influenza

Surveillance studies on influenza in the population were continued. Sporadic cases of influenza were seen throughout the year. Investigation of suspected cases (mainly staff members) was carried out. From cases in Bombay, a few isolations were made. These were all of A₂ Hong Kong/68 type virus.

The work of influenza surveillance has been summarized below :—

Throat washings and swabs collected for diagnosis	..	49
Positive isolations for influenza (All were A ₂ /Hong Kong/68 type).		13
Number of throat washings under process	..	23
Number negative for influenza	..	13

A serological survey was made of 95 sera collected from individuals attending as blood donors at the Blood Bank. The results obtained on 95 sera were given below : Table 11.

TABLE 11

Antigens		No. of sera showing haemagglutination titres of						
		1·10	1·20	1·40	1·80	1·160	1·320	
A ₂ /India/47/57	..	..	19	18	7	5	5	..
A ₂ /Taiwan/64	..	..	4	25	20	22	16	4
A ₂ /Tokyo/67	..	..	12	28	27	15	8	2
A ₂ /Hong Kong/1/68	..	..	9	14	21	15	14	11
A ₂ /Bombay/35/68	..	..	13	18	22	16	14	4
A ₂ /Bombay/23/68	..	..	17	18	16	14	5	2

These results on sera, collected during the early part of the year, did not show a marked increase of antibodies against the Hong Kong type virus in the population tested.

Investigation of sub-epidemic outbreaks of influenza

During November, December 1969 and January 1970, reports of influenza in epidemic form were received from various parts of the country and from other parts of the world.

During this period, with the help of the local authorities, outbreaks of influenza were investigated in two areas.

In Aurangabad town and district, large numbers of influenza cases were reported in January 1970. A staff member visited the area and, with help from the Medical College, Aurangabad, and the Deputy Director of Public Health Services made an on-the-spot study. It was found that, both at clinics in the city and at the Rural Health Unit at Paithan (40 miles from Aurangabad), there were a large number of cases of upper respiratory disease. In the opinion of the medical officers concerned, however, the increase in numbers of such cases, over the normal incidence, was not such as to constitute a regular epidemic. Throat gargles and blood samples were collected from 20 representative cases and brought back to Bombay for study. From 7 of the 20 throat washings

collected, A₂/Hong Kong/68 type virus has been isolated ; 13 are still under study. Paired serum samples were obtained from 6 cases of which 5 showed a fourfold or greater rise in titre of haemagglutination-inhibiting antibodies against A₂ Hong Kong virus. Included in these five were two of the cases whose throat washing yielded an isolate of A₂ Hong Kong virus.

A second outbreak of influenza, which was reported in Dahanu, was investigated by a team from the department at the end of March, 1970. Several cases were seen, many of whom, however, had symptoms of long duration, not typical of influenza. Throat washings were collected from 12 suspected cases and brought back to Bombay for study. No blood samples could be obtained.

Sero-epidemiology of measles

Serological studies on measles antibodies in the normal population were continued. Sera of 50 children each, in the age groups 3 to 5 years and above 5 years were examined. The results are summarized in the Table No. 12. It will be seen that 40 per cent of children in the younger and 70 per cent of the older group showed high titres of antibody suggestive of past infection.

Tests of commercially prepared samples of gamma globulin for high antibody titres were also continued. The total number of samples tested and are summarised in Table No. 13. It is seen that such gamma globulin preparations could be used for prevention in special circumstances.

Measles vaccine trial

The measles vaccine trial as described in the last report was carried out using Melvilin (Glaxo)—a vaccine containing the Schwarz attenuated strain of measles virus. The trial was planned in two phases. In phase I, a double blind trial, wherein 600 coded vials of vaccine or placebo were to be used by each participating centre, i.e. Bombay, Indore and Trivandrum. The participants were instructed to bleed each child before, and one month after the administration of the vaccine ; to observe the vaccinated children daily for 30 days for any reactions and to subsequently follow up the children up to 1 year (the next winter season, when measles cases are usually prevalent) to record any vaccinees developing the disease. The paired sera collected from these vaccinees were to be sent to this Institute for testing for antibodies. In Phase II, 1,000 doses of plain vaccine were to be used by each centre, reactions to be noted and the children followed up, as in phase I, for one year.

A large number of children appeared to have suffered from measles at an early age. Hence, sufficient numbers of children without history of measles were not available. Participating centres were unable to vaccinate or to bleed the number of children planned and the actual numbers were small. Paired sera received were as follows ; from Bombay, 56 pairs ; from Indore, 194 pairs and from Trivandrum, none. Due to these difficulties vaccine for Phase II

was distributed to many workers other than the original participants, mainly in Bombay. The utilization was as follows :—

Bombay (various centres) 600 doses, Indore 1,000 doses, Trivandrum 600 doses, Palghar (Rural Health Unit) 300 doses and Aurangabad (Rural Health Unit) 200 doses.

From Aurangabad, paired sera of vaccinees were received from 74 cases. The results of antibody tests against these are shown in Table No. 14 which indicate a good antibody response to administration of vaccine.

Few reactions were noted and the incidence and severity of reactions after measles vaccine were very much lower than those reported with the same vaccine in other parts of the world. These findings will be properly assessed after the statistical analysis of Phase I is over.

TABLE 12

Presence of high antibody titres against measles

Number of sera	Age group No. + ve/No. tested		Antibody titres		
			HAI	CF	NT
2			1 : 40	1 : 4	1 : 64
3			1 : 40	1 : 8	1 : 64
5	3 to 5 years	..	1 : 80	1 : 8	1 : 128
5	(20 / 50)	..	1 : 80	1 : 16	1 : 128
4			1 : 160	1 : 16	1 : 256
1			1 : 160	1 : 32	1 : 256
20					
10			1 : 40	1 : 8	1 : 64
10	Above 5 years	..	1 : 80	1 : 16	1 : 128
9	(35 / 50)	..	1 : 160	1 : 16	1 : 256
5			1 : 320	1 : 32	1 : 256
1			1 : 320	1 : 32	1 : 512
35					

TABLE 13

Measles antibodies in commercial preparation of gamma globulin

Gamma globulin from		Antibody titres		
		HAI	CF	NT
Placental extract				
2	..	160	16	128
3	..	160	16	256
2	..	160	32	256
1	..	320	32	256
1	..	320	32	512
1	..	320	64	512
Venous blood				
4	..	80	8	128
3	..	160	16	128
9	..	320	32	256
1	..	320	64	512
8	..	640	64	512

All dilutions are expressed as reciprocals.

TABLE 14

Rise in measles antibody titres of pre (A) and post (B) vaccinated sera from Aurangabad

Number of paired sera showing rise in antibody titres		Antibody titres					
		HAI		CF		NT	
		A	B	A	B	A	B
4	..	10	40	4	8	4	64
14	..	10	80	4	8	4	128
1	..	20	80	4	8	32	128
15	..	10	160	4	16	4	256
16	..	10	160	4	32	4	256
20	..	10	320	4	32	4	512
3	..	10	320	4	32	4	512
1	..	40	320	4	32	32	512

Total .. 74

All dilutions expressed as reciprocals.

Screening of preparations for chemotherapy and chemoprophylaxis of vaccinia

Twentyfive compounds, most of which were supplied by the departments of Pharmacology and Chemotherapy, were screened for activity against vaccinia, by reduction of pocks on chick embryo chorioallantoic membrane. The vaccinia virus used for challenge gave counts of 80-100 pocks in untreated controls. Fifty per cent or more reduction in pock count was taken as significant of anti-viral activity.

Each compound was tested in different dilutions and, at times, in different solvents. Tests were repeated at least twice before evidence of activity against vaccinia was accepted.

Twelve of the 25 compounds tested during this period showed anti-viral activity. These will be studied further.

Studied on rats as possible vectors of human virus infections

The study to assess the role of household rats in the spread of human virus diseases was continued. The details of procedure have been described in previous reports.

The total studies made so far are given in the Table No. 15 below :—

TABLE 15

			Rats from rural area collected in June, July, August, 1968	Rats from urban area (Kamathipura September- December, 1968)
Number of tissue specimens collected	..	..	325(65 × 5)*	200(40 × 5)*
Number of tissue specimens inoculated	..	..	235	100
(i) in mice (infant and adult)	..	..	135	20
(ii) in MKTC	..	..		
Number of tissue specimens showing agents pathogenic				
(i) to infant mice but non-pathogenic to adult mice	..	..	2	1
(ii) to infant and adult mice and MKTC	..	..	20	5
Number of tissue specimens showing no pathogen for mice				
(infant and adult)	..	..	191	64
Number of tissue specimens under study in infant and adult mice and/or MKTC	..	..	60	40
Heat inactivation test				
(i) unstable at 37°C 1 hr and 56°C for 1/2 hr.	..	..	1/21	1/11
(ii) stable at 37°C for 1 hr and unstable at 56°C 1/2 hr.	..	..	11/21	7/11
(iii) stable at 37°C 1 hr and 56°C 1/2 hr.	..	..	9/21	3/11
Ether sensitivity test	..	..	7(-ve)	2(-ve)
Bile salt sensitivity test	..	..	21(-ve)	11(-ve)
Strains isolated identified as EMC	..	..	13	5

*5 tissue pools from each pair of rats.

An interesting feature of studies made so far was that several pools (22 per cent) of rats of the village Kalundri in Panvel area showed the presence of a virus, whereas only 4 per cent of pools studied from the adjacent village of Palaspe (about 4 miles distant) yielded similar isolations (see Table No. 16). This regional aspect is to be studied further in the current year.

TABLE 16
Rate of virus-isolations from rat pools of two villages

Rat tissues			Kalundri village	Palaspe village
Liver	..	..	6/30*	0/18
Spleen	..	..	7/30	1/18
Stool	..	..	14/30	2/18
Brown fat	..	..	3/30	0/18
Brain	..	..	3/30	1/18
Total	..	..	33/150 (22 %)	4/90 (4.4 %)

*No. of specimens+ ve/No. of specimens processed.

Studies on rubella in Bombay

During recent years, there has been increasing consciousness of the teratogenic sequelae of rubella infection in pregnant women. We have received calls for investigation and advice on suspected cases and also for advice regarding termination of pregnancy in such cases. It was, therefore, felt that a survey of rubella in Bombay and the status and severity of the disease should be studied.

Two aspects were taken up for study ; (i) a serological survey, covering various age groups of the population and (ii) a case study of abortions, still-births and of children born with congenital defects.

For serological survey, 598 sera have been collected from various age groups, including suspected cases of rubella syndrome and their parents and siblings.

In the case study of infants suspected of rubella syndrome, 12 cases have been collected from the city hospitals. Two of the infants died, autopsy was done and tissues (lung, liver, kidney, suprarenal and heart) collected for virus isolation. One infant was operated for congenital cataract and the lens material was collected at operation. From the rest, blood samples have been collected.

The sera collected and tests done so far for haemagglutination inhibiting (HI) antibodies against rubella are summarised in the Table No. 17. It will be seen that a majority of adults and children above the age of 6 years show haemagglutination inhibiting antibodies against rubella.

TABLE 17.

Serological survey of rubella in Bombay

Age group (years)				No. of sera collected	No. /No. +ve/tested
0-5	..	..	..	6	1/4
6-10	..	..	..	226	151/185
11-15	..	..	..	27	21/22
Adults (16 and over)	..	..	..	339	23/23
Total ..				598	

POLIOMYELITIS

Paralytic illness among vaccinated children

The Enterovirus Unit detected 84 cases of paralytic illness among vaccinated children in Greater Bombay. Greater emphasis is being laid by the Unit in studying these cases. For virological studies, multiple samples have been collected (on an average 3 stool samples and 3 rectal swabs on 3 consecutive days). This procedure has reflected in the increased isolation rate as compared to those in the previous years. A total of 67 cases were studied virologically.

Out of the 67 cases with history of oral polio vaccine in the past, 13 cases had one dose, 18 cases had two doses and 34 cases had three doses. In 2 cases, the number of doses administered was not known.

Clinically, 61 cases presented a typical picture of flaccid paralysis, while in 6 cases the clinical picture was atypical.

Age and monthly distribution of these cases are shown in accompanying Tables Nos. 18 and 19.

A total of 47 cases (70 percent) showed presence of virus during the acute stage of the illness. Details of virus isolation are shown in the Table No. 20. These studies are in progress.

TABLE 18
Paralytic illness among vaccinated children
 Age Distribution
 1969

Age					Number of cases
0—6 months	..	..	..	..	2
7—12 months	..	..	..	..	14
1—2 years	..	..	..	..	32
2—3 years	..	..	..	..	13
3—5 years	..	..	..	..	3
5 years	..	..	..	..	3
Total ..					67

TABLE 19
Paralytic illness among vaccinated children
 Monthly Distribution

Months					Number of cases
January	..	..	..	..	2
February	..	..	..	..	2
March	..	..	..	..	0
April	..	..	..	..	1
May	..	..	..	..	0
June	..	..	..	..	3
July	..	..	..	..	20
August	..	..	..	..	12
September	..	..	..	..	11
October	..	..	..	..	6
November	..	..	..	..	4
December	..	..	..	..	6
Total ..					67

TABLE 20

Paralytic illness among vaccinated children, 1969

H/O	O. P. V.	No. of cases	Polio virus			Mixed/ non-typed	No isolation
			type 1	type 2	type 3		
1 dose	..	13	3	1	0	4	5
2 doses	..	18	2	1	1	11	3
3 doses	..	34	8	6	0	14	11
Unknown	..	2	..	..	..	1	1
Total		67*	13	8	1	30	20

* Some patients yielded more than one isolate.

Studies on efficacy of oral polio vaccine (Bombay)

The objective of the above study is to determine the excretion pattern of the vaccine virus and its effect on the antibody pattern in the susceptible child population. The study also aims at determining the role of wild enteric viruses in causing interference with the vaccine viruses in the intestinal tract.

For the study, very young children (children below 3 years) attending "Well Baby Clinic" at two paediatric Centres in Bombay were selected.

From each child, a pre-vaccination blood sample and a rectal swab were collected following which the first dose of pretested trivalent Oral Polio Vaccine (containing approx. 1 million TCID₅₀ of Poliovirus) was administered. Attempts were then made to collect a daily rectal swab from the vaccinees for a period of 8 days. All these collections were made by making home visits. Mothers were told in advance to visit the well Baby Clinics on specified dates for receiving the second and third doses of vaccine. Each time the baby visited the clinic, attempts were made to collect blood samples for serological studies.

During the period under report, 110 babies were recruited for the above study. Till to date, 824 rectal swabs and 130 blood samples have been collected.

Virological studies on the first 60 vaccinees have been made. Further studies are in progress. Analysis of the results obtained so far showed the following features :—

- (i) Of the first 60 babies recruited and undertaken for study, the collection of serial rectal swabs (6 or more samples) was possible in 42 children. Six babies dropped out from the second day.

(ii) Of the 60 vaccinees, 8 (13 per cent) showed presence of a wild virus in the gut before administration of the oral polio vaccine.

(iii) During the first 10 days after administration of the first dose of oral polio vaccine, 19 vaccinees showed excretion of poliovirus. Of these 19, sixteen showed excretion of one type, 2 showed excretion of two types, while one vaccinee showed excretion of all the three types of poliovirus.

(iv) Of the 8 vaccinees who had a wild virus in the gut before administration of the vaccine, 6 failed to excrete poliovirus during the first 10 days. Two vaccinees showed excretion of poliovirus.

Correlation between virus isolation and serological response in paralytic poliomyelitis

The Enterovirus Unit receives materials for virological studies from out-stations. Materials are requested to be sent in neutral glycerol saline. Samples thus despatched arrive non-refrigerated. It was to study the results of virus isolation and correlate these with the results of serological tests of the corresponding samples. For this study, materials from 45 cases of paralytic polio sent from Colombo, Ceylon, were selected.

Summary of the results is shown below :—

Total number of cases studied	45 cases.
Isolation of Poliovirus	9 cases.
Isolation of nonpolio/mixture of viruses	12 cases.
Serological response to type-1	19 cases.
Serological response to type-2	2 cases.
Serological response to type-3	5 cases.
Serological response to mixed (more than one type)	3 cases.
Serological response to none	16 cases.

It will be seen from the above results that out of the 45 cases of paralytic poliomyelitis, virus could be isolated from 21 cases. Of these, only 9 proved to be poliovirus. In contrast, the serological response to poliovirus was seen in 29 cases. In 15 cases, there was no serological response to poliovirus as well as no poliovirus was isolated. In three cases, the serological response observed was against multiple type of poliovirus.

Enterovirus and kidney disease

There are a large number of published works which indicate viral involvement in diseases of urinary tract. Viral involvement in kidney diseases is further suggested by such animal infections as canine distemper and hepatitis, avian bronchitis and aleutian mink disease in which marked renal pathology is part of the disease syndrome.

During last one year, the Enterovirus Unit has been exploring the possibility of any role played by Enteroviruses in cases of nephritis in children. A total of 38 cases were investigated. A total of 95 samples, consisting of recta-swabs, throat swabs and samples of urine were collected and tested. Till to-date no agent has been isolated from these cases.

Study of simian intestinal viruses

Since almost all the virological investigations are being carried out in the use of monkey kidney tissue culture and it is known that monkeys frequently harbour viruses in their gut as well as various tissues, it was decided to study the viral flora of the intestinal tract of the rhesus monkey.

Intestinal contents of 100 rhesus monkeys were collected for virological studies. As many as 56 (56 per cent) monkeys were found to harbour a cytopathogenic agent in the intestinal tract.

These cytopathogenic agents could broadly be grouped into two types according to the morphological changes induced by them in tissue cultures, viz., (a) those which produce CPE like human adenoviruses (b) those which produce CPE like human enteroviruses. Group 'b' could be further classified as (i) those agents which produce total destruction of cells and (ii) those which produce only partial destruction of the cell sheet leaving large number of cells unaffected.

One strain, 26 R, which produced adenovirus like effect in tissue culture, on testing for plaque producing capacity under agar overlay, showed inability to produce plaques. In contrast, two strains, 9F and 15F, gave rise to small and diffuse plaques with hazy borders. Antisera in rabbits are being prepared against strains 26 R and 9 F. Further characterization of these simian enteroviruses is in progress.

Nagpur encephalitis

Encephalitis like syndrome in epidemic form, especially affecting children, has been observed in and around Nagpur since the last 10 years. The increase in the incidence of these cases is noticed during April to July each year. The disease is characterised by the sudden onset of high fever followed by convulsions and semi-consciousness or unconsciousness, leading to a high case fatality rate.

Absence of any positive findings on bacteriological examination of the stool, blood or C. S. F., collected from these patients suggested that the disease was possibly of viral aetiology.

During the period under review, there were 57 cases of encephalitis admitted in the Government Hospital.

First case was recorded on 17th April 1969. During the month of April, there were four cases, in May 35 cases and in June there were 18 more cases.

Of the above 57 cases, 34 (60 per cent) proved to be fatal. It was possible to perform autopsy studies on 15 fatal cases; in 9, complete autopsies were performed, while in 6 permission was granted only for partial autopsy examination.

The age and sex distribution of the 57 cases and fatalities among these were as follows (Table No. 21):

TABLE 21

Age Group			No. of cases	Males*	Females*
0—1 year	..	..	19	12(8)	7(4)
1—2 years	..	..	8	4(2)	4(2)
2—3 years	..	..	5	5(3)	0
3—4 years	..	..	6	5(4)	1(1)
4—5 years	..	..	3	3(1)	0
5—10 years	..	..	12	7(6)	5(0)
10 years	..	..	4	2(2)	2(1)
Total			57	38(26)	19(8)

*Figures within brackets indicate number of fatal cases.

Materials for virological studeis were collected soon after cases were admitted to the hospital. All the materials thus collected were transported to the laboratory under cold storage, and were stored in a frozen state at -50°C pending inoculation.

Materials collected from clinical cases are as follows:—

(a) *Antemortem*:

C. S. F.	..	..	57 samples
Blood	..	..	85 (57 acute samples 28 convalescent samples)
Throat swabs	..	..	56
Rectal swabs	..	..	56
Urine	..	..	7
Total			261

(b) *Post-mortem* (from 15 cases)

Samples of C. N. S.	..	..	107 Samples
Other organs	..	..	101 "
Total			208

Materials collected from contacts of clinical cases and non-contacts (collected by the epidemiologist).

Rectal and throat swabs	..	..	351
Blood samples	..	..	90
Total			441

All the studies carried out so far have been aimed at the possibility of enterovirus aetiology.

The isolation work in tissue culture was carried out at Nagpur. Since it was not possible to create adequate animal house facilities at Nagpur, all the animal inoculations were done at Bombay. The samples from clinical cases, both antemortem and postmortem, and those collected by the epidemiologist from contacts and non-contacts, were tested both in tissue culture and infant mice. All the viruses isolated in tissue culture were tested for infant mouse pathogenicity, haemagglutination activity and infectivity end points. For identification of these isolates, pooled as well as individual reference antisera against poliovirus, Coxsackie A₉ and B₁—B₈ and all the available ECHO types were used. Plaque morphology and marker tests (temperature marker) were carried out on selected isolates.

Using neutralization test in tissue culture, all the sera collected from fatal cases were tested against identified as well as unidentified agents.

Paired sera from cases which survived, were similarly tested.

Detailed results of the studies carried out are shown in the Table No. 22.

Comments

(i) Of the 57 cases selected for study, 16 cases showed presence of an enterovirus.

(ii) There is no significant difference in the isolation rates between fatal and surviving cases.

(iii) Poliovirus type 3 was the predominant isolate.

(iv) Of the 15 cases studied at autopsy, two cases showed presence of virus in the CNS material. Both cases showed poliovirus type 3. In both these cases, no virus could be isolated from samples collected antemortem.

(v) Serological studies carried out on sera from fatal cases and paired sera (in surviving cases) did not show any significant rise in titre against the isolates.

(vi) Analysis of seroimmune patterns against poliovirus has revealed a normal pattern in the fatal cases.

(vii) The virological and serological studies carried out on 57 cases of Nagpur Encephalitis did not show any evidence of enterovirus aetiology.

TABLE 22

Nagpur Encephalitis

Summary of Virological Results

		FATAL M+F=T*	SURVIVING M+F=T*	TOTAL M+F=T*
Cases studied	..	26+8=34	12+11=23	38+19=57
Virologically positive	..	9+1=10	4+2=6	13+3=16
Polio-3	..	4+1=5	3+1=4	7+2=9
Cox B ₅	..	1+0=1		1+0=1
Echo 7	..		1+0=1	1+0=1
Echo 14	..	1+0=1		1+0=1
N. I. @	..	3+0=3	0+1=1	3+1=4

*M=Male

F=Female

T=Total

@N.I.=Not identified.

DEPARTMENT OF ZOONOSIS

Plague epidemiological studies were carried out in Thana and Nasik districts. Apart from these two districts, studies were also made in 'A', 'F' and 'R' wards of Greater Bombay.

Two places were selected for studies from each of the twelve talukas of Thana district, one urban and one rural locality, covering an area of about 120 square miles with a population of about 5 lacs. During these studies, 2,640 rats were collected out of which 87 percent were *R. rattus*, proving it to be the most predominant rat species in the area. Average rat density for Thana district was found to be 30.5.

From these rats 3,087 fleas were collected. Average flea index for Thana district was 1.8. *X. cheopis* was observed to be the predominant flea. Studies on susceptibility status of fleas from different locations in Thana District to DDT, Dieldrin and Malathion were conducted. The observations showed Dieldrin had the highest degree of susceptibility.

Rats collected from these areas were also challenged with plague bacteria for assessing their susceptibility. Rats from Thana District showed a high degree of resistance ranging from 60 percent to 80 percent, except 36 percent at Talasari. Serological studies with rat blood did not show any evidence of *P. pestis* infection.

Studies in Nasik district were carried out on similar lines as in Thana district. Two thousand seven hundred and ninety-three rats were collected, out of which 99 percent were *R. rattus*. The rat density in Nasik area was observed to be 44.6. About 97.6 percent of the fleas collected from these rats were *X. cheopis*. In all, 6,620 fleas were available for studies. The flea index was observed to be 2.3.

Rats from Nasik District are being challenged to assess their susceptibility to plague and their sera are being examined for evidence of *P. pestis* infection.

In Greater Bombay, studies in 'R', 'F' and 'A' wards showed a considerable variation in the incidence of rodent fauna. In 'F' and 'A' wards, *B. bengalensis* were predominant. *X. cheopis* was observed to be the prevalent flea harboured by these rodents followed by *X. astia*.

Studies in ascariasis

Epidemiological investigations were carried out to determine the intensity of infection. At K. E. M. Hospital, 108 out of 500 (21·6 percent) stool samples of in-door patients examined showed the presence of *Ascaris* eggs. Similar surveys carried out on 2,978 children between the ages of 0-11 years at Bai Jerbai Wadia Children's Hospital indicated the presence of 33·58 percent infection. It was also observed that the intensity of the infection was highest in young age groups especially in pre-school children below 5-6 years. Survey at R.M. Bhatt High School is in progress. Out of 112 faecal samples examined so far 23 (20·53 percent) have shown the presence of infection. *Ascaris* eggs were successfully cultured in different media at room temperature and their development pattern was studied.

Rodenticide trials

Rodenticide trials with MR-100— a compound containing warfarin and sulfaquinoxaline vis-a-vis a control rodenticide containing only warfarin—were concluded. The former had a flavour enhancer added to it.

These trials were conducted against four species of rats, viz *Rattus rattus*, *Bandicoota bengalensis*, *Rattus norvegicus* and *Rattus norvegicus* (albino). MR-100 bait showed superior acceptance in comparison to the control rodenticide. Both the compounds were found to be effective and the difference as regards their efficacy was not significant.

Thiosemicarbazide

This compound was studied for its efficacy as a rodenticide. Trials were carried out against four species, of rats, viz. *Rattus rattus*, *Bandicoota bengalensis*, *Rattus norvegicus* and *Rattus norvegicus* (albino).

It was observed that the bait, when used as 2 percent formulation, was quite effective against the species of rats tested. When orally fed, a dose of 50 mg/kg gave 100 percent mortality in *B. bengalensis*, 75 percent in *R. norvegicus* and 50 percent in *R.rattus*. However, it was felt that a higher dose of 100 mg/kg could be very useful in the field. Field trials conducted with 0·5 percent concentration gave very promising results, as 97 rats were found dead in 4 days. It was, however, noted that the consumption decreased on successive days which could be due to bait shyness. Trials conducted by using bread pieces soaked in bait solution were also encouraging.

Studies on venomous animals

Studies on anatomical peculiarities of salivary glands of various poisonous and non-poisonous snakes showed that in case of the latter, mucus cells were uniformly distributed all over the gland while, in case of the former, the major portion of the gland was occupied by serous cells and the mucous cells showed sparse distribution. Most of them were restricted to the anterior ampullar region of the duct.

Studies on snakes

Studies were carried out to find out if the specific polyvalent antivenin gave protection against poison of snake other than those utilized for the manufacture of antivenine, viz. cobra, viper, krait and echis. It was seen that though protection was given, in general, it differed in its degree among different snakes used, namely, monocellate, acellate, cobra, king cobra, green and brown pit viper, banded krait and sea snake.

Studies on the respiratory organs of some snakes indicated that they consisted of a vascular and a sacular lobe in poisonous and non-poisonous snakes other than in the family of Boidae (non-poisonous) snakes where these were bifurcated.

PART III
ROUTINE ACTIVITIES AND MISCELLANEOUS
ACTIVITIES

ROUTINE ACTIVITIES

ANALYTICAL AND CONTROL DEPARTMENT

The department continued to work for the major part of the year in the old K.F.D. block and in the two rooms provided near the Pharmacology Department. The inadequacy of space, which has been a handicap for the expanding activities of the department, is being partly removed by providing space in the Nutrition Department in exchange for the old K.F.D. block.

Shri F. L. Saldanha of this department was awarded Ph. D. degree in Microbiology and left on a one-year W.H.O. Fellowship to U.S.A. for advanced training in 'Drug Analysis and Biological Standardisation'. Shri N. Ramani was promoted to officiate as Junior Scientific Officer in place of Dr. F. L. Saldanha, Kum. K. P. Tipnis, Kum. M. T. Shah, Kum. T. J. Bapat and Shri P. S. Chavan joined the department as Senior Technical Assistants.

Methods for standardisation of tablets of di-iodohydroxy-quinolin and for analysis of solutions for peritoneal and kidney dialysis were developed.

Routine

The number of different tests conducted by the department during the year are tabulated below (Table No. 23).

TABLE NO. 23

Products	Tests					No. of samples tested
Sterility						
Bacterial vaccines ...	..	..	..	..	..	13,453
Antirabic vaccine ..	..	..	..	..	..	6,988
Parenteral bulk solutions	..	..	..	..	..	7,962
Antitoxins, sera, etc.	..	..	..	..	..	18,695
Miscellaneous ..	..	..	..	..	..	768
Total					..	47,866

TABLE NO. 23—*contd.*

Products	Tests				No. of samples tested
Pyrogen					
Parenteral, bulk solutions and blood products, etc.	...	...	...	...	1,359
Chemical and physical examination					
Parenteral bulk solutions	..	...	...	...	1,377
Vitamin preparations	..	..	...	...	306
Sulpha drugs	..	..	...	...	201
Miscellaneous	..	...	...	...	150
Toxicity					
Antitoxins, toxoids, vaccines, etc.	..	..	...	...	645
Absence of infective virus					
Viral vaccines	..	..	...	...	258
Potency					
Antitoxins	..	..	...	...	82
Antivenine	..	..	...	...	38
Toxoids	..	...	...	...	18
Triple antigen	..	..	...	..	4
Preservatives					
Vaccines, antitoxins, etc.	..	...	...	...	226
Free Formaldehyde					
Bacterial vaccines, toxoids	..	...	...	..	203
Microbiological assays					
Pemon	...	...	...	...	103
Vitamin preparations	...	..	...	..	83
Total				..	52,919

DEPARTMENT OF BACTERIOLOGY

During the year under review, the department continued the work on stability of vaccines at high altitudes for which the Ministry of Defence Research and Development Organisation, sanctioned a grant of Rs. 12,846 to extend the scheme up to May 1971.

The George Williams Hooper Foundation, San Francisco (U.S.A.), has proposed, through the World Health Organisation, a collaborative study for testing plague vaccine (both killed and live) in langur monkeys to assess their efficacy. The new Haffkine plague vaccine will also be included in the study.

Shri Y.S. Nimbkar, Assistant Director, is co-opted as a member of the Advisory Committee of the Small Industries Research Institute, Poona.

The World Health Organisation Scholarship awarded to Shri Khleifat Hussein in 1968 was extended upto September 1970.

Maharashtra Government Scholarship awarded to Kum. J. M. Kikani has been extended for a period of one year to continue her research work.

Shri R. P. Bhamaria, M. Sc., joined the department in July 1969, as Junior Scientific Officer in the Research section of the Department. Smt. P. S. Ranadive, B.Sc., D.M.L.T., was appointed as a Senior Technical Assistant from February 1970.

Vaccine section

Production

The production of Cholera-El-Tor vaccine was continued. As decided in the meeting of the manufacturers of Cholera El tor vaccine held in the Office of the Director-General of Health Services during the month of September, the strength of Cholera-El-Tor vaccine was increased from 8×10^9 organisms per ml. to 12×10^9 organisms per ml. The following quantities of different vaccines were produced during the year (Table No. 24) :

TABLE 24

Products	Quantity (litres)
Cholera-El-Tor vaccine	10,901807
T.A.B. vaccine	7,65763
Plague vaccine	48660

In addition to the production of vaccine, four samples of frozen shrimps were taken up for bacteriological examination at the request of one of the exporters of marine products.

MEDIA SECTION

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

The manufacture of parenteral solutions also continued in full swing.

In addition to the above, the section was provided with general facilities for sterilization, cleaning of glassware etc. The ampoule section supplied cut and sterilized ampoules to other departments. The production figures of the different items are presented below (Table No. 25).

TABLE 25.

Products	Quantity (litres)
<i>Parenteral solutions—</i>	
Dextrose and Sodium Chloride Injections, U.S.P.	71,692·00
Dextrose Injection, I.P. 5%, W/V	84,955·00
Dextrose Injection, I. P. 25%	3,522 00
Sodium Chloride Injection, I.P.	59,252·00
Water for Injection, I.P.	8,854·00
Sodium Lactate Injection, B. P.	9,751·00
<i>Culture Media—</i>	
Casein hydrolysate agar	23,101·00
Meat digest agar (Typhoid)	734·00
Agar medium for cholera research	110·00
Sterility Test Media	13,858·00
Sabouraud's medium, U.S.P.	4,322·00
Peptone water	213·50
Nutrient broth	327·20
Sterility test medium (acid broth)	25·00
Cooked meat medium (in tubes)	80·00
Nutrient agar	670·5
Acid digest of meat agar	1,240·0
Hartley's broth	7·00
MacConkey's medium	17·50
MacConkey's agar	104·00
Löffler's medium	30·00
Lowenstein and Jensen's medium	8·00
Sabouraud's medium	51·50
Glucose broth	47·50
Taurocholate medium	32·50
Citrate saline	7·50
Lab. Lemco broth	20·00

Products					Quantity (litres)
<i>Sugar for biochemical tests—</i>					
Glucose	..	..	..	..	35.50
Lactose	..	..	..	..	36.50
Sucrose	..	..	..	..	35.00
Mannite	..	..	..	..	35.00
Maltose	..	..	..	..	1.20
Arabinose	..	..	..	..	0.30
Xylose	..	..	..	..	0.50
Dulcitol	..	..	..	..	34.50
Potato agar	..	..	..	..	4.00
D. E. C. medium	..	..	..	..	85.00
Glucose phosphate	..	..	..	..	10.50
Selenite broth	..	..	..	..	12.00
Keser's citrate medium	..	..	..	..	12.00
Thioglycolate medium	..	..	..	..	72.00
<i>Diagnostic Reagents</i>					
Benedict's solution (qualitative)	..	..	..	..	868.00
Albert's stain No. 1	..	..	..	..	12.00
Albert's stain No. 2	..	..	..	..	11.00
J. S. B. stain No. 1	..	..	..	..	18.00
J. S. B. Stain No. 2	..	..	..	..	32.00
Leeffler's Methylene blue	..	..	..	..	19.00
Carbol fuchsin	..	..	..	..	91.00
Gram's stain No. 1	..	..	..	..	25.00
Gram's Stain No. 2	..	..	..	..	43.00
Hayem's fluid for R. B. C.	..	..	..	..	12.00
Methylene blue	..	..	..	..	61.00
Oxalic acid solution for W. B. C.	..	..	..	..	12.00
Saffranine 0.5% (aqueous)	..	..	..	..	20.00
Basic Fuchsin	..	..	..	..	6.00

DEPARTMENT OF BLOOD BANK

Shri V. M. Patankar, Shri P. R. Dichwalkar and Shri A. A. Parelkar, Senior Technical Assistants were awarded degrees in M.Sc. of the Bombay University.

The following papers were presented by the department at conferences:—

“Studies on the antibody pattern against common infections in Normal Human Immunoglobulin prepared from the plasma of Indian origin”, by Shri V. M. Patankar and Dr. K. V. Kulkarni, at 3rd International Conference on Global Impacts of Applied Microbiology held in Bombay in December 1969.

“Studies in Bacterial contamination of Banked Blood” in Annual Session of Indian Association of Pathologists at Jamnagar, January 1970, by Shri P. R. Dichwalkar and Dr. K. V. Kulkarni.

Training in the field of Blood Group Serology and Blood Banking Technology was afforded to the medical and para-medical personnel deputed by various Governments and Medical Institutions and the following members were trained:—

Shri D. M. Pandya of the Civil Hospital, Ahmedabad; Dr. C. H. Sharma of the Patna Medical College, Patna; Dr. Kulbir Singh; Shri S. N. Bhalerao and Shri N. V. Bhat.

Production

During the year, the routine production activities of the department, viz., collection of blood from blood donors, processing and lyophilization of plasma, fractionation of human plasma and production of blood grouping reagents was carried out as usual (Table No. 26 and 27). With the appointment of a Public Relations Officer, attached to this department, the drive for the enrolment of voluntary blood donors was intensified and extended to wider areas in the State and collection of blood from this source showed a marked increase. The Public Relations Officer has established contacts with many establishments like educational institutions, factories etc. and mobile blood collection sessions were held at these institutions and large numbers of voluntary donors donated their blood.

TABLE 26

Names	No.
Institutions/colleges, factories, etc.	78
Institutions/colleges, factories etc., where blood collection sessions (mobile van) were held.	29
Voluntary donors enrolled	3,844
Blood Donors—	
Voluntary	3,307
Prisoners	2,176
On payment	3,953
Total	
	9,436

Blood plasma and by products

Name of product	Packing	Produced	Supplied
Dried plasma		548.75 lit.	648.25 lit.
Plasma for emergency		95.20 lit.	
Human gamma globulin	1 ml. ampoules	13,308	13,720
Human fibrinogen	150 ml. bottles	280	280
Human fibrinogen	10 ml. ampoules	43	43
Fresh plasma	250 ml. bottles	3	3
Cryoprecipitate plasma		15	15
Human serum albumin		25 gms.	25 gms.
Bovine albumin		200 gms	200 gms
Red blood cells packed		544 bottles.	544 bottles.

Blood grouping reagents

Name of product	Packing	Produced	Supplied
Anti — A	1 ml. ampoule	28,892	27,897
Anti — B	"	29,537	27,971
Anti — A + B	"	2,338	2,090
Coomb's reagent	"	817	803
Bovine albumin	"	1,226	1,218
Anti — C + D	"		23
Anti — D	"	4,875	4,730
Anti — A ₁ (Lectin)	"		28
Anti — H (Lectin)	"	110	73
Anti — M (Serum)	"	80	52
Anti — N (Serum)	"	27	11

Rh tests carried out ..	..	..	330
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Total number of anti-venine ampoules lyophilized (10 ml.)	62,463
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DEPARTMENT OF CHEMOTHERAPY

Good progress was maintained in all the research problems in hand. In the field of synthesis of compounds for possible activity against mycobacteria, the work comprised of the study of ethylene diamine derivatives and the synthesis of new Mannich bases of monosubstituted piperazines. In the area of cancer chemotherapy, new azaspiro compounds and the other Schiff-bases were synthesised and studied in collaboration with Cancer Research Institute. Lead for the synthesis of newer antiprotozoal substances was pursued. On the basis of the random screening results, efforts were directed towards the synthesis and study of triazole and thiadiazole derivatives for their possible hypoglycaemic activity.

Shri J. D. Modi and Shri D. R. Shekawat continued to work as Government of Indian Junior Research Fellow and Unichem Research Fellow respectively. Shri H. V. Patel was the recipient of U. G. C. Fellowship.

During the year under review, Sarvashri R. S. Sheth, D. J. Vadodaria and J. D. Modi obtained the Ph.D. degree of University of Bombay.

Eight scientific papers were published and two more have been accepted for publication.

Routine

The microanalytical section of the department carried out over 2,050 elemental analyses as detailed below :—

Carbon-Hydrogen	694
Nitrogen	559
Sulphur	500
Halogen	305

Production

The production and supply of clinical products for the use of Government hospitals, E. S. I. S. and Public Bodies was maintained at full level. Production of new formulations was taken up as per request of E. S. I. S. authorities. The list of items and their production is given in the following Table No. 28.

TABLE 28

Name of the product	No. of tablets manufactured
Vitamin A. D (P)	91,78,000
Vitamin B. C	1,47,23,000
Vitamin B ₁ 10 mg.	10,92,300
Vitamin B ₁ 50 mg.	10,92,300
Vitamin B ₂	3,89,200
Vitamin C	11,94,800
Nicotinic Acid	7,12,100
Vitamin BC—3	7,15,600
Multi-vitamin	1,51,44,000
Vitamin AD (Coated)	15,50,800
Calcium with Vitamin D ₄	21,89,400
Vitamin B. Complex with Vitamin C and Folic Acid	54,40,700
Polyvitamin	1,53,94,770
Vitamin K	1,95,270
Isoniazid	40,86,100
Iodochlorohydroxyquinoline	17,97,400
Di-iodohydroxyquinoline	15,60,900
Sulphadimidine	1,49,75,500
Sodium p-aminosalicylic acid	1,30,40,400
Thioacetazone-Isoniazid	18,93,700
Sulphadiazine	61,50,000
Sulphaguanidine	90,39,500
Tolbutamide	7,93,300
PAS/INH	600
Chlorpropamide	15,300
Phthalylsulphathiazole	1,90,200
Total	12,25,55,140

Pilot plant activity for the production of tolbutamide, isoniazid and Pemon antiseptic was maintained (Table No. 29).

TABLE 29

Name of the product	Quantity
Tolbutamide	519.0 Kg.
Isoniazid	365.0 Kg.
Pemon	42,420 Litres

DEPARTMENT OF CLINICAL PATHOLOGY

The department continued epidemiological studies on the following diseases like—diphtheria, leptospira and Beta-haemolytic streptococcal infections.

The bacteriological analysis of 100 specimens each of ice-cream, sweetmeats and sugar-cane juice was completed.

Shri K. N. Joishy, who is working on bacterial genetics of mycobacterium, continued to receive the Government of India Scholarship.

Indian Council of Medical Research grant has been received for the project on leptospirosis. Shri A.C. Desai and Kum. H. K. Dange respectively are the Research Assistant and Senior Technical Assistant appointed for this work.

Two papers were presented at the 29th Annual Conference of Indian Association of Pathologists held in Jamnagar this year.

Three papers were presented at the International Conference on Global Impacts of Applied Microbiology at Bombay this year.

Dr. M. V. Sant participated in the I.C.M.R. Cholera Seminar at Calcutta.

Dr. S. Mukerjee has been appointed as Senior Scientific Officer (Class I) for the Section of Diagnostic Re-agents.

Eight trainees students completed a short-term training course in Clinical Pathology Department.

TABLE 30

Routine investigations carried out during the year 1969-70 are presented below (Table No. 30.)

				Total
<i>Histopathology</i>				
Histopathological Examination	..	..	..	3,776
<i>Haematology</i>				
Haemoglobin estimation	..	..	..	348
Total R. B. C. count	..	..	..	365
Total W. B. C. count	..	..	..	414
Differential W. B. C. count	..	..	..	424
P. C. V. estimation	..	..	..	7
E. S. R. estimation	..	..	..	84
Prothrombin time	..	..	..	4
Platelet count	..	..	..	2
Bleeding time	..	..	..	5
Clotting time	..	..	..	5
Reticulocyte count	..	..	..	2
Blood smear for malaria	..	..	..	5
Blood smear for filariasis	..	..	..	5
Bone marrow smear examination	..	..	..	1

Clinical Microscopy

Urine for routine examination	..	..	..	464
Stool for routine examination	..	..	..	312
Semen for examination	..	..	..	3
C. S. F. for cytological examination	..	..	..	143
Other fluids for cytological examination	..	..	..	43
Pregnancy Tests (Qualitative)	..	..	..	2682
Pregnancy Tests (Quantitative)	..	..	..	385

Bacteriology

Blood for cultural examination	..	..	..	63
Urine for cultural examination	..	..	..	326
Stool for cultural examination	..	..	..	5
C. S. F. for cultural examination	..	..	..	6
Body fluid for cultural examination	..	..	..	
Throat swab for cultural examination	..	..	..	24
Antibiotic sensitivity tests	..	..	..	157
Autovaccine	..	..	..	47
Blood for typhoid cultural examination	..	..	..	1525
Specimens for tuberculosis cultural examination (sputum, urine).	..	..	..	107
Specimens for guineapig inoculation for T. B.	..	..	..	27
Smear for acid-fast bacilli	..	..	..	32
Swabs for diphtheriae, cultural examination	..	..	..	315
Material for tetanus, cultural examination	..	..	..	112
Tissue for autolysate preparation	..	..	..	23
Stool for cholera cultural examination	..	..	..	8017
Urine for leptospira examination	..	..	..	7
Smear examination for gonorrhoea	..	..	..	
Miscellaneous cultural examinations	..	..	..	272
Sputum for cultural examination	..	..	..	46

Mycology

Specimen for fungal cultural examination	..	..	..	1
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Serology

Widal tests	..	..	..	1578
Weil Felix tests	..	..	..	1578
Test for brucellosis	..	..	..	1578
Paul-Bunnell test	..	..	..	17
Test for leptospirosis	..	..	..	162
Kahn test	..	..	..	467
V.D.R.L. test	..	..	..	617
Washerman	..	..	..	196
Casoni's (skin) test	..	..	..	4
Frei's (skin) test	..	..	..	1
Schick (skin) test	..	..	..	98

Diagnostic Reagents Section

Rideal Walker Coefficient	..	..	..	45
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Interesting histopathology specimens

Granular cell Myoblastoma in a newborn child
 Urethral Rhinosporidiosis

Production.

The Diagnostic Reagents Section continued its useful activity of production and supply of various reagents (Table 31). The demands are steady. The new items introduced are high titre sera, shigella *Boydii*, *E. coli* 0102 and 'O' ¹¹²

TABLE 31

				Production (ml)	Supply (ml)
Dead emulsion	..	..	..	433755	507655
High-titer sera	..	..	..	5263	5180
Kahn antigen	..	..	..	10520	11460
W. R. antigen	..	..	..	1960	1500
Casoni's antigen	..	..	..	460	410
Cobra venom solution	..	..	..	1985	1643
R. V. venom solution	..	..	..	749	717
<i>E. coli</i> sera	..	..	..	1342	930
<i>S. typhi</i> Vi sera	..	..	..	192	84
Biological stains	..	..	..	1306800	1309500

DEPARTMENT OF IMMUNOLOGY

During the year, the Department produced various antisera and toxoids as usual. The demands for tetanus toxoid showed a steady increase. The Department is experiencing difficulty in obtaining new horses for increasing production of antisera. At the Pimpri farm, the work of construction of 2 new stables and 48 class IV quarters is nearing completion. The construction of 12 class III quarters has started.

Shri J. M. Mehta, Junior Scientific Officer and Shri R. J. Malgaonkar, Senior Technical Assistant resigned. Shri A. D. Kulkarni was promoted to the post of Junior Scientific Officer. Smt. R. V. Jaykar joined the department as Senior Technical Assistant.

The Atomic Energy Commission has given a grant of Rs. 1,665 to conduct work on "Cellular aspects of antibody production and immunoparalysis".

Dr. G. D. Dimitrov of the Institute of Chemical Technology, Sofia, Bulgaria worked in the department on the problem of "Isolation of biologically important factors from Russell's viper venom", for about 1 year and left in November.

TABLE 32

Production

	Products			Quantity (Litres)
Tetanus Toxin—				
(a) Peptic digest medium	..	..	..	700
(b) Protein-free medium	..	..	..	1203
Tetanus Toxoid—				
(a) Peptic digest medium	..	..	..	138
(b) Protein-free medium	..	..	..	1058
Diphtheria Toxin—				
(a) Papain-digest medium	..	..	..	156
(b) Protein-free medium	..	..	..	246
Diphtheria toxoid—				
(a) Papain-digest medium	..	..	..	45
(b) Protein-free medium	..	..	..	226
Adsorbed tetanus toxoid	..	..	..	31
Diphtheria-tetanus toxoid	..	..	..	41
Adsorbed diphtheria toxoid	..	..	..	35
Antivenin	..	..	..	190
Tetanus antitoxin	..	..	..	1554 M. U.
Diphtheria antitoxin	..	..	..	205 M. U.

DEPARTMENT OF NUTRITION AND BIOCHEMISTRY

Nutrition Section

The section is engaged in practical nutrition work, applied dietetics and various problems connected with improving the nutritional status of the people which includes diet and nutrition surveys and several aspects of field studies. The laboratory continued to carry out research work and test food samples for identification, nutrient content and purity.

During the year, the field staff actively participated in the applied nutrition programme launched through the combined efforts of UNICEF and FAO. The programme, as adopted by the State Government is, in essence, to promote education and training in the production, preservation and consumption of nutritionally valuable foods.

The post of the Medical Officer (Class II) remained vacant and the 4 Regional Nutrition Units could not be started.

The research scheme on "Studies in lipid metabolism", sponsored by the Government of Maharashtra, was continued during the year. Shri P. Divakaran, Senior Research Fellow on the Scheme, who had completed his research work for the doctorate degree, left the department to join the research section of Hindustan Lever. His thesis entitled "Biochemical studies on dietary fats" was accepted for the Ph. D. degree of the Bombay University. Shri A. J. Modak, Junior Research Fellow, resigned to join the Indian Navy. In his place, Shri V.D. Rane was taken up and has been given the necessary preliminary training.

The Senior Research Fellowship has not been filled up yet.

Shri A.M. Bijur from the Analytical and Quality Control Department and Shri V.M. Patankar from Blood Bank Department received their M.Sc. degrees in Biochemistry under the guidance of Dr. S.D. Ambegaokar. Shri S. S. Karkhanis from the Pharmacology Department has registered for the M.Sc. degree.

FIELD UNIT

Applied Nutrition Programme

Dietary studies.—Lady Nutritionists continued to visit different areas covered under the A.N.P. with a view to train the non-technical personnel in techniques of conducting diet surveys, to provide reasonably accurate data regarding levels of food consumption amongst the farming population.

During the year, five villages in three blocks were covered. In each village, various functionaries, such as the Sevak/Sevikas, have been trained. They submitted dietary records to the department for verification and analysis. The data were received from the following four villages: Nala village of Bassein

Block (Dist. Thana) Chargaon of Gondia Block (Dist. Bhandara); Shirol and Inzala of Ghatanji Block (Dist. Yeotmal).

Analysis revealed the following.—Bassein Block : Caloric intake was very low, being less than 1900 per C.U. per day; yet the protein consumption was fairly satisfactory, being 55g. mostly derived from foodgrains.

Gondia Blocks—Caloric intake was extremely high, viz. over 3,600 calories, being the highest observed so far. Levels for all the nutrients were high; for instance, protein average is 94g. Ghatanji Block : Caloric intake was quite high, viz. 3100-3300 calories with over 100g. of proteins.

Industrial Canteens.—The Factory Canteen Adviser of the department was entrusted with the responsibility of the scientific study and improvement of the food services for workers through canteens in industrial units, throughout the State.

During the year, 45 canteens in the industrial establishments in Greater Bombay and Thana and Poona Districts were visited in order to evaluate the nutritional merits of the food service to the workers. Based on the detailed study of the existing practices and the nutritional evaluation of the meals and snacks served, reports incorporating suggestions for improvement were sent to the concerned managements.

Nearly 40 per cent of the canteens were run departmentally and the rest either by co-operative societies or private contractors, but appreciably subsidised by the respective managements.

The caloric value of the full meals served in most of the canteens ranged between 800-1100 calories. Most of the meals and snacks needed improvement particularly in respect of vitamins A and C and in certain cases calcium also. In addition to suggestions for improvements in respect of different nutrients through the use of leafy vegetables, combinations of pulses and groundnuts, etc., suggestions were also given in respect of menus for providing variety, sprouting of pulses, palatable salad mixtures, cooking of vegetables etc., to obtain better nutritive value from the foods served.

On account of prohibitive prices of sugar, hydrogenated oils and fats, the preparation of sweet items was either discontinued or limited in many canteens.

The study of the food service in the canteens was followed by nutrition education of the workers regarding the importance of foods and proper food habits, by way of exhibition of instructive posters and charts, talks and cooking demonstrations. Coloured printed posters were also presented for the purpose to interested managements..

Bombay Port-Trust Canteens.—Preparation of a number of popular snacks and meal items was conducted at the main Port Trust canteen to demonstrate practical ways of implementing the suggestions made earlier for improving the nutrient composition of food preparations served through the different canteens under Bombay Port Trust.

Feeding the Toddlers in the Creche.—In response to interest evinced by the management of a textile mill, the meals and snacks served to the toddlers at the creche were studied. In view of the special nutritional requirements of this age group and their poor diets at home, introduction of some nutritious food items like eggs, pulses, groundnuts, vegetables and seasonal fruits in different combinations was suggested as variety and for improved nutrition.

Square Meal Canteen, Sachivalaya, Bombay.—Routine monthly visits were paid to the Square Meal Canteen to check and ensure that the quality and the quantity of the balanced meal served to over 1,000 dinners daily was maintained. The menus were examined for variety, the cooked food was tasted and necessary suggestions were given from time to time on related matters such as food hygiene and cleanliness in the kitchens and the pantry.

With the ever-rising trend in the cost of foodstuffs, the price of the meal was increased from 55 to 60 paise per meal since the middle of the year, to cover the food expenses.

Due to restricted milk supply from Government, the quantity of milk served in the meals (in the form of butter milk) fell short of the recommended allowance (viz. 85 ml.) by about 25-35 ml. It was, therefore, suggested that defatted edible groundnut cake flour may be incorporated in the meal to partly compensate for shortage of milk proteins.

A consumer acceptance trial was conducted by incorporating groundnut cake flour at 10 per cent level in *Chapaties* and also in Cudhi. As both the items were found acceptable, groundnut cake flour was subsequently introduced in the daily preparation of *Chapaties* served in the meal. Arrangements were made with a local oil mill to obtain regular supply of this defatted edible groundnut cake flour for the purpose.

HOSPITAL DIETS

Formulation of new diet scales.—The newly formulated schedule of diet scales for patients suffering from hepatitis, reported earlier, was finally accepted by Government.

Surprise check of diet management.—Eighteen hospitals (nine in the districts and nine in Greater Bombay) were visited. Although most of the hospitals were trying to follow the recommended allowances of dietary items, there is still lot of scope for improvement, particularly in cooking (baking of *Chapaties* etc.) and food service in the wards. The general complaint from most of the patients was with regard to the monotony of food as served. Due to financial limitations, non-vegetarian foods were served less frequently.

Social Dietetics.—Dietary conditions in two Government institutions were studied by correspondence. In both, the management was following, as far as possible, the diet schedule prescribed for the inmates, consistent with the food allowance granted to them.

Education and Publicity.—The Directorate of Publicity having completed the translation of the popular English brochure "Towards Better Nutrition" into Marathi, the latter is now in the press.

Combine taste with nutrition.—Proper techniques with unfamiliar but nutritious food combinations are being evolved by Lady Nutritionists as part of their efforts to improve the nutritional status through persuasive education.

To meet the demand from nutrition-minded women, such recipes have been published (both in English and Marathi) in the quarterly bulletins of the Consumer Guidance Society (Registered), Bombay.

Experimental Cookery.—While information on the nutritive value of individual foods, as purchased, was readily available, there was paucity of information on the calories and nutrient content of food combinations as prepared, served and consumed by different communities. Our effort to provide the necessary source material was continued by standardising a few more recipes during the year.

Education and extension work.—Cookery demonstrations : With the rising spiral of food prices, the popularisation of ' nutritious-cum-palatable ' recipes from locally available foodstuffs has been restricted to a few organisations only. In all, 17 cooking demonstrations were arranged during the year.

At the instance of the Gandhi Smarak Committee, a programme of two cooking demonstrations, illustrated by talks, was arranged at the Mahila Training College, Ratnagiri.

Popular talks with food display.—In response to requests from social welfare organisations, talks on subjects of topical interest were arranged, catering to the needs of a broad spectrum of health-conscious group, such as students of social sciences, social workers, the middle-class intelligentsia pursuing various vocations etc. These talks had proved effective since, even the so-called non-labouring middle-class finds it difficult to provide adequate and satisfactory diets to their families, without basic knowledge about the nutritional merits of foods.

Malnutrition ameliorative programme:—Under the auspices of Bombay Mothers' and Children Welfare Society, and Maharashtra State Anti-tuberculosis Association, a camp was organised at Ashta, District Sangli, with a view to (i) detect cases of T. B. and treat them, (ii) immunize the child population with B.C.G. and Polio etc., (iii) detect diabetes and (iv) educate the mothers on matters of nutrition and child care.

The nutritionists of the department participated in the programme to bring home to the women the necessity of suitable diets for pre-school children who were found malnourished and gave cooking demonstrations of about 8 recipes. In addition, a talk on nutrition in Marathi was given to the nurses who attended the camp.

Diabetic camp at Lonavala.—At the invitation of the Diabetic Association of India, a Senior Lady Nutritionist, who is also a member of the Managing Committee, participated in the three-day camp at Lonavala by arranging a diet exhibition and giving talks to the diabetics on planning diabetic diets etc.

Exhibitions

Nutrition education stalls with display of low cost nutritious recipes were organised at four centres in Greater Bombay. Display of weighed quantities of foods rich in vitamins A and C to indicate how best one could obtain the daily requirements of these two essential nutrients was a novel feature introduced during the year.

Poster display and a wide range of over 15 nutritious food combinations were specially arranged at the public Health Institute, Nagpur, on the occasion of the Fourth State Level Meeting on Applied Nutrition Programme.

Training in Practical Nutrition

Training of various functionaries mostly Auxiliary Nurse-Midwives under the A. N. P. in practical nutrition and applied dietetics was carried out for several batches, at the Public Health Institute, Nagpur.

Nutrition Camp under A. N. P.

A nutrition camp was held at Bailwadi village in Nagpur Division for the house-wives. A talk on nutrition, along with cooking demonstration, was included in the programme. About ten nutritious preparations were demonstrated. Nearly 70 women from the villages attended the camp.

Radio Support for A. N. P.

An additional feature of the programme was the initiation of radio support for the A. N. P. In this connection, the field staff participated in the special training course organised for teachers and gramsevikas and presented talks on different topics from the Poona Station of All-India Radio.

The field staff also participated in the following courses :

A series of ten sessions in Advanced Dietetics and Practical Public Health Nutrition work for the medical graduates of D. P. H. Course of the University of Bombay, which included visit to a primary school to acquaint the post-graduates with rapid survey technique.

Two talks (with food display) were given to the trainees attending a course of the Social Service League, Bombay.

A post-certificate course in Pediatric Nursing at the J. J. Hospital, Bombay. It consisted of nine tutorials and a session on practical dietetic work, viz : cooking demonstration of nutritious snacks and weaning foods.

Training of medical graduates at two Rural Health Training Centres in the State. The medical internees at these centres—called Health Units (now under the control of the Surgeon-General) were acquainted with the techniques of evaluating the role of nutrients and dietary deficiencies in the causation of nutritional ill-health in the rural communities. At the same time, base-line data were also collected from each Health Unit regarding the levels of dietary intake and the incidence of malnutrition which was meant for the ultimate use by the paramedical staff of the respective Health Units.

Wherever possible, Public Health Nurses, Sanitary Inspectors and the Health Educators were also acquainted with the technique for the study of dietary habits and the approach to nutrition education programme for the farming population.

Consultative service

The department continued to render help in an advisory capacity to the K. E. M. Hospital, the Diabetic Association of India, the Pediatric Centre of the J. J. Hospital, S. N. D. T. University and the like organisations. Technical advice continued to be given (in person or by correspondence) to individuals regarding their queries on nutritional merits of food problems in, practical dietetics, such as food selection, suitability of food combinations in health and diseases and so on.

Acceptability Tests with Leaf Protein Flour

At the request of the Central Food Technological Research Institute, Mysore, an acceptability test with leaf protein was carried out in the department. About 30 staff members of the Haffkine Institute participated in the test.

Three varieties of wheat products containing leaf protein were received for the purpose; (i) Leaf protein incorporated wheat flour, (ii) Leaf protein incorporated wheat rawa and (iii) Leaf protein incorporated wheat maida. The three products provided about 15 per cent of proteins.

The object was to find out how far common food preparations made out of the three varieties of leaf protein were acceptable. Keeping in mind the above objectives, 12 common food preparations (9 salty and 3 sweets) were prepared from the abovementioned material in three different ways, i.e.:

- (i) Plain .. by conventional method
- (ii) Plain + artificial colour by using conventional method but adding green colour in order not to distinguish by colour the leaf protein preparations.
- (iii) Leaf protein.

All the preparations were examined for appearance, taste and odour. None of the consumers was aware or given any indication of the presence or absence of the leaf protein in the different preparations. The test lasted for about 3 weeks.

Analysis of the results revealed that the lower limit of acceptability had appreciably fallen with leaf protein items. Considering the hypothesis that an item is acceptable if accepted by 64 per cent and above, both plain and coloured proteins were acceptable. In the case of leaf protein items, 25 per cent items were acceptable by appearance, 33 per cent by taste and only 17 per cent by odour. Thus, the main factors for the lower acceptability of leaf protein items are odour and appearance.

Apparently, the dark green colour of the leaf proteins was largely responsible or the unacceptability of the leaf protein recipes. Secondly, most of the leaf

protein recipes did not seem to be so palatable as their plain counterparts. Here again, the three salty snacks containing a liberal amount of 'masala', were not so disliked, confirming the role of palatability in any unfamiliar food material, however, high it might be in nutritive value.

Routine

A total of 350 testes were carried out on 88 samples of foods and others, (Table No. 33). The tests included Vitamin A, folic acid, vitamin B12, proximate principles, R. M. value, milk fat and acidity.

TABLE 33

Samples	No.	No. of Tests	Nature of analysis
Butter	2	10	Moisture R. M. Value, milk fat, acidity and Furfuraldehyde test.
Milk	6	18	Sp. gravity, fat and solids.
Skim milk powder	2	8	Physical properties, sediment and acidity.
Breast milk	2	2	Vitamin B12
Wheat flour	1	4	Moisture, gluten, ash and acidity.
Groundnut oil	2	12	According to P. F. A. Rules.
Sunflower oil	1	4	Moisture, acid value, colour and insoluble materials.
Human serum	1	1	Folic acid.
Cattle serum	3	3	Vitamin A
Horse feed	3	24	Proximate principles and minerals.
Cattle feed	69	276	Proximate principles.
Total	88	362	

Biochemistry Section

This department continued to render effective service to the city hospitals and private parties. The E.S.I.S. Hospital referred some samples in addition to ordering of biochemical reagents for their laboratories.

Shri M. J. Modak, Diamond Jubilee Research Fellow, was awarded the Ph. D. degree of the Bombay University for his work on "Biochemistry of the Salmonella". He has been continuing his research work in the department as a Haffkine Institute Diamond Jubilee Senior Research Fellow. Kumari Shantha Nair's thesis on "Biochemical studies on *Vibrio* and El-Tor cholera with special reference to berberine action" was accepted for the Ph. D. degree of the Bombay University. Kumari Ranjana Buit, M.Sc., has joined the Department as a Diamond Jubilee Junior Research Fellow. Dr. M. J. Modak and Dr. (Smt.) Shanta Modak attended the Pharmaceutical Congress at Chandigarh and presented three papers. Dr. M. J. Modak attended the 2nd National Seminar on cholera at Calcutta and presented a paper. Shri U. J. Dumaswala attended the Lipid Conference organised by the V. P. Chest Institute at Delhi and read a paper.

Biochemical Tests (Table No. 34)

TABLE 34

Name of the test	Total number
<i>Blood and Serum—</i>	
Bleeding time	2
Clotting time	2
E. S. R.	2
Icteric Index	153
Van den Bergh	79
Amylase	12
Bilirubin	322
Calcium	114
Chlorides	51
Creatinine	58
Cholesterol	250
N. P. N.	18
Acid phosphatase	55
Alkaline phosphatase	279
Phosphorus	119
Total proteins	279
Albumin	204
Globulins	200
Sugar	606
Thymol turbidity	164
Urea nitrogen	81
Uric acid	55
Total	3,105
<i>Body fluids—</i>	
C. S. F. chlorides	129
C. S. F. Proteins	153
C. S. F. Sugar	112
S. M. [test]	48
Pandy's test	2
Total	444

Name of the test					Total number
<i>Urine and stool—</i>					
Acetone bodies	..	..	..	..	2
Bence-Jones proteins	..	..	..	..	2
Bile salts and pigments	..	..	..	..	229
Occult blood (stool)	..	..	..	..	1
Homogentisic acid	..	..	..	..	1
Urobilinogen	..	..	..	..	7
Albumin	..	..	..	..	5
Chlorides	..	..	..	..	1
Porphyryns	..	..	..	..	3
Sugar	..	..	..	..	96
Porphobilinogen	..	..	..	..	3
Calcium	..	..	..	..	10
Uric acid	..	..	..	..	3
Total					363
<i>Special tests—</i>					
Basal metabolic rate	..	..	..	..	32
Iodine (serum)	..	..	..	..	22
Gastric function	..	..	..	..	12
Glucose tolerance test	..	..	..	..	68
Iron binding capacity (serum)	..	..	..	..	1
Iron (serum)	..	..	..	..	4
Fatty acids (serum)	..	..	..	..	3
Phospholipids (serum)	..	..	..	..	3
Lange's test (C. S. F.)	..	..	..	..	62
Potassium (serum)	..	..	..	..	46
Protein-bound iodine	..	..	..	..	56
Cholinesterase (serum)	..	..	..	..	1
Protein fraction paper (electrophoresis)	..	..	..	..	18
Sodium (serum)	..	..	..	..	64
17-Ketosteroids (urine)	..	..	..	..	35
S. G. O. T. (serum)	..	..	..	..	541
S. G. P. T. (serum)	..	..	..	..	545
Urinary stone	..	..	..	..	7
Total					1,520
A total of 5,432 tests were carried out during the year.					
Blood	..	..	..	..	3,105
Urine, stool	..	..	..	..	363
Body fluids	..	..	..	..	444
Special tests	..	..	..	..	1,520
Total					5,432

DEPARTMENT OF PHARMACOLOGY

During the year under review, Kum. H. A. Shah, B. Sc., was appointed as Junior Scientific Officer in place of Smt. Susy Cherian promoted to Senior Scientific Officer Class II in the Department of Bacteriology. Smt. V. S. Joshi, B. Sc., joined as Senior Technical Assistant. Kum. Veena Seshadri, B. Sc., and Kum. Usha M. Shah, B. Sc., resigned as Senior Technical Assistant.

Shri S. N. Iyer was awarded the degree of M. Sc. (Applied Biology) by University of Bombay. The demand for short-term training in drug analysis continued to be very popular. Training was extended to (i) Kum. Usha Mitra from Juggat Pharma Private. Limited. ; (ii) Kum. P. Krishnakumari from Narmiat Powers & Co., Calicut ; (iii) Shri S. K. Rao from Incons, Bombay ; (iv) Shri D. V. Ishwar from Hoechst Pharmaceuticals Ltd., Bombay ; (v) Kum. Vinodini G. Karkhanis and (vi) Kum. Aliamma Cherian.

Routine activities

As in the previous years, testing of drugs, chemicals, fine chemicals, *Ayurvedic* drugs and patent medicines continued.

The collaborative assay of heparin for the establishment of National Reference Standard was undertaken and reported to the Central Drugs Laboratory, Calcutta.

The total number of samples tested for the period under report was 3,400. This number was more than the previous year's total of 2619. The percentage of non-standard drugs for the period under review reached nearly 18 per cent.

Government Analysts' Section.—Since August 1968, the Pharmaceutical Section of the drug testing laboratory has been transferred to the new laboratory established under the Drug Control Administration. The number of the samples received for tests in the department pertained only to the Microbiological and Biological Sections. The number of samples reported during the year under review, under the Drugs and Cosmetics Act was 704. The samples of non-standard quality was 200, which was 28.7 per cent. Out of the 533 samples analysed, in the Microbiological Section, 348 samples were vitamin preparations. One-hundred seventy-one samples came under the purview of antibiotics and the rest as biologicals. The percentage of non-standard samples reported showed that the bulk of the samples came from vitamin preparations. This indicated that greater attention should be paid by the pharmaceutical concerns in stabilizing the vitamin preparations. Preparations containing Vitamin C and its salts showed the highest percentage, i.e. 30 per cent, which were classified as non-standard.

There was an increasing tendency amongst manufacturers to include various enzymes in the digestive preparations. A very high percentage of mixtures of enzymes, on analysis, was found to be failing in one ingredient or another.

It seems that manufacturers should pay greater attention to stabilizing such preparations before marketing.

Samples of antibiotic eye ointments contained large particles and were gritty. A sample of antibiotic, claimed to contain penicillin, did not contain penicillin at all. A sample of triple antigen (diphtheria, pertussis and tetanus) did not comply with the test.

Samples received from Commercial and Government Agencies.—The commercial concerns have made good use of the special facilities afforded to them by Government for testing their products. Samples received in this category were mainly those, where biological methods had to be adopted in determining either their activity or toxicity. Samples of synthetic oxytocins, insulins, toxids, antitoxins, antibiotics formed the bulk of such samples in this category. The number of samples reported was 738.

Samples received from Police and Prohibition Departments and other Government Agencies

With the enforcement of the Prohibition Act in the State, samples of spurious medicinal preparations alleged to contain medicinal ingredients used to be sent to this department for analysis and tests. However, from October 1969, samples of 'toddy', which is sold in the licensed shops in the State, were sent to us for detection of harmful ingredients or other adulterants. The number of spirituous preparations tested was 934 and the number of 'toddy' samples tested was 312. Samples of ganja, bhang and opium continued to be received in this department as in previous years.

The Government Medical Stores Department also referred a number of pharmacopoeial and non-pharmacopoeial preparations for analysis. The number of samples in this category was 362. The Director, Small-scale Industries, Poona, had also taken advantage of the testing facilities for distilled water manufactured by him.

Toxicological and other Miscellaneous Studies

Anti-allergic agents, such as dexamethasone, amoebicidal agents, such as dehydroemetin hydrochloride, anti-convalescent drugs, such as sodium salt of diphenyl hydantoin, parenteral preparation containing coagulating enzymes isolated from Botrapose (Jamaica), parenteral preparations containing enzymes, such as trypsin and chymotrypsin, antibiotic agents like ampicillin, were a few amongst a wide range of drugs and pharmaceuticals reported on toxicity tests.

Three-hundred samples comprising of antibiotics, such as streptomycin, chloramphenicol and its preparations, tetracycline hydrochloride, were tested for their safety or undue toxicity tests.

Four samples of extracts of plants, assumed to possess hypoglycaemic activity, were tested. The extracts showed only slight hypoglycaemic activity.

Five samples of iron sorbitol and other iron preparations containing vitamins were tested for iron absorption and toxicity tests. Only one sample failed to comply with the iron absorption test.

Local anaesthetic activity of sample of Lignocaine hydrochloride injection was determined biologically and was found to be less active than the claim made.

Amongst a wide variety of hormones and their preparations received for test, adrenaline, synthetic oxytocins, pituitary (posterior lobe) and insulins formed a major part. Out of 90 samples received for adrenaline assay, two pink coloured samples of adrenaline hydrochloride injections showed fifty per cent of activity.

Fifty-eight samples of oxytocin, synthetically prepared, were tested for their oxytoxic activity. All the samples were found to be of standard quality.

Eight samples of pituitary (posterior lobe) were tested for their potency, vasopresin activity and antidiuretic properties. All of them were found to comply with the test. Insulin was being regularly received from some manufacturers. Thirty-nine samples were tested during the period under report and were found to contain the requisite amount of activity.

Two-hundred and thirty-four parenteral preparations were tested for absence of pyrogens. Only six samples failed to comply with the test.

DEPARTMENT OF VIROLOGY

The measles vaccine trial was completed. Serological studies are in progress. Meanwhile, some collaborative studies on measles epidemiology, wherein this department will be responsible for laboratory studies, have been started with two of the participating medical colleges.

Dr. K. H. Dave, Senior Scientific Officer and Officer in-charge of the Enterovirus Unit of the Indian Council of Medical Research, has been appointed a member of the Virus and Arthropod Borne Diseases Expert Committee of the Indian Council of Medical Research.

Shri S. M. Desai, Junior Scientific Officer, attended a one-week W.H.O. Seminar on the Laboratory Diagnosis of Small-pox, at Bandung, Indonesia.

In the months of November and December 1969, and January, 1970 outbreaks of influenza were reported from several areas, both in India and abroad, and there were newspaper reports and forecasts of an epidemic wave. A meeting of concerned officers was called by the Secretary to the Government of Maharashtra, Urban Development, Public Health and Housing Department, to discuss the situation in the State. Another meeting was held on the same day at the Municipal Corporation to discuss the influenza situation in the City. Both these meetings were attended by the Director and the Assistant Director, Department of Virology, representing this Institute. The reports obtained from various authorities and from private practitioners in Bombay were all in favour of the view that there was a rise in the incidence of upper respiratory infections but that it was not of epidemic proportions. It was agreed that Influenza vaccine should be produced on a limited scale for the key personnel only.

Routine

Rabies diagnosis.—Twenty-one brain specimens were referred for diagnosis of rabies. Of the 18 specimens tested, 3 specimens were positive for Negri bodies, by direct microscopy, 9 specimens (inclusive of the above 3) were positive for virus isolation by mouse inoculation test. Most of the negative specimens were from cases where no suggestive history was available and the test was requested to rule out the possibility of rabies. The results are given in Table.

Sub-acute progressive encephalitis.—Measles has been shown to be an aetiological factor in several cases of this condition. Hence, sera of such cases were referred for assessment of antibodies against measles. Ten cases were studied for antibodies to measles. Two of these showed high titres suggestive of recent acute measles infection, 2 cases showed minimal haemagglutination inhibition titres similar to those found in adults with a history of measles in childhood. The number of cases studied is too small for assessing the significance of these findings in relation to the clinical syndrome of subacute progressive encephalitis.

Poliomyelitis in Bombay During the period August 1969 to April 1970, 410 cases of paralytic poliomyelitis were recorded in Greater Bombay. Of these, 305 case (74%) were recorded in children between the age groups 7 months to 2 years. The monthly and age distribution are shown in the Table No. 35. There was no change in the epidemiological pattern of the disease.

TABLE 35

Case incidence by month			Case incidence by age		
Month	No. of cases		Age	No. of cases	
August, 1969	..	103	0-6 months	..	18
September, 1969	..	84	7-12 months	..	139
October, 1969	..	50	1-2 years	..	166
November, 1969	..	36	2-3 years	..	44
December, 1969	..	37	3-4 years	..	14
January, 1970	..	25	4-5 years	..	4
February, 1970	..	24	5 years onwards	..	25
March, 1970	..	33			
April, 1970	..	18	Total	..	410
Total	..	410			

Poliomyelitis in Ceylon

During the period under report, materials for virological and serological studies from 94 cases of paralytic poliomyelitis were received by the unit from Ceylon. Virological studies have been completed on 50 cases. The details of the results are as follows:—

Type	No. of cases	
Poliovirus type 1	..	17
ECHO virus type 7	..	2
ECHO virus type 12	..	1
Untyped	..	7
No virus isolated	..	23

Production

Production of anti-rabic vaccine	..	2,912,678	ml.
Production of canine vaccine	..	22,848	ml.
Production of hyperimmune rabies serum	..	400	doses
Production of polio vaccine	..	1,282,815	doses
Production of influenza vaccine Type A ₂ /Hong Kong/68.		2,500	doses

DEPARTMENT OF ZOONOSIS

The Department of Entomology was converted into the Department of Zoonosis in February 1970.

Plague epidemiological studies were carried out in Thana and Nasik districts and were concluded in March 1970.

Shri A. R. Arwari from the office of the Executive Engineer, Hydraulic Branch, was trained in the identification of parasites from water samples.

Smt. J. R. Bharucha continued to work as a Government of India Scholar.

Dr. G. C. Chaturvedi was promoted as Senior Scientific Officer, Department of Entomology and took over from September 1969.

Routine

During the period under report, vacuum method was tried to milk the Krait snakes and this method was found to be advantageous and yielded about four times more venom in comparison to the old method.

The Table No. 36 details the number of snakes and other items received from outside for identification and advice.

TABLE 36

Animals						Number of samples
Snakes	..	..	..	..	..	67
Rodents, insects etc.	..	.	..	..	..	33

Production :

Venoms				Production (gms)	Supply (gms)
Krait	..	..	..	85.44	56.78
Cobra	..	..	..	152.45	69.72
Viper	..	..	..	62.46	39.74
Echis	..	..	..	51.39	4.05
Total				351.74	170.29

Scorpion venom

Name		Production (gms)
Black <i>Palamaneus gravimanus</i>	..	1.54
Red <i>Buthus tamulus</i>	..	0.17
Total		1.71

ANIMAL FARM

The production and supply of different species of laboratory animals were satisfactory and as per the requirements of the departments during the year under report.

Students of the D.M.L.T. and Technicians' Training Course were trained in general management of laboratory animals. Trainees of the Laboratory Animals Technicians' Training Course conducted by the Laboratory Animals Information service (I. C. M. R.) were provided facilities for practical work in connection with their course. Dr. M. M. Joshi of Chowgule and Company, Bombay ; Shri Deshpande of Executive Engineer, Field Unit Division, Bombay Shri Gopal Raju and Shri K. Bhaskar Achari of Biological Evans, Hyderabad, attended the training in breeding, maintenance and management of animal house.

The following figures show the number of different species of laboratory animals bred in the farm during the year under review.

White mice	..	..	..	..	2,32,454
Albino rats	..	..	..	..	12,013
Rabbits	..	..	..	..	3,287
Guineapigs	..	..	..	..	4,506
Hamsters	..	..	..	..	1,510
Cotton rats	..	..	..	..	38

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

LIBRARY SECTION

Scientific literature, especially in the fields of chemistry, biology and medicine, is increasing in geometric progression, while the facilities of space, staff and equipment have remained more or less stationary over the last many years. This has made it necessary to carry out reorganisation from time to time, so that the library can cater to the needs of research workers. The necessity for increase in staff and facilities is acutely felt.

During the year under review, some reorganisation was carried out, especially in the current journals and books sections. The current journals were rearranged and the rooms were equipped with better lighting and ventilation. However, there is need to air-condition this room so that readers will make better use of the vast and ever-increasing literature being published in scientific journals. There is also need for a seminar room where small groups can hold scientific discussions. This is being given effect to and it is hoped that during the coming year, the seminar room will be ready for use.

Hours of works.—Hours of work were extended during the year by about two hours and the library remained open till 7-00 p.m., to enable our research staff and outside readers to make use of the facilities of the library.

Library services.—The library participated in the inter-library loan service with other scientific libraries. It collaborated in exchange programmes sponsored by the 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 visiting the library	..	2,500
Number of books and periodicals issued to our staff	..	1,100
Number of books and periodicals issued under inter-library loan service.		225
Books and periodicals obtained for our staff from other libraries		115

Additions to library

Number of books added during the year	..	..	250
Number of volumes bound	..	..	465
Number of current periodicals subscribed	..	..	145
Current periodicals received free of charge	..	..	105
Reports of scientific institutions received	..	..	40

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- Baxi, A. J., Parikh, N. P. and Jhala, H. I. :
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Absorption, distribution and excretion of berberin (1969).
Indian Journal of Medical Research, 57, 2128.
- Bhide, M. B. and Modak, M. J. :
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Nature, 223, 842.
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- Chaturvedi, G. C. and Deoras, P. J. :
Colour-phases of *Rattus rattus* and their flea-infestation in two dock areas of Bombay Port Trust (1969).
Pesticides, 3, 41.
- Chaturvedi, G. C., Deoras, P. J. and Rao, N. R. :
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- Dutta, N. K. :
Haffkine Institute : Its contribution towards services and research in the country (1969).
G. I. A. M. Souvenir.
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Cholera-genic Toxin—A new factor in the causation of cholera (1969).
Journal of Scientific and Industrial Research, 28, 334.
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A study on the antifertility action of VIDR-2GD :
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Iyer, L. S. and Sant, M. V. :

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5, 235.

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Anonymous mycobacteria from human and animal sources (1969).

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5, 238.

Joishy, K. N. and Sant, M. V. :

Some observations on a typical mycobacteria from tuberculous meningitis in children.

Part I : Isolation, Cultural, Biochemical and Biological Studies (1969).

Indian Journal of Tuberculosis, 14, 106.

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Indian Journal of Tuberculosis, 14, 118.

Kulkarni, B. S., Fernandez, B. S., Patel, M. R.,

Bellare, R. A. and Deliwala, C. V. :

Chemotherapy of Tuberculosis, Part IX : Synthesis and Screening of New Thiazolyl Thiocarbanilides (1969).

Journal of Pharmaceutical Sciences, 58, 852.

Modak, M. J., Modak, Shanta and Venkataraman, A :

A simplified MAK column procedure for the separation of RNA subfractions (1969).

Analytical Biochemistry, 32, 284.

Modak, M. J., Nair, S. and Venkataraman, A :

Studies on the Fatty Acid Composition of the Salmonella (1970).

Journal of General Microbiology, 60, 151.

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'Properties of RNA containing bacteriophage f4 (1969).

Experientia, 25, 1027

- Mokashi, S. J., Dave, J. K. and Sant, M. V. :
A three-minute immunologic slide test for pregnancy (1969).
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- Parikh, N. P., Baxi, A. J., Jhala, H. I. and Kulkarni, K. V. :
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3-Azaspiro (5·5) undecanes (1969).
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- Williams, E. M. Vaughan, Dohadwalla, A. N. and Dutta, N. K. :
Diarrhoea and accumulation of intestinal fluid in infant rabbits infected with *Vibrio cholerae* in an isolated jejunal segment (1969).
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Indian Journal of Medical Research.
- Bhide, M. B., Aroskar, V. A. and Dutta, N. K.
Release of active substances by cholera toxin.
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Journal of Infectious Diseases.
- Bundeally, Amina and Rao, S. S.
The nature of the protective antigen of *Bordetella pertussis*.
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Effect of increased DDT tolerance on the duration of life cycle in *Xynopsylla cheopis*, Roth. *Pesticides.*
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A note on high incidence of flea infestation in *Rattus rattus*.
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- Chavan, A. S. and Sant, M. V.
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 Deliwala, C. V.
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- Modak, M. J., Aroskar, V. A. and Bhide, M. B.,
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 inactivating agent.
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 Effect of berberine on the fatty acid composition of vibrio and *El tor* cholera.
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 Preparation and purification of fibrinogen.
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 Possible origin of syrian Christians in India on the basis of distribution o
 blood groups and other genetical characters,
Indian Journal of Medical Research,
- Rao, S. S., Patki, V. M. and Kulkarni, A. D.,
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Indian Journal of Biochemistry.
- Rumao, L. M. and Sant, M. V.
 Study of streptococci isolated from scabies cases.
Indian Journal of Medical Sciences.

MAINTENANCE ENGINEERING AND CARPENTRY SECTION

During the year under review, the Section was fully busy with increased workload and maintenance of equipment etc.

It is gratifying to record that we could undertake the repairs and maintenance of multi-stage ultra-low temperature (-700°C) deepfreezing, including the cutting open of a stator of a sealed compressor, got it rewound and fitted back. We could also attend to the defects in micro-vac pump of the freeze-drying plant and effect the replacement of mechanical shaft seals.

A completely new job handled included the Deminorolit plant of the Immunology Department. Its metafilter had got choked, the conductivity bridge was out of commission and feed pumps were damaged. All these were set right after proper detection of faults.

Other reconditioning jobs done included repairs to badly scored cylinder head of air compressor. This was got rebored and by using oversize piston rings, the compressor could be put back into working condition.

The two strumia freeze-drying machines received from the Army as scrap were dismantled and after getting the necessary components etc., reassembled into good working units.

In addition, a large amount of installation work of refrigeration machinery equipment both for laboratory and production and fabrication of and repairs to innumerable items like ovens, centrifuges, compressors, various shaking machines, stirrers, pharmaceutical machinery etc., was carried out.

The carpentry section also contributed substantially to the yearly output. Specially required wooden items for research and production, such as animal dispatch cages, crates for 8, 12 and 20-litre pyrex jars, laboratory racks, partitions etc., were made.

Also the annual hydraulic testing of all the equipment and machinery that works under pressure, as required under Factory Act, was carried out.

