



सत्यमेव जयते

GOVERNMENT OF MAHARASHTRA

**HAFFKINE INSTITUTE
BOMBAY**

**ANNUAL REPORT
1967-68**

Printed in India by the Manager, Government Central Press, Bombay,
Published by the Director, Government Printing, Publications and
Stationery, Maharashtra State, Bombay-4

CONTENTS

PART I

	PAGE
1. GENERAL REVIEW	3

PART II

A. RESEARCH PROJECTS—

(a) Cholera Project	11
(b) Diphtheria Project	14
(c) Enterovirus Project	16
(d) Plague Project	19

B. OTHER DEPARTMENTAL RESEARCH—

1. Department of Bacteriology	22
2. Department of Blood Bank	28
3. Department of Chemotherapy	31
4. Department of Clinical Pathology	35
5. Department of Entomology	36
6. Department of Immunology	39
7. Department of Nutrition and Biochemistry	43
8. Department of Pharmacology	49
9. Department of Virology	52

PART III

ROUTINE ACTIVITIES

1. Analytical and Control Department	61
2. Department of Bacteriology	63
3. Department of Blood Bank	66
4. Department of Chemotherapy	68
5. Department of Clinical Pathology	70
6. Department of Entomology	71

	PAGE
7. Department of Immunology	72
8. Department of Nutrition and Biochemistry	74
9. Department of Pharmacology	79
10. Department of Virology	82
11. Animal Farm	84
12. Library Section	84
List of Research Papers Published	85
13. Maintenance Engineering Section	88

PART IV

MISCELLANEOUS

1. Critics Club	91
2. Distinguished Visitors	93
3. Academic Courses and Training of Health Personnel	96
4. Fellowships and Scholarships	102
5. Guest Workers and Trainees	105
6. Grants-in-aid Research Schemes	105
7. Business Side	107
8. Welfare Activities	109
9. Haffkine Institute Co-operative Credit Society Ltd.	110
10. Dispensary	110

STAFF

THE HAFFKINE INSTITUTE, BOMBAY

Director

Dr. H. I. Jhala, M.D. (Bom.), F.C.P.S. (Bom.), D.T.M. (Cal.), F.A.M.S., F.A.,
Sc. Path. (London).

Assistant Directors

Dr. N. K. Dutta, D.Sc. (Oxon.), D.Phil. (Oxon.), M.B.B.S. (Cal.), F.N.I.,
Assistant Director in charge, Department of Pharmacology.

Dr. P. J. Deoras, M.Sc. (Nagpur), Ph.D. (Durham), F.E.S.I., LL.B.
Assistant Director in charge, Department of Entomology.

Dr. C. V. Deliwala, M.sc. (Bom.), Ph.D. (Bom.)
Assistant Director in charge, Department of Chemotherapy.

Dr. K. H. Dave, M.D. (Bom.),
Assistant Director in charge, Department of Clinical Pathology and
Diagnostic Reagents (up to 25th September 1967).

Dr. M. V. Sant, M.D. (Bom.), F.C.P.S. (Bom.) Assistant Director in charge,
Department of Clinical Pathology and Diagnostic Reagents (from
26th September 1967).

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

Dr. (Smt.) Alamela Venkataraman, B.A. (Madars), M.Sc. (Madras), Ph.D.
(Madras).
Assistant Director in charge, Department of Nutrition and Bio-
chemistry.

Dr. S. S. Rao, M.Sc. (Banaras), Ph. D. (Toronto).
Assistant Director in charge, Department of Immunology (on deputation
from 5th September 1967 to 17th December 1967).

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

Shri Y. S. Nimbkar, B.Sc. (Bom.), Dip. Bact. (Manchester),
Assistant Director in charge, Department of Bacteriology (Additional
charge of Assistant Director in charge, Department of Immunology
from 5th September 1967 to 17th December 1967).

Dr. M. K. Habbu, B.Sc., Ph.D. (Bom.),
Assistant Director in charge, Department of Analytical and Control
(Up to 18th July 1967).

Dr. R. B. M. Colah, M.Sc., Ph.D. (Bom.),
Assistant Director in charge, Department of Analytical and Control
(from 5th October 1967).

Heads of Sections

Dr. A. E. Kulkarni, G.B.V.C. (Bom.)
Senior Scientific Officer (Class I), in charge Animal Farm.

Administrative and Auxiliary Sections

Shri T. V. Ranganathan, B.A. (Madars),
Personal Assistant to the Director.

Shri P. K. Sholapurwala, B.Sc. (Bom.), LL.B. (Bom.),
Business Manager.

Shri P. K. Godbole, B.A., B.Com.,
Cost Accounts Officer.

Shri A. T. Maniar, B.Sc.,
Stores Officer.

Shri M. T. Jagatani, B.A.,
Stocks and Supplies Officer.

Shri A. R. Dhavlikar, G.D.C.,
Special Officer (Recoveries).

Smt. S. P. Gaitonde, B.A.,
Labour Welfare Officer (from 1st March 1968).

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

Shri M. R. Chaudhari, Dip-Tech. (Electrical), A.M.I.E. (India),
Assistant Engineer (Maintenance).

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

PART I
GENERAL REVIEW

GENERAL REVIEW

The year 1967-68 witnessed industrial slacking and an all-round recession, as a result of which there has been considerable slowing in the production activities of many manufacturing organisation. Haffkine Institute did not escape the adverse effects of it. The purchasing power of the various Government institutions, where most of the Haffkine Institute products are sold, was on a restricted scale. Our industrial activity showed some increase, but the expenses were high. The sales of the Institute had reached 93 lakhs which were 14·15 lakhs more than the previous year. The high cost of raw packing materials, increased freight charges and increased dearness allowances for the staff, reduced our profit.

During the year under review, the Institute was greatly honoured by the award of Sir Shanti Sarup Bhatnagar Memorial Award to Dr. N. K. Dutta, the present Director. The presentation ceremony for the award of S. J. Watumull Foundation Award in Medicine for the year 1965 to Dr. Dutta took place in November 1967. The Prime Minister presided over the occasion. Dr. S. S. Rao, Assistant Director in-charge Department of Immunology, went abroad on a W.H.O. Fellowship. He spent about 3 months visiting different laboratories in U.S.A. and Canada. The Institute spared the services of Kum. M. S. Kotnis and Kum. Shanti Arora of the Nutrition Department to the F.A.O. on special assignments to African countries. Dr. P. J. Deoras attended an international "Symposium on Endocrinology" held in New Delhi. He also attended the meetings of the Transport of Live Animals Committee under the Indian Standards Institution.

Many scientists from different parts of the world visited the Institute. They came from Australia, Czechoslovakia, U.S.S.R., Philippines, Switzerland, Israel, U.S.A., Norway, Japan, Sweden, Romania, East Pakistan, Yugoslavia, Northern Ireland and West Germany.

The Institute continued to attract fellowships and scholarships from many sources like Council of Scientific and Industrial Research, Government of Maharashtra, Unichem Laboratories Ltd., Sir Dorabji Tata Trust, University Grants Commission, Indian Council of Medical Research, besides from the Haffkine Institute Diamond Jubilee Fund. It also received grants for researches from World Health Organisation, Indian Council of Medical Research, Council of Scientific and Industrial Research. Six guest workers came from abroad (Teheran, Jordan, Philippines and Kabul) under the W.H.O. Fellowship and had spent varying periods for studies. One Pool Officer of the Council of Scientific and Industrial Research joined the Institute.

There were 15 recognised post-graduate teachers of the Bombay University under whom the students have been working for M.Sc. or Ph.D. Three officers of the Institute are also recognised by the College of Physicians and Surgeons of Bombay for training candidates in F.C.P.S and D.P.B. The number of students who obtained M.Sc. was six. Eight students were awarded Ph.D. of the Bombay University. The total number of students who joined the Institute for undergoing short training courses numbered 30. Seven students were trained under the Technicians' Training Course and all of them passed. Twelve students were admitted for D.M.L.T. Course (University of Bombay) and they received the Diploma at the end of their training period (one year).

The members of the Critics Club had an opportunity of listening to the lectures of nine distinguished scientists from abroad.

The library continued to serve the scientific public with facilities for study. One hundred and three new books were added during the year. Reports of sixty scientific institutions were received. One hundred and fifty-one periodicals were on the subscribed list.

Forty-four research papers covering various research branches of medicine, chemistry and bio-chemistry were published. One of the most significant contributions made during the recent years was of the finding of a drug against small-pox and chicken-pox. A chemical substance was isolated from the seeds of Wild Banana plants which is popularly known as *Chawai* and is found in abundance in the Western Ghats. Small-pox is a great scourge in the South-East Asia. In India, about 30,000 people die annually and an equal number suffers from ill effects. Those who survive are disfigured and may even go blind. Powdered seeds of Banakadali (botanically identified as *Ensete superbum* Cheesm) are popular with Ayurvedic practitioners for preventing and treating small-pox and chicken-pox. But it is for the first time that the value of the indigenous drug has been confirmed in scientifically controlled experiments. One has to go a long way before the pure substance, isolated from the seed, could be used on man.

One interesting work came out in the field of cholera. It may be recalled that a new toxin has been isolated from the cholera vibrio here. This is responsible for the diarrhoeal symptoms in man. A question arose as to whether this toxin acts locally in the intestines or is absorbed into the blood and then exerts its effect. Dr. Vaughan Williams of the Department of Pharmacology, Oxford, who had specially come here to tackle this problem, showed that this toxin was absorbed through the intestines into the blood and this was a very important factor in the causation of the disease.

The Institute has developed a potent plague vaccine which is expected to be used as a reference standard for assay of other plague vaccines. A new method for the standardisation of cholera vaccine using intracutaneous permeability of the cutaneous vessels in the rabbit was developed. This method gave unreplicable results and, therefore, could be used for the assay of cholera vaccines.

A major problem facing our country in the field of nutrition is the protein deficiency. In order to meet the situation, a search for a new and cheap protein-rich food from any plants and live-stock sources has been going on for quite some time. In this context, "Gawar" meal, a protein (48 per cent.) rich by-product of a gum producing industry, was investigated for its nutritive value. Based on the feeding experiments on rats, it was found that the nutritive value of "Gawar" was deficient in two amino-acids (final breakdown of a product of protein). If "Gawar" has to be used as a meal, it will have to be supplemented by the two amino-acids, namely typtophane and methionine.

Cyclamate is now being used widely in place of the sweetening agent—our common sugar. The effect of sodium cyclamate was studied on pregnant rats. It was found to have no adverse effect on the fertility rate, nor on the litter size, nor on the birth rate and the survival of the young ones. It has no bad effect on the growth of the rat too.

It has been previously shown that berberine hydrochloride—an alkaloid isolated from an Indian medicinal plant—is effective in controlling diarrhoea and acute cholera. This drug has been introduced in the market by four or five firms of our country. The mechanism of the anti-diarrhoeal action was studied. It was found to reduce the effect of histamine, 5-hydroxy-tryptamine and bradykinin, thereby suggesting interaction between these substances and berberine in reducing the diarrhoeal effects. The effect of berberine on incorporation of certain precursors of protein—phenyl alanine, leucine and glycine—on the log phase of the cholera vibrio was studied. About 75 per cent. inhibition as indicated by specific activity and total radioactive counts in the total cells was observed. These studies showed that berberine interfered with the protein synthesis. This might be one of the possible reasons of benefit of the drug in acute cholera.

In our previous report, it was mentioned that considerable progress has been made in the manufacture of acetone-dried typhoid vaccine. This type of vaccine is likely to replace the conventional vaccine that we have been using now. More batches of acetone-dried vaccine were prepared. It was observed that the moisture content in the vaccine played an important role on its potency. When moisture exceeded certain level,

there was deterioration of the potency. It was necessary to have further experimental and clinical data of the vaccine before it could be released for general use.

Considerable research had to be done to fractionate gamma globulin from blood plasma. Now, this was successfully done and the manufacture of gamma globulin begun. Placental plasma was one of the sources of supply of gamma globulin. Efforts are now being made to manufacture albumin, which is used in the cases where plasma is contraindicated. Various tests are being carried out before its recommendation for human use.

For several years, work has been going on "Anonymous mycobacteria.". These organisms belong to the class of *Mycobacterium*, of which the classical tubercle bacilli is an example. They showed typical characters of *M. tuberculosis* yet, they do not produce pathological lesions like tuberculosis. The investigation, which was completed, showed that these organisms did not produce typical lesions as those of the classical tubercular bacilli and were more resistant to the common anti-tubercular drugs. They never produced severe types of lesions.

The epidemiological studies of leptospirosis, both in man and in animals, (zoonosis) have shown that this disease is prevalent in 10 per cent. of the human specimens examined and in sheep and dogs. It is one of the frequent causes of abortion in animals.

In order to find out a potent tetanus bacilli, which would give more toxin than the present one in use, systematic studies were undertaken. Nutritional requirements of the tetanus bacilli were subjected to scrutiny. It was found that the bacteria could grow and produce a fair amount of toxin if acid hydrolysed casein medium in which it was grown was supplemented with cystine, tryptochane, vitamins (seven in number) and salts. An attempt is being made to extend this work on a pilot scale, so that this particular bacilli could be used for the preparation of anti-toxin.

The culture of tissue cells is one of the important tools of virus investigation. Studies on the effect of temperature of fully-grown monkey kidney cells showed that cell suspension could be stored at 0-4°C up to 15 days; at room temperature up to 2 days, and at 37°C up to 7 days.

The study of rats as a carrier of human virus infection was a new line of work taken up during the year. Rats have been known to be reservoir of plague, leptospirosis and other bacterial and parasitic diseases. Field teams of the Haffkine Institute trapped rats in the country side and brought them alive for these studies. Examination of the blood of some of these captured rats showed the presence of virus. The identification of the virus was being carried out.

The Department of Virology manufactured about 4 million ml. of antirabic vaccine. About 70 lakhs doses of oral polio vaccine were processed. The Institute supplied polio vaccine to the Bombay Municipality, to the various institutes of the State, and to various places in India.

The breeding and supply of laboratory animals were maintained at a satisfactory level. People came from far away places to visit our Animal House. Two scientific workers were trained in the production and rearing of laboratory animals. One of them came from Ludhiana and the other from Kerala.

The social and welfare activities of the Institute were continued with greater enthusiasm. In the field of sports, besides the usual games that were conducted, one "drama" was staged for the collection of funds. The Institute proposed to have a Sports Pavillion, where mostly indoor games will be played. This was a long-felt need. The members of the staff also collected money on the Flag Day and for the Koyna Disaster. Children below six continued to get free supply of skim milk from UNESCO. The residents of the Chawls voluntarily conducted free classes in the evening for the children studying in the I to VIII Standards. The Co-operative Credit Society advanced Rs. 3.2 lakhs as loans to the employees.

PART II

A. RESEARCH PROJECTS

B. OTHER DEPARTMENTAL RESEARCH

A. RESEARCH PROJECTS

(a) CHOLERA PROJECT

Department of Pharmacology

(i) *Bioassay of live cholera vaccines and their antigenic inter-relationship by infant rabbit model.*—Efficacy or otherwise of live vibrio cholera vaccines is demonstrated in a passive immunisation test in the infant rabbit against homologous and heterologous challenge. Adult rabbits were immunised with different types of live vibrio vaccines and the antisera obtained from these animals were used for passive immunization of a group of infant rabbits that were subsequently challenged intra-intestinally with live vibrios. The results were subjected to statistical analysis. In infected rabbits treated with antisera, three distinct responses were recognised. Some treated animals did not live longer than the untreated ones, some died but their death was delayed, and some survived the challenge. The potency of a vaccine was calculated according to two given formulæ depending upon whether the animals survived or not. When the 95 per cent confidence intervals for the mean survival time between two groups of animals do not overlap, their mean survival times are taken as significantly different. Monovalent live vibrio vaccines of *V. cholerae Inaba*, *V. cholerae Ogawa*, *V. eltor Inaba*, *V. eltor Ogawa* and NAG strains demonstrated good antigenic value against homologous challenge but not against the heterologous challenge, except in the case of the vaccine containing *V. cholerae Inaba* 569B which showed significant protection against homologous and heterologous challenge with *V. cholera Inaba*, *V. cholerae Ogawa*, *V. eltor Inaba*, *V. eltor Ogawa* and NAG. The method is recommended to be studied for assay of cholera vaccines.

Departments of Pharmacology and Bacteriology

(ii) *Attenuation of V. cholerae with a view to produce live Cholera Vaccine.*—On repeated sub-culture in MacConkey's broth, it was observed that *V. cholerae* 569B (Inaba) strain lost its virulence. After 30 passages through MacConkey's broth, the LD₅₀ in mice of the passaged strain rose from 1000-2000 organisms to 10⁶ organisms. This strain also lost its cholera-genic property in the infant rabbit.

Department of Bacteriology

(iii) *Live vibrio cholera vaccine.*—The strain C14 S5 of *V. Cholera Ogawa* obtained from Dr. Bhaskaran of Central Drug Research Institute was studied for its virulence to mice. The LD₅₀ of this strain in N. I. H. strain mice was found to be 16×10⁶ organisms in comparison to 3 organisms of the virulent strain 5321 Ogawa.

Department of Pharmacology

(iv) *Laboratory assay of field-tested Cholera Vaccines by infant rabbit method.*—A quantitative assay of vaccines used in the field in Dacca in 1963 and in Calcutta in 1965 has been carried out as recommended in the bioassay technique given above. The laboratory assay values were compared with the relative effectiveness of the vaccines in the field trial.

The five cholera vaccines, one from Dacca (1963 field trial) and four from Calcutta (1965 field trial) that have been studied in the field were tested for potency by the infant rabbit protection test. The results of the rabbit tests and those in field trials were found to be comparable.

Department of Bacteriology

(v) *Standardisation of cholera vaccine.*—The comparative studies on one batch of lyophilised monovalent vaccine (*V. cholera* Ogawa) by using F. P. and S. H. tests and their modifications were continued. Eleven tests were completed. Statistical analysis of some of these tests showed that F. P. tests gave a 5–6 times higher ED_{50} than S. H. test and the dose response line of F. P. had a higher mean slope indicating that F. P. test may give a more reliable value of ED_{50} . Further detailed statistical analysis is in progress.

Department of Pharmacology

2. (i) *Studies on cholera toxin.*—Suitable media for production of cholera toxin. The preparation of a potent cholera toxin in relatively pure form, requires that the organism be cultivated in a liquid medium. This is beset with difficulties to find out suitable media which will give a high yield of toxin. Several factors were considered and put to the test, viz., temperature of incubation, oxygen supply and time of incubation, rate of shaking during cultivation etc.

The following media were tried for preparation of the toxin :—

(i) Cohen and Wheeler's medium (modified by Bundeally and Rao) where several modifications were introduced. (ii) Finkelstein and Lankford's medium.

The above media were modified by using (a) acid hydrolysed casein, (b) pressure hydrolysed casein.

Results.—The media of Cohen and Wheeler (or its modification) in which *V. cholerae* were grown, were centrifuged. The supernates and the

sonicated residual cells of these media did not produce choleraemic signs and symptoms in the infant rabbits.

(b) The modified medium of Finkelstein and Lankford (acid hydrolysed casein) in which *V. cholerae* was grown, was centrifuged. The supernate and the sonicated residual cells of this medium produced in the infant rabbits typical signs and symptoms of cholera.

(c) The modified medium of Finkelstein and Lankford (pressure hydrolysed casein) in which *V. cholerae* was grown, was centrifuged. The supernate did not produce any diarrhoeal symptoms in the infant rabbits. The sonicated residual cells produced only slight diarrhoea and some fluid accumulation in the large intestine.

(ii) *Intra-intestinal route of administration of ultra-sonic toxin.*—Intra-intestinal route of administration of ultra-sonic toxin in the infant rabbit was tried. Much lower dose of the toxin was required for producing diarrhoea in these animals as compared to oral feeding of the toxin. Only 1 ml/100 g. of body wt. was found effective in producing profuse diarrhoea within 8 hours of administration as compared to 5 ml/100 g. of body weight when given orally.

(iii) *Purification of choleraemic fraction by chromatography.*—Sonicate toxin was prepared in distilled water and lyophilised. This material was used for the purification. The toxin was dissolved in very small quantity of saline and passed through G-200 Saphadex column. The column was eluted with normal saline and fractions of 5 ml. each were collected and spectrophotometric readings were taken. The fraction collected under different peaks were pooled, dialysed lyophilised and reconstituted to suitable volumes and tested in animals. The fractions collected under 80 ml. to 190 ml. showed choleraemic activity in infant rabbits. The fraction collected under first 75-80 ml. showed antigenic activity as shown by Ochterlony's double diffusion technique.

(iv) *Absorption of toxin into blood from separated jejunal segment.*—One of the major unsolved problems in cholera research is whether the toxic agents primarily responsible for the diarrhoea are confined to the intestinal tract, or are absorbed into the blood. To solve this problem an attempt was made to prepare intestinal fistula with blood supply intact, in infant rabbits. A definite answer might be found to the question whether one of the important routes for the distribution of toxin was blood. Vibrios, or their lysate were introduced into the separated segment with one end blind and the other opening to the exterior as a fistula. From thirty successful experiments it could be said that in infant rabbits a toxin of major importance was absorbed into the blood from *V. cholerae* in a separated segment of jejunum, and caused characteristic signs of cholera in the whole animal.

(b) DIPHTHERIA PROJECT

Haffkine Institute is interested in investigating problems of public health importance. Improvement of public health requires the preparation of suitable diagnostic reagents and of prophylactic agents against diseases. The problems selected for study in the diphtheria project, therefore, concern some aspects of epidemiology of the disease as well as all those aspects which help in the preparation of better diagnostic reagents and prophylactic agents.

1. *Use of acriflavine for the removal of the toxinogenicity of C. diphtheriae and of phage.*—Acriflavine is known to disinfect bacteria from cytoplasmic genetic factors. This reagent was, therefore, used to find out whether the toxinogenic character of *C. diphtheriae* can be removed to convert it into a non-toxinogenic strain. It was found that acriflavine, added to the growth medium in a concentration of 5 ug/ml, was able to convert the toxinogenic strains into non-toxinogenic strains. Such strains produced not even a trace amount of toxin. Acriflavin in a concentration of 5 ug/ml. was also able to remove prophages from lysogenic strains.

Different concentrations of acriflavine were then used to see whether it could convert toxinogenic phage *in vitro* into a non-toxinogenic phage. The toxinogenic phages P and B were induced from corresponding C7(P) and C4(B) by irradiation with ultra-violet light. The phage lysates were then concentrated by centrifugation and purification with ammonium sulphate. These were then treated with 0.5 ug/ml. of acriflavine in cold for 18 hours and then mixed with suitable indicator strains and plated out. It was found that most of the plaque forming phages had lost the ability to convert non-toxinogenic strains into toxinogenic strains, while the phage which was not treated with acriflavine had not lost its toxinogenic character. As far as is known, this is the first time that a transducing phage is treated *in vitro* to remove the transmissible factor.

2. *Role of iron in the control of toxin production.*—It is well-known that the amount of iron in the medium should be adjusted to narrow limits for production of maximum toxin by the PW8 strain. When no iron is added or when iron is added in excess, low yields of toxin are obtained. A number of theories have been put forward to explain this (see previous report). However, there has not been any satisfactory explanation.

The isolation of a strain of PW8 which could grow and produce toxin on a simple medium containing only glutamic acid, cystine, nicotinic acid and salts indicated the suitability of this strain for the study of the role of iron in toxin production. In the absence of iron, it required a narrow optimum around 0.01 g. of cystine in 100 ml. of medium for maximum

toxin production. At cystine concentration of 0.004 g. or more per 100 ml., there was very little growth and no toxin production. However, in the presence of iron, the toxicity of cystine was nullified and it did grow and produce toxin. At cystine concentration of 0.01 g/100 ml. and in high concentrations of iron (0.4 mg/litre), the organisms could grow but produced very little toxin. However, if the cystine concentration was increased to 0.04 g/100 ml., then normal amount of toxin was produced. These results have suggested that cystine is an important requirement for growth and toxin production, but excess amounts are toxic and iron in medium in small amounts can nullify the toxicity of cystine but amounts lower the amount of cystine available to the organism for production.

3. *Conversion of non-toxinogenic strains into toxinogenic strains the mediation of phage.*—Attempts were made to obtain the DNA for production from the PW8 strain using Marmur's method. One was done to see whether the DNA could induce toxin production in strain. Results were negative. Further experiments are continuing.

4. *Phage typing of C. diphtheriae.*—So far, about 81 strain of *diphtheriae* from the suspected clinical cases were studied along with clinical history. Some of these are from the Infectious Diseases Bombay. About 37 were gravis and 26 were mitis types. No type could be isolated. However, 7 strains of *C. hoffmanii* were Both *in vitro* and *in vivo* toxigenicity tests (Elek's and Rabbit's neous) carried out on 63 cultures showed 36 to be reactive by *in vitro* and 38 by *in vivo* test.

The lytic reactions were studied against the P, B and r phages by over-layer and spot technics using homologous strains C_7 and C_4 . No definite typing could be made, as the titre of the phages would not high enough to give clear-cut results. However, efforts are made to the R.T.D. of these phages.

Recently, 19 indicator strains and 24 phages of *C. diphtheriae* have obtained from Rumania (W.H.O. International Reference Centre for thera phage work). So far, these phages did not give confluent lysis phage typing. Attempts are being made to improve the titre of these by adopting the recommended procedures and media.

The epidemiological work has been going on since November 1967. strains were isolated from the clinical cases, 2 more carries amongst contact were discovered when 172 throat swabs of the contacts were In 72 children under the age of 7 years Schick test was done. Of six were positive and two were false positive.

5. *Study of cellular constituents of C. diphtheriae*.—Study of cellular constituents may throw light on the metabolism of *C. diphtheriae*. It is still not known as to how the PW8 strain could produce large amounts of toxin while other strains infected with the same toxin inducing phage would produce less toxin. The results may also be useful in finding out the factor which enables particular strains to form pseudo-membranes. The protein and lipid metabolism of some of the standard strains will be examined.

(c) ENTEROVIRUS PROJECT

1. *Gastroenteritis in children*.—A large number of investigations carried out to resolve the problem of etiology of gastroenteritis in children have shown that a good proportion of cases fail to show any known bacterial or protozoal pathogens. In view of this, attention has been focussed on the virological component.

In the present study, cases of gastroenteritis not showing any known bacterial or protozoal pathogens were taken up for virological investigations.

In view of the hypothesis that these cases of gastroenteritis might be the resultant of symbiotic action of an enterovirus and enterocoli, the present study was extended to study of the pattern of *E.coli* in the selected cases.

Materials from selected cases were collected for virus isolation and serotyping of *E.coli* and study of antibody patterns against viruses isolated whenever blood samples were available.

Virological studies on 177 cases of gastroenteritis and 100 controls (individuals without gastroenteritis) have shown both qualitative and quantitative differences. (Table 1).

2. *Relationship to age groups*.—There was no correlation between age groups and incidence of gastroenteritis. There has been a fair uniformity as regards incidence from the age of 3 months to 5 years and above. This uniformity has also been noted as regards isolation of viruses from cases of gastroenteritis. (Table 2).

3. *Spectrum of viruses encountered*.—As many as 24 serotypes of enteroviruses were encountered during the present study (22 serotypes in cases of gastroenteritis while 14 types in controls). Two serotypes were found predominant in cases of gastroenteritis, Coxsackie B₃—27 cases, and ECHO 7—72 cases. No such significant predominance of any virus type was encountered in the case of controls.

4. *Multiplicity of viral types*.—A significant number of cases of gastroenteritis showed presence of multiple serotypes in their gut, 41 out of 128 positive in contrast to 2 out of 28 in case of controls. (Table 3). Significance of presence of multiple types is not known.

5. *Seasonal distribution of enteroviruses in gastroenteritis*.—It is well known that enteroviruses appear in the gut of apparently healthy children as transient viral flora and this is particularly so during the summer months. During the remaining part of the year, the incidence of enteroviruses in healthy children is fairly low. In contrast to above, it has been observed that the incidence of enteroviruses has been significantly high in the cases of gastroenteritis throughout the year.

6. *Correlation with E.coli*.—The Bacteriological studies carried out have failed to show any correlation between presence of an enterovirus and an enterocoli.

7. *Serology*.—Acute and "convalescent" sera collected from these cases are under study for antibody titres. However, most of the "convalescent" sera are collected before discharge from the hospital 3 to 5 days after admission. A significant rise in antibody titre is not expected in 5 days. Hence, this information may not contribute much at present.

8. *General remarks and future plan*.—It is well known that enterovirus "infections" may be present in normal, asymptomatic children, and may stimulate a rise in antibody titre also. However, the pattern of infection in diarrhoea cases differ markedly both in quantity and quality from that found in non-diarrhoea controls as also from that found in surveys of asymptomatic children; several questions arise out of these studies :—

(a) Is the enterovirus a causative factor in diarrhoea? If so, can any enterovirus produce diarrhoea under favourable conditions? What are these conditions?

(b) Is the enterovirus an associated phenomena, i.e. is the presence of diarrhoea, presumably with some shedding of epithelium, a predisposing factor to virus infection? If so, what role, if any, does the virus play in the continuation of diarrhoea?

If the virus plays any part in the diarrhoea process, the recovery may be accompanied by rise in antibodies and disappearance of the virus infection from the gut. Further work will be designed to answer these questions.

9. *Aseptic meningitis*.—More samples from aseptic meningitis were received during the year under study.

Serology has yet to be undertaken.

Further interpretation will be possible after a sufficient number of cases have been studied serologically.

TABLE 1
Gastroenteritis virus studies.

	Total cases studied	Total No. virologically +ve	+ve only in TC	+ve only in infant mice	+ve in both TC & mice	-ve both in TC & mice
Gastroenteritis	177	128	57	4	67	49
Controls	100	28	8	11	9	72

TABLE 2
Virus Studies Agewise distribution of cases of gastroenteritis and controls.

	Cases	Controls
1 month	1/1*	2/4
1-2 months	4/5	1/3
2-3 months	3/3	1/6
3-6 months	16/24	2/8
6-9 months	14/23	3/4
9-12 months	33/42	7/15
1-2 years	21/34	0/13
2-5 years	25/28	6/22
5 years	9/13	6/25
Age not known	2/4	—
Total :	128/177	28/100

*No. of individuals virologically positive/total studied.

TABLE 3
Gastroenteritis Multiplicity of isolates

	Cases of Gastroenteritis.	Controls.
Total No. studied	177	100
Total No. virologically positive	128	28
Isolation of one type	76	22
Isolation of two types	41	2
Isolation of three types	5	—
Isolation of more than 3 types	1	—
Unidentified	5	4

(d) PLAGUE PROJECT

Panvel rat elimination project.—Rat control operations initiated in the four villages, i.e. Kalundre, Kolkhe, Kon and Palaspe of Panvel Taluka were concluded. The work was compared at the end in two more villages, which were kept as controls, i.e. Shridhon and Nawade. The trapping operations taken up on the experimental village Kalundre yielded no rats on the day of the termination of experiments, while in the village Shridhon 53 rats were trapped. One month after the closure of the work, i.e. in November 1967, another village, Nawade, was taken up for comparison with the results of the experimental village. Only 3 rats were collected at Kalundre and 115 rats at Nawade showing that the population of rats had not increased at Kalundre the village where rat control operations were carried out.

The date of different species of rats trapped and dead due to rodenticide trials in all the experimental and control villages and their sex ratio are summarised below :—

Rats collected by trapping .

Rat species		Total	Male	Female.
<i>Rattus rattus</i>	...	2055	864	1191
<i>Bandicota gigantia</i>	...	1	...	1
<i>Mus musculus</i>	..	6	3	3
<i>Leggada booduga</i>	...	1	1	...

Dead rats collected by baiting

<i>Rattus rattus</i>	..	78	26	52
<i>Bandicota gigantia</i>	...	13	3	10
<i>Mus musculus</i>	...	4	2	2

In all, 851 fleas were recovered from the rats collected by trapping. Of these, 802 were *X. cheopis* and 49 were *X. astia*. Of the *X. cheopis* collected, 458 were males and 444 females. In the case of *X. astia*, it had 20 males and 29 females. The sex ratio of the collection, therefore, was 1:1.2 for *X. cheopis* and 1:1.4 for *X. astia*.

Rattus rattus was the predominant rat and it showed a sex ratio of 1:1.4 in the collection. The male rat had more fleas than the female

rat and on an overall basis the females of both species of fleas predominated on both the sexes of rats.

It was observed that rats from Kalundre showed a resistance to *P. pestis* to the extent of 53 per cent, while those from Nawade were highly susceptible. The results are interesting, the distance between these two villages being only 10 Kms.

Blood films of 14 rats from Panvel area showed the presence of Trypanosomes. Intestinal contents of 35 rats showed the presence of intestinal parasites like cysts and ova of amoebae, ova of *Trichuris trichur* *Hyenolepis diminuta* and larvae of some nematodes.

Rat control campaign at Niphad.—With a view to control rats in wheat growing areas, work was taken up in Niphad Taluka of Nasik district. The object was to know the species of rats in existence and their habits. This work carried out at the agricultural Research Station, initially and was later extended to Kundewadi and Rowlas villages.

The house to house trapping at the Agricultural Research Station, Kundewadi village and Rowlas village yielded 136 rats in 230 traps. Of the rats collected in houses, 126 were *Rattus rattus* and 10 *Mus musculus*. Among 33 rats collected from fields, 28 were *Golunda ellioti* and 5 were *Bandicota bengalensis*.

In all, 253 fleas were collected from these rats. These fleas were found to be *X. cheopis*. The sex ratio in the collection was observed to the 1 : 1.3.

Zinc phosphide baiting was undertaken in 108 acre of fields with standing crops. About 10.75 kgs. of bait were offered at 1150 bait stations, out of which 525 gms. of bait was consumed by rats. In all, 26 dead rats were collected. All these rats belonged to the species *Golunda ellioti*.

Plague project studies at Mahad.—In the town of Mahad 1,337 rats were trapped. Out of these, 1,288 were *Rattus rattus* (528 males and 760 females), 7 *Bandicota bengalensis* (1 male and 6 females), 2 *Bandicota gigantia* (males), 39 *Mus musculus* (16 males and 23 females) and 1 *Leggada booduga* (male). In addition to this, 33 *Suncus caeruleus* (12 males and 21 females) were also trapped. *Rattus rattus* was observed to be the most predominant species, being 96 per cent of the total catch, and their sex ratio was noted to be 1 : 1.8. Rat density for Mahad town was 22.3. About 3,829 fleas of different species were recovered from these rats, bringing the flea index to the level of 2.9.

Trapping operations carried out near 4 surrounding villages had resulted in the collection of 62 rats. All these rats belonged to the species *Rattus rattus* (23 males and 39 females). The sex ratio was observed as 1:1.7. Rat density in this area was found to be 7. In all, 95 fleas were collected from these rats, bringing the flea index to 1.5.

Zinc phosphide baiting campaign was taken up in the town of Mahad. In all, 95 kgs. of wheat were used for prebaiting and 16.24 kgs. of zinc phosphide bait were used for the baiting campaign. One hundred and fifteen rats were recovered dead. Out of these, 111 were *Rattus rattus*, 1 was *Bandicota bengalensis* and 3 were *Mus musculus*. This campaign was taken up after arousing much public interest.

Frequency of rat population.—This study was carried out in the Kurla lake area with the help of single door traps for 14 days. Eight rats were collected. Out of these, 3 rats were *Golunda ellioti* (2 males and 1 female) and 5 *Tatera indica* (3 males and 2 females). Seven fleas were recovered from these rats in addition to 4 ticks and mites.

Trapping operations were undertaken in the field area to collect as many rats as possible. Twenty-four rats were trapped in 15 days. Out of these, 4 rats were *Tatera indica* (2 males and 2 females), 1 *Bandicota bengalensis* (Male), and 19 *Leggada booduga* (10 males and 9 females). Seventeen fleas were recovered from these rats and 11 other ectoparasites were collected.

Susceptibility of rats to plague infection.—The work of testing susceptibility of rats to infection with *P. pestis* collected from different areas of Maharashtra State was continued. House rats were collected from two districts, viz. Nasik (Niphad) and Kolaba (Panvel and Mahad). It was observed that about 50 per cent of the rats in Niphad and Mahad and one village in Panvel taluka were susceptible to plague. The other villages in Panvel taluka showed a higher susceptibility of 64 per cent. This susceptibility of the rats in all these places was higher as compared to that of rats from Bombay city or Marathwada districts. It compares favourably with that of the Urban areas of Vidarbha region.

The sera of 50 rats collected from Mahad were tested by titination test to detect the presence of plague antibody. Eight have shown presence of plague antibody, though in lower titre.

Development of a standard plague vaccine.—Biological tests used assay of prophylactics generally involved comparison of the activity the test sample to that of a standard. The design of the assay

included at present in Indian pharmacopoeia does not include a reference vaccine. Therefore, it was proposed to develop a plague vaccine which would be representative of the vaccines generally manufactured in the country and would serve as a reference standard. Tests were carried out on four different batches. One of them, now lyophilized, was subjected to further tests for detailed study. The results of these tests, when completed, will show the relative potency of this vaccine with routinely manufactured batches and also the limits of error. This vaccine can be adopted as a reference after further studies on its keeping qualities.

B. OTHER DEVELOPMENT RESEARCH

(1) DEPARTMENT OF BACTERIOLOGY

1. *Studies on the growth of S. typhi in shaken and submerged cultures.*—Experiments to develop a suitable medium for the growth of *S. typhi* in shaken and Stationary culture were continued. Casein hydrolysate medium containing 300 mg. nitrogen per 100 ml., fortified with 4 mg. per cent dl-tryptophane, 0.1 per cent. glycerol, 0.5 per cent. Na CP. glycerophosphate, 10 per cent v/v yeast dialysate and 1 per cent. w/v yeast extract gave best results.

Vaccines were prepared by growing *S. typhi* in this medium by three different methods viz., (1) in stationary cultures in 500 ml. flasks containing 100 ml. of the medium, (ii) shaken cultures in same containers with the same volume per flask, (iii) in a fermenter with 3 litres of medium in a 10-litre fermenter vessel with agitation and without agitation. The culture were grown for 4 days. After every 24 hours, samples were tested for viable count, pH and optical density. Vaccines were prepared by formalising the cultures. Phenyl mercuric nitrate was used as a preservative. The vaccines were adjusted to contain $1,000 \times 10^6$ organisms/ml. The vaccines passed the tests for sterility and freedom from abnormal toxicity. The stationary and fermenter culture vaccines prepared from 24 and 48 hour growth were tested for their potency by active mouse protection test using an acetone-killed dried vaccine as control. The results are given below :—

Vaccine	Growth	ED ₅₀
B ₁ (stationary)	... 24 hrs.	0.0088
B ₂ (fermenter)	... 24 hrs.	0.0096
B ₃ (stationary)	... 48 hrs.	0.0097
B ₄ (fermenter)	... 48 hrs.	0.025
Acetone-killed and dried 3/1967 (AKD).		0.0080

It will be observed that the vaccines prepared by both the methods from 24-hour growth are comparable with the control AKD vaccine, while the fermenter vaccine from 48-hour growth was slightly lower in potency.

Experiments were also carried out to determine the production of H and O agglutinogens under the three conditions of growth. It was observed that when the culture was grown in the basal medium, it gave the same H & O agglutination titre up to 48 hours in both stationary and shaken flask cultures. After 96 hours, however, the shaken flask cultures gave higher titres against both H and O antisera. Fermenter culture with agitation but without aeration using a complete medium showed poor O and Vi titres on the first two days. Similar results were also observed in stationary cultures. The O and Vi titres of stationary cultures showed some rise, but the fermenter cultures showed lower titres even on the 3rd day. However, the four-day old fermenter cultures showed higher O and H titres. It is suspected that the low titres against O serum may be due to covering of the O antigen by Vi antigen in the first three days while probably, Vi antigen formation decreased by the fourth day and higher O titres were obtained. This assumption was confirmed by using boiled cultures. With boiled cultures, higher O titres were obtained from the first day and reached the maximum by the 3rd or the 4th day.

2. *Acetone-killed and dried typhoid vaccine.*—During the period, two batches of these vaccines (Batch No. 2/1967 and 3/1967) were prepared. These batches were tested for potency, freedom from abnormal toxicity and sterility along with the previous batch. The moisture content of these batches was also determined. All the batches passed the sterility test and tests for freedom from abnormal toxicity. The results of the potency test and moisture content are given below :—

Batch No.	ED ₅₀	Relative potency taking Walter Reed Army Institute of Research vaccine as 1.	Moisture per cent.
1/1967	... 0·0079	0·36	18·93
2/1967	... 0·0053	0·547	17·59
2/1967	... 0·0063	0·460	4·1
Walter Reed	... 0·0029	1	...

It will be observed that the batch 1/1967 had a relative potency of 0·36 in comparison to Walter Reed Army Institute of Research vaccine. It showed a potency of 0·6 about 7 months ago. The vaccine lot 2/1967 had also shown a fall from 1·903 to 0·547. The moisture content of

those lots was also increased, showing that rubber closures used for vials breathed moisture. It is likely that the fall in potency might be due to moisture. The last lot prepared was dried over a longer period and had a moisture content of 4.1 per cent. This was about the same as that reported for vaccines used in Yugoslav field trials. Attempts to obtain leak-proof rubber stoppers to reduce moisture content to a lower level are in progress.

3. *Growth comparison of V. cholerae (Strain 5321 Ogawa) under conditions of continuous growth.*—This particular project has been undertaken with a view to compare the potency of the vaccines produced from a continuous culture with that of the conventional stationary phase culture.

The design of the apparatus 'chemostat' is very similar to that illustrated by Novick and Szilend except that the reservoir for the medium is a 500 ml. separating funnel which feeds fresh medium to the growth vessel through a graduated burette. The flow rate of the medium is thereby controlled and accurately measured. The constant volume maintained in the growth vessel is 35 ml. Aeration of the medium is achieved by means of a small aquarium pump (usually used to aerate domestic fish tanks), the air being first passed through phenol water trap before entering the growth vessel. The organisms were grown in lactose glutamic acid medium (Nerurkar), the growth being dependent upon the concentration of glutamic acid in the medium. Initial experimental runs carried on a culture provided with aeration but without the addition of fresh medium showed a lag period of 1-1/2 to 2 hours. Hence, for the continuous growth culture experiments, the addition of fresh medium was commenced after an incubation of 1 hour at 37°. Attempts to keep the *V. cholerae* under conditions of continuous growth were partly successful, i.e. in maintaining a steady rate of growth over a maximum period of 24 hours. The culture was found to attain a steady rate of growth condition after about 8 hours through 24 hours and thereafter showing a slight tendency towards degeneration.

4. *Autolysis of Vibrio cholerae.*—Agar-grown cholera vaccines were standardised on the basis of the total number of organisms contained in a unit volume at the time of preparation by determining the capacity of the culture. However, as the cholera organism undergoes considerable lysis in a liquid menstium within a short time, the vaccine shows less opacity and consequently lower strength after a short storage. In the absence of any other suitable accepted method for standardisation of cholera vaccine, if lysis could be inhibited, the potency of the vaccine could be determined any time by comparing it with standard opacity tubes. It was, therefore, thought of interest to study the lytic phenomenon in *V. cholerae* in detail.

(a) *Location of the lytic principle.*—It was observed that repeatedly washed cultures of *V. cholerae* were less susceptible to lysis than unwashed cultures. This suggested that the lytic substance may be present in the culture supernatant. The cell-free culture supernatant was then precipitated with saturated $(\text{NH})_2\text{SO}_4$ and dialysed in cold for 24 hours. The dialysate was then added to fresh *V. cholerae* cultures, both washed and unwashed. This resulted in an increase in the rate of lysis and showed that the active principle that was responsible for lysis was present in the culture supernatant.

(b) *Spermine and autolysis.*—Mager & Tabor *et al* have reported that spermine and related polyamines have a stabilising effect on the cell wall and prevent lysis in certain organisms. Hence, it was decided to study the effect of spermine on the lysis of *V. cholerae*. Varying concentrations of spermine were used in liquid synthetic medium as well as casein hydrolysate agar medium which were used for growing *V. cholerae*. The addition of spermine to the medium did not have any stabilising effect in the case of *V. cholerae*.

5. *Preparation and standardization of Typhoid Vaccines.*—This work was primarily undertaken to study if any locally isolated strains of *S. typhi* could be used for the preparation of potent typhoid vaccines. However, as no reliable laboratory method is available for the assay of typhoid vaccine, it was considered that a new method based on the intracutaneous reaction in rabbits could be developed and used for the comparative study. Thus, the whole problem was divided into two main facets :—

(I) (a) Study of local strains with regard to their serology and virulence in mice and in rabbits.

(b) Preparation of phenolized and formalized vaccines from these strains along with vaccines using standard strain and testing these vaccines by conventional methods, such as agglutinin production and active protection tests in mice.

(II) To standardize a method for measuring rabbit skin response by active infection or develop a neutralization test and use these methods for assay of typhoid vaccines prepared as above and see if these methods could distinguish between different vaccines.

As work on some of these aspects is completed, a short summary of the work is given below.

Three strains, isolated in the Clinical Pathology Department from cases reported, were selected after carrying out all the biochemical and serological tests for ascertaining their identity. In addition to this, the virulence of these organisms was determined on mice as well as on the rabbit skin by method.

Though the LD₅₀ of each strain in terms of organisms varied, all the strains were virulent in mice. LD₅₀ in mice and M.R.D. (minimum and reactive dose) on rabbits of three strains are shown in the Table.

Strain	LD ₅₀ in mice No. of organisms.	M.R.D. in rabbits No. of organisms.
<i>S. typhi</i> Ty ² (standard) ...	10	180·000
<i>S. typhi</i> 93 ...	10	1700·000
<i>S. typhi</i> 624 ...	727	1600·000
<i>S. typhi</i> 698 ...	45	600·000

It would be observed that the order of virulence in mice and rabbits was not identical. Though strain 93 was extremely virulent in mice (as virulent as the standard strain) it had the highest M.R.D. in rabbits and was hardly 1/10 as virulent as Ty₂. It had also the same virulence in rabbits as strain 624 though, in mice, it was 72 times virulent than strain 624. Similarly, strain 698 was nearly 16 times more virulent than strain 624 in mice, though it was hardly about 2·5 times virulent in rabbits. These results indicate that though mucin was used in both the tests for enhancing virulence, the route of inoculation affected the virulence.

It was observed that agglutination tests with H, O, and Vi antigenus did not differentiate the vaccines prepared from local and standard strains. Active mouse protection tests, however, clearly showed that the vaccines prepared with local strains had significantly less potency. However, the strain 93 which was as virulent as Ty₂ gave a poor formalized vaccine, but phenolized vaccine prepared from the same strain had much higher potency as compared to formalized vaccine. Though mouse protection tests did not clearly distinguish between vaccines prepared from the standard strain, it graded the vaccines prepared from strains with probably different antigenic make-up.

As regards the second part of the work, a method of intracutaneous challenge in rabbit which gives reproducible results with the same challenge dose has been standardized. Local strain vaccines assayed by this method showed lower potency and the acetone-dried vaccine of the Walter Reed Army Institute of Research showed highest potency, thus indicating that it might be possible to use this reaction for assay of typhoid vaccine.

6. *Studies in P. pestis in relation to virulence and immunogenicity.*—The nutritional requirements of both virulent and avirulent strains of *P. pestis* possessing different antigenic characters were studied with special referenc to their action requirements for growth at 27°C and 37°C. The

variation in morphology and amino acid composition of the cells and cell walls was studied. It was observed that though the amino acid composition of cells at two different temperatures differed, the amino acid composition of cell wall remained practically the same, indicating the identity of basic cell wall structure, viz., the R layer in both virulent and avirulent strains. During studies on growth rate in aerated liquid cultures, lysis of *P. pestis* cells was also observed.

With a view to elucidate the role of divalent cations, the effect of some lytic system on the cells of five different strains of *P. pestis* was studied by using two techniques. The effect of lysozyme alone was studied by using technic of Grula and Hartsell and that of EDTA and Lysozyme were studied by Repaske's technique. The results showed that the lysozyme substrate was present in the cells of all the strains grown at 27°C. However, the technique of Grula and Hartsell involving the use of drastic acid and alkali treatment was unsuitable for the cells grown at 37°C which lyse by themselves in the absence of lysozyme. This indicate the weakened nature of the cell wall grown at 37°C which was also observed under normal growth condition leading to ultimate lysis. Therefore, Repaske's technique making use of EDTA and lysozyme in balanced proportion was attempted. It was observed in this case also that the cells grown at 37°C were converted into spheres by the use of EDTA alone without reaching the stage of complete lysis, indicating absence of true sphereoplast for formation. This phenomenon suggested that the role of Ca and Mg might be required for maintenance of rigidity of the cell walls.

Strains having an antigenic make-up similar to 195/P or EV were used for the preparation of either killed or live plague vaccines. Claims for superiority of live and killed vaccines were put forward from time to time. However, very little data was available regarding protection offered by strains varying in their antigenic composition. With a view to elucidate some of these aspects of the problem, protection offered by killed virulent strain vaccines and by live and killed vaccines prepared from avirulent strains on mice was compared, using two challenge strains 195/P and M 17. The strains 195/P contained the full complement of virulent antigens (F1+VW+P+). It was observed that killed vaccines prepared from either virulent or avirulent strains protected mice against a challenge by 195/P, but much lower protection was obtained against a challenge by M 17. On the other hand, live EV (avirulent) strains gave good protection against both the challenge strains. It was evident that F1+ strains vaccines (killed) might not offer sufficient protection against a F1- strain, but a live vaccine would do so, This observation

is important in the face of the fact that a strain named Bryan which is F1— was isolated in the field from case of plague in U. S. A. importance of selecting a suitable strain and a method for vaccine production which would give a sufficient degree of protection against an infection by both F1+ and F1— strains was brought out. It was also observed that a killed vaccine prepared from a virulent F1— strain did not protect mice against a homologous or heterologous challenge.

Though the result stated above threw some light against the protective ability of different vaccines against F1+ and F1— infecting strains in mice, the problem of protection offered to guinea-pigs and monkeys still remained unanswered. Earlier reports on this aspect did not give unequivocal evidence on this aspect. It was, therefore, proposed to undertake initially the study of protection in guinea-pigs after obtaining a suitable strain of guinea-pigs.

(2) DEPARTMENT OF BLOOD BANK

1. *Studies of genetical characters in some endogamous groups in Indian population.*—In all, 1,228 samples of blood and saliva were collected from certain population groups, viz., Audich Brahman, Lad Vania, Visa Oswal, Mahars and Syrian Christian and studied.

They were investigated for blood groups, viz., A, A₂, BO, MN, Rh (including genotyping), Duffy and Kell systems, secretor status in saliva, glucose-six-phosphate dehydrogenase deficiency (G-6-PD), hæmoglobins with special reference to sicklings, haptoglobins and transferrins, colour-blindness and ability to taste phenyl-thio-carbanide. The data were statistically analysed for calculating gene frequencies and thereby to find out the similarities and differences between groups.

Interesting observations, such as absence of A₂ gene in Audich Brahman, insignificant sex-differences in all characters except the sex-linked characters, viz., G-6PD and colour blindness, the similarity in genetic pictures between Audich Brahman and Syrian Christians, have contributed to the understanding of the genetic variations among these groups. The results were also compared with those of earlier workers who have studied some genetic parameters in some of the population groups which are selected in the present study. The present study is more extensive with many more parameters and covering 3 more endogamous groups not studied earlier. Generally, all results tallied with those of earlier workers, although little variations in very few cases was observed. However, these variations failed to show any significant difference when they were subjected to (chi-square) test.

2. *Bacterial contamination of banked blood and methods of reducing the same.*—Investigation to trace the possible sources of contamination in blood collection were carried out.

Samples were collected by taking swabs of the donors' arms prior to bleeding, before and after swabbing with antiseptics and after the bleeding operation was over. Usually, the donors' skins were not sterile, but could be rendered sterile in majority of the cases by cleaning.

The top surface of the bleeding bottles was tested and found to be sterile.

The agar petri dishes were exposed to see the ærial contamination at the place of bleeding. In some places, it showed contaminations.

The bottles containing A. C. D. solutions and bleeding sets remained sterile even after transport over long distances.

There was no connection between hæmolysis and contamination.

Isolation of organisations in pure culture from contaminated blood was done and their identification was carried out. About 230 samples were tested, the major contaminants being staphylococci, pseudomonas and fungus.

3. *Plasma fractionation.*—The collection of placental blood has been started from maternity hospitals in the city of Bombay. The first batch of plasma has been taken up for fractionation.

Gamma globulin, which is manufactured from a large pool, is tested for the antibodies against poliomyelitis, diphtheria and antistreptolysin "O". The titres are as follows :—

Antibodies	Antibodies in Plasma (Units)	Antibodies in gamma globulin (Units)
Antistreptolysin "O" ..	333	833
Diphtheria ..	—	0.01
Polio virus neutralizing—		
Type I ..	316	3162
Type II ..	316	3162
Type III ..	316	562

The human gamma globulin (produced in this Institute from pooled plasma and placental plasma) is also being analysed by immuno-electrophoresis and gel diffusion techniques in order to study IgG, IgA, IgM components. The gamma globulin produced from whole plasma showed a fair degree of purity, almost showing a single line indicating pure IgG content in immuno-electrophoresis, whereas some batches were contaminated with traces of alpha and beta globulins and albumin. The placental gamma globulin also showed single line in immuno-electrophoresis indicating that it is as pure as gamma-globulin produced from plasma.

4. *IgG (anti-D).*—Recent progress in the prevention of Rh iso-immunisation through heterospecific pregnancy has been the administration of IgG (anti-D) sera immediately after delivery to such women. Successful trials have been reported both from U. S. A. and U. K. after 6 years' study. In order to make this sera available in this country, a batch of anti-Rh sera, obtained from deliberately hyperimmunised blood donors, has been fractionated to produce Anti-Rh gamma globulin. This product is being analysed further for its purity as IgG. On immuno-electrophoresis, it gave a single band.

5. *Cryoprecipitates for treatment of hæmophilia.*—The recent treatment of hæmophilia consists of the use of cryoprecipitates from fresh human plasma. Utilising siliconised bottles, these precipitates are being prepared according to the method of D. L. Brown. The precipitates are being used in clinical trials at the K. E. M. hospital, Hæmatology department.

6. *Anti-Rh Serum.*—Nine new donors (Rh negative) together with 5 old, have been given injections of 'O' Rh+ve (Ror) blood with a view to induce Rh antibody production. Each donor has been given 2 to 18 injections. So far, only two donors have been found to form the Rh antibody. One has a titre of 1:8 and the other has a titre of 1:64. The latter has been bled once.

Two other donors, who were Rh negative, have been given 12 injections each of O R¹ R¹ blood. No antibody was formed.

Ten donors, who were previously immunised, have formed Rh antibodies of 1:128 titre or more.

7. *Anti-human serum (Coomb's reagent).*—The storage of (Coomb's serum) Anti-human serum over a long period poses a problem, as the serum is diluted manifold in saline and, consequently, the protein content

of the final product is very low. This affects both its keeping quality and also its avidity. To get over this drawback, the following method has been devised. It was found that if normal rabbit serum is used as diluent, the avidity and the keeping quality of this anti-human serum is improved.

(3) DEPARTMENT OF CHEMOTHERAPY

1. *Anticancer Agents.*—In view of the significant antitumor activity in experimental test systems displayed by several of our previously synthesised compounds, the work on the synthesis of nitrogen mustards has been continued further. During the period under review, 45 new nitrogen mustard derivatives were prepared and 17 of these were submitted to Cancer Chemotherapy National Service Centre, Bethesda (U.S.A.) for their anticancer evaluation against L-1210 lymphoid leukemia, Walker carcinosarcoma-256 (intramuscular) and toxicity studies.

The screening reports so far received had indicated that several of our compounds exhibit significant antitumor activity. The work on nitrogen mustard derivatives could be grouped as follows :—

(a) *Schiff bases from 4-(p-aminophenyl)-5-methyl-2-substituted thiazoles.*—In a series of schiff bases derived from various aminophenyl thiazoles, high antitumor activity was observed, where 4-p-aminophenyl-2-methylthiazole was condensed with unsubstituted benzaldehyde nitrogen mustard.

Hence, it appeared that among the schiff bases derived from 4-(p-aminophenyl) thiazoles, compounds substituted at 2-position exerted great influence on the antitumor activity. In an attempt to prepare compounds with higher antitumor activity and to study structure-activity relationships, in this series, twenty new Schiff bases were synthesised from 4-p-aminophenyl-5-methyl-thiazoles, having various substituents at position 2.

These schiff bases were obtained by the condensation of various 4-(p-aminophenyl)-5-methyl-2-substituted thiazoles with appropriate N, N-[bis(2-chloroethyl)amino] benzaldehydes.

Five representative compounds in this series were submitted for antitumor evaluation and reports of these compounds are awaited.

(b) *Schiff bases from 4-(p-amino-2-chloro) phenyl 2-substituted thiazoles.*—Among the schiff bases from substituted anilines synthesised and studied by us, it was observed that a derivative from m-chloroaniline demonstrated high antitumor activity. Hence, it was hoped that incorporation of a chlorine atom in the *meta* position to the phenyl ring of 4-p-aminophenyl-2-substituted thiazoles and condensing them with benzaldehyde and substituted benzaldehyde nitrogen mustards might enhance the antitumor activity further.

The synthesis of such compounds involving the condensation of various mustard aldehydes with 4-[(p-amino-2-chloro)phenyl]-2-substituted thiazoles was, therefore, undertaken.

Twenty schiff bases were prepared and seven of them were submitted for antitumor evaluation. During this period, screening reports of some of the compounds of this type sent earlier were received. Two compounds viz. NSC-107637 and NSC-107639 displayed good activity against lymphoid leukemia L-1210.

(c) *Nitrogen Mustards from 4-(p-aminophenyl)-2-substituted thiazoles.*—Nitrogen mustard derivatives such as chlorambucil, cyclophosphamide, sarcolysin etc. are widely used in the treatment to cancer. Since thiazole is a biologically important moiety and several of its derivatives are useful chemotherapeutic agents, it was felt worthwhile to attach nitrogen mustard grouping on phenyl thiazole in an attempt to study whether these compounds would also serve as useful anticancer agents. After considerable trials, nitrogen mustard grouping was introduced in 4-p-aminophenyl thiazoles having various substituents in position 2. These compounds were obtained in fairly good quantity.

Two representative samples of these compounds were submitted to C. C. N. S. C. for antitumor screening and toxicity studies.

Two of four compounds, viz., R-1314 (NSC-104982) and R-1315 (NSC-104983) which had successfully completed the three-stage sequential tests underwent further screening at several Cancer Chemotherapy National Service Centre Laboratories. Reports from all these laboratories confirmed the antitumor activity of these compounds.

2. *CNS Depressants*—N¹-[*r*-(3, 4, 5-trimethoxy-benzoyl)propyl]-N¹ (substituted) piperazines and the corresponding alcohols.

The Mannich bases synthesised by us in this department from 3, 4 5-trimethoxyacetophenone and various N-monosubstituted piperazines showed promising CNS depressant activity.

In view of this and the established CNS activity of the butyrophenone class of tranquilizers, the corresponding butyrophenone derivatives were synthesised. These compounds displayed structural features similar to GABA, a substance having a key role in the biochemistry of brain function.

The butyrophenone derivatives were synthesised by the fusion of γ chloro 3, 4, 5 trimethoxybutyrophenone and requisite N-monosubstituted piperazines. The required γ -chloro 3, 4, 5 trimethoxybutyrophenone, hitherto unknown, was prepared in good yield, from α -3,4,5-trimethoxybenzoyl-butyrolactone by treating it with hydrochloric acid and fused zinc chloride. Few butyrophenone derivatives were also reduced to the corresponding alcohols.

All the compounds synthesised were submitted for primary screening in experimental animals for their CNS depressant activity. Report of few compounds were received. Two compounds showed significant CNS depressant activity. These are being taken up for further pharmacological study. Butyrophenone derivatives were found to be less active than propiophenones. Results of other compounds are awaited.

3. *Synthetic Diuretics*.—This scheme is supported by a grant from C. S. I. R. At present, most of the synthetic diuretics in clinical use are being imported and the patent monopoly enjoyed by these products has kept the prices very high. We have been engaged in the synthesis of newer diuretics. The existence of facilities for screening of diuretics with the C. S. I. R. screening unit had been helpful in getting leads for further modifications and evaluation of newer series.

During the period under review, the work was carried out on the following lines :—

(a) *Analogues of R-37, (2-amino-4-m-chloroanilino-5-triazine)*.—In pursuance of getting better clinical results than those obtained with R-37 synthesised by our group and found effective in experimental diuresis, 4-m-fluoro and 4-m-trifluoro anilino-2-amino-s-triazine analogues were synthesised and screened for their diuretic activity.

A series of substituted 2-(4'-chloro-5'-sulfonamido phenyl)-quinoline-4-carboxylic acids were synthesised and tested for their diuretic activity. These compounds were obtained by the condensation of various substi-

tuted Isatines with 4-chloro-5-sulfonamido acetophenone in presence of alcoholic KOH.

4. *Hypoglycaemic Agents.*—(a) 3, 4-Di-substituted-5-carboxymethyl-mercapto-1, 2, 4-(H)-triazoles as possible hypoglycaemic agents.—About 15 compounds in the 1, 2, 4(H)-triazole series prepared earlier were tested for their hypoglycemic properties. Some of these compounds showed significant activity. In view of this, further work on newer derivatives of this class is taken up.

(b) *Synthesis of m-substituted benzene-sulphonylureas as hypoglycaemic agents.*—No systematic study in this meta-substituted class of benzenesulphonylurea has been reported so far although a few scattered compounds have been mentioned in literatures. The synthesis and experimental study of this class of compounds were taken up. The substituents used in the phenyl ring were chloro, fluoro, methyl and trifluoromethyl. The amines utilized were, *n*-propyl-, *iso*-propyl, *n*-butyl, *iso*-butyl and cyclohexyl. In this series, only one compound showed significant hypoglycemic activity. Further pharmacological evaluation is in progress.

5. *Drug Screening Unit.*—The antimicrobial screening of compounds for possible activity was continued. Instead of the previous practice of screening compounds against only those organisms for which the compounds were synthesised, the department took up a broad screening programme wherein all the available compounds were put up against as many micro-organisms as possible. This became possible with the provision of a grant from C. S. I. R. We have also now taken up additional *in vitro* screening against *E. histolytica* and for antimicrofilarial activity using (*Darofilaria*) as the test organisms.

During the period under review, 315 synthetic compounds were screened against various pathogenic bacteria, fungi, *E. histolytica*, *Dirofilaria* etc.

During this broad screening programme, we also screened all the compounds synthesised in the department as CNS depressant agents and were surprised to find one series of compounds in this class exhibiting significant antitubercular and antifungal activity but having no marked action against other pathogenic bacteria. These compounds were found to be devoid of any significant CNS action and having a very low order of toxicity. We came across a new class of substances hitherto not studied as possible antitubercular agents and it was proposed to take this lead to synthesise further series of compounds for antitubercular screening.

TABLE I

Name of the micro-organism	Total number of compounds screened	200 μg/ml	Number of compounds showing antimicrobial activity in the different concentrations					
			100 μg/ml	40 μg/ml	20 μg/ml	10 μg/ml	5 μg/ml	
M. tuberculosis	..	315	25	49	82	29	14	4
S. typhi	..	315	8	4	..	3	1	..
Staph. aureus	..	315	22	9	12	12	3	..
V. comma	..	315	11	9	7	4	1	1
C. albicans	..	315	..	3	1	1	..	..
T. mentagrophytis	..	315	5	22	6	2	12	11
T. rubrum	..	315	9	14	10	4	6	13
E. histolytica	..	220	17	1	1	..	..	..
Dirofilaria	..	442	..	..	..	..	..	..

(4) DEPARTMENT OF CLINICAL PATHOLOGY

1. *Epidemiological Study*.—Thirteen *C. diphtheriae* strains were isolated from clinical cases. Two hundred and thirty-four contacts of these cases were screened and 3 carriers were detected. Samples from twelve children below the age of 7 were tested and six out of these were Schick positive and 2 were false positive.

2. *Studies on haemolytic streptococci*.—Three hundred and fifty-seven samples were collected from different hospitals. The source of the streptococci was mostly from the throat swabs. Out of these samples, 93 were *B. haemolytic streptococci*. Eight belonged to Group A.

Bacitracin disc was used for the identification of group A.

3. *Studies on leptospira*.—Serological studies in man and animals were continued by S. E. L. (Sensitized Erythrocyte Lysis) and M. A. (Microscopic Agglutination) tests. In the meantime, anti-human-globulin conjugate was prepared to try the F. A. (Fluorescent antibody) technic by

indirect method for the diagnosis of leptospira. Of the 100 human samples tested, 11 sera gave positive results by both the (S. E. L. and M. A.) tests. In 90 samples of sera from aborted sheep, 18 were reactive (titre greater than 1/100) and of the 90 stray dog sera, 35 were (titre greater than 1/100) found to be reactive.

4. *Diagnosis of enteric fevers.*—The work on the studies in the diagnosis of enteric fever as compared with the routine conventional method of fluorescent antibody technic and the haemagglutination technic has been completed. The haemagglutination technic proved to be more sensitive and reliable than the conventional agglutination or fluorescent antibody tests. Similar studies will be undertaken by employing "Bentonite fluorecculation Test".

5. *Anonymous mycobacteria.*—The study on anonymous mycobacteria with reference to various microbiological aspects was completed. It seemed that these anonymoustrains form a biological link between typical and saprophytic types. A new observation with respect to chromogenesis of Group I Strain on nitrate agar in the absence of a source of light was also made.

6. *Evaluation of immunological test for pregnancy.*—An attempt is being made to compare the immunological Ratex agglutination inhibition (Gravindex) test with the biological rat test that is being carried out in the department. So far, majority of them showed complete agreement as far as the pregnancy is concerned. Still, there are few cases showing discrepancy between the two tests. These are being followed up and investigated further. Work is in progress to estimate the hormone level of chorionic gonadotropic hormone in i. u. in 24 hours' urine sample to diagnose pregnancy and obstetrical disorders.

(5) DEPARTMENT OF ENTOMOLOGY

1. *Studies on Musca Sorbens.*—Studies on *M. sorbens* were concluded. Collections from Haffkine Institute compound showed that *Musca domestica nebulosa* and *M. sorbens*, shared the same habitat. Attempts were made to breed *M. sorbens*, Wied, under laboratory conditions, and to study their biology. Successful breeding of *M. sorbens* was achieved by using sugar-cane cubes and agar medium.

These two species could be differentiated morphologically and biologically. Internal morphological studies gave a large number of differentiating characters. However, in spite of these differences, the tissue grafts of the *Corpora allata* from *M. sorbens*, Wied to *M. nebulosa*, Fabr, and vice versa

readily took place, but there was no development of ovaries in the allatectomised females of these species. The transplantation of *Corpus allatum* in the same species effected the development of ovaries and fat metabolism, as was seen here in *Musca nebulo*.

2. *Breeding of different species of rats in the laboratory.*—*Tatera indica* collected from wild environment was bred in the laboratory up to seven generations so far. It was noticed that if the same male is used for insemination, the gestation period is reduced by a number of days. *Rattus rattus* also was successfully bred under laboratory conditions. The breeding of *Golunda Sp.*, *Tatera Sp.* and *Rattus mackenziei*, collected from Gevrai and Daulatabad, under laboratory conditions, was being continued.

3. *Rat collection.*—It was noticed that on stone paved Port Trust area, the predominant species of rat collected was *R. rattus*, while in the crowded localities of Bombay town, it was *B. bengalensis*. Collections were also made at Sewri near the Haybunder docks. These trappings were carried out in the Chawls as well as in fish godowns. In the Chawls, 58 rats were collected in 4 days (35 were male and 23 females). Further, here the rat *R. rattus* predominated and *B. bengalensis* was confined to ground floor collection only. The predominant flea was *X. cheopis*. In the godowns, during the same period, 251 rats were collected in which *B. bengalensis* were 52.5 per cent and *R. rattus* 26 per cent. Total fleas collected were 386, of which *X. cheopis* were only 36.5 per cent. and *X. astia* 63.5 per cent.

4. *Rat feeding.*—Experiments were carried out on a 24-hour basis to find out the amount of food and water consumed by rats in the laboratory. It was noticed that *R. rattus*, when given a dry food ate, on an average, 9.54 gms., when continuously exposed to light and 13.1 gms. when kept for 12 hours in dark. Eosin mixed with food showed that the excretion of pellets starts 6 hours after food intake and continues until 7 hours after the stoppage of the colouring matter in food.

The average output of pellets per hour was 7.2, maximum being in the early morning hours between 3 and 4 a.m. The average water consumption in 24 hours was 19.5 ml.

5. *Rodenticide trials.*—Experiments with Ratafin, an anti-coagulant rodenticide based on fumarin, were terminated. The results indicated that it was more effective on *R. norvegicus* (albino) than on *B. bengalensis* and *Rattus rattus*; average time of death was found to be 4.5, 6.7 and 6.5 days respectively at 0.025 per cent concentration—the concentration normally recommended for use in field operation. However, at the lower concentration of 0.0125 per cent. it was observed that while all the *B. bengalensis* and *R. norvegicus* (albino) rats died within 6 days of feeding. only 40 per cent. *R. rattus* died within that time.

Studies were also conducted on the blood-clotting time of rats after feeding them with an anti-coagulant rodenticide Warfarin, in a concentration of 0.025 percent for a varying number of days. The blood was collected by venipuncture after opening the rat and the clotting time with the help of capillary and slide methods. The table below the results obtained by these 2 methods.

Days	Methods	Normal	Clotting time in Seconds after feeding					
			1	2	3	4	5	6
<i>rattus</i> ,	Slides	107.8	125.0	167.5	201.0	215.0	244.0	282.5
	Capillary	126.00	145.0	177.5	192.5	230.0	237.5	315.0

6. *Studies on Oestrous cycle*.—Studies were conducted on *R. rattus*, *bengalensis* and *R. norvegicus* (albino) in connection with the duration of their oestrous cycles by taking vaginal smears at different intervals with the help of a sterile pipette. Periods of duration of oestrous cycle observed in different species of rats were *R. rattus* 117 hours, *B. bengalensis* 114 and *R. norvegicus* albino 95 hours.

7. *Studies on the host specificity of X. cheopis and X. astia. B. bengalensis*.—It was seen by releasing the fleas in *B. bengalensis* burrows for breeding. Before being released in the burrows, these rats were kept in the laboratory for more than 45 days to eliminate the possibility of a female rat being already pregnant at the time of release in the burrows. In all the three sets kept, *B. bengalensis* was seen to breed within 4 months from the date of release. It was also observed after one month that the number of fleas collected was less from the body of the adult rat than that from young rats. Burrows excavated showed that *X. astia* was collected more and that it has bred in the burrow, showing the specificity of *astia* to *B. bengalensis*.

8. *Studies on salivary glands of snakes*.—Anatomical studies of these were undertaken in both poisonous and non-poisonous snakes with a view to observe the modification that may be present in the gland of poisonous snakes, viz. Cobra, Krait and Viper. Studies were also undertaken to know the toxicities of such secretions of salivary glands of these for their comparative evaluation. Results showed that mucous portion predominates in non-poisonous snakes and in the poison- it was the serous portion that predominated.

(6) DEPARTMENT OF IMMUNOLOGY

1. *Isolation of Clostridia from soil, dung etc.*—This work was continued. In addition to the twelve strains isolated, as already mentioned in the previous report, two more have been isolated. The technique of isolation was the same, namely, use of "Differential reinforced clostridial medium" containing bacto-peptone, yeast extract, beef extract, soluble starch, sodium acetate, glucose and cystine. Sodium sulphite and ferric citrate are added just before plating out. Colonies of clostridia are easily identified by their ability to produce black iron sulphide. The black colonies were plated out again and further checked by spore formation, fermentation reactions, etc. The two newly isolated strains were labelled CK-1 and CK-2.

Strain CK-1 fermented glucose and maltose with gas formation. It did not ferment lactose, sucrose and mannitol. Gelatin was liquified. Methyl red test, V. P. test and indol tests were all negative. The biochemical tests showed it to be a strain of *Cl. sporogenes*.

Strain CK-1 was tested for the presence of bacteriophage and for bacteriocine. It was found to produce bacteriocine against *Cl. tetani* but harboured no phage. The strain CK-1 was itself lysed by the bacteriocine produced by C3. This is an interesting strain which produces a bacteriocine against *Cl. tetani*, but is itself lysed by a bacteriocine produced by C3 which is also a strain belonging to *Cl. sporogenes* group.

2. *Transfer studies of bacteriocinogenic factor from C3B⁺ to C3B⁻.*—In the previous report, the removal of the bacteriocine producing property C3B⁺ and its conversion to C3B⁻ strain was reported. This was possible by the combined action of penicillin (0.075 units ml.) and acriflavine (lugiml.) It was now of interest to find out if C3B⁺ can transfer the B⁺ factor to C3B⁻. Before this was attempted, the generation time of the donor (C3B⁺) and the recipient C3B⁻ were determined from opacity increase in thioglycollate medium. The generation time of both the strains was the same, being 36 minutes; the curves were also parallel. Starting with inocula of constant opacity, the strains C3B⁺ and C3B⁻ were mixed in the proportion 1 : 20 respectively and incubated at 37° C. Samples were taken out immediately after mixing and at intervals 5, 15, 30, 60 minutes and 24 hours, diluted appropriately and plated out on a lawn of Harward Strain of *Cl. tetani* to observe plaque formation. The proportion of colonies showing haloes to those not showing haloes (C3B⁻) was found out. It varied from 1 : 5 to 1 : 20. The increased number of (C3B⁺) could possibly be due to the distribution error. Though transfer of B⁺ was considered a possibility, could not be definitely established in these experiments. The use of markers like antibiotic resistance or nutritional requirements was thought to

3. *Transfer studies using syneromycin marker*.—Cultures of C3B⁺ and the indicator strain (Harward, *Cl. tetani*) were made resistant to 0.003 ug/ml. syneromycin by culturing them in increasing concentrations of the antibiotic. C3B⁺ was susceptible to this concentration of the antibiotic. C3B⁺ and C3B⁻ (Ros) of equal opacity were mixed in equal proportions. Samples were taken out immediately after mixing and at intervals 5, 15, 30 minutes and 24 hours and plated out on thioglycollate agar containing 0.003 ug/ml. syneromycin and spread on a lawn of *Cl. tetani* + (Resistant) colonies of C3B⁺ could not be isolated, indicating that B⁻ factor does not infect by transfer.

In order to see whether B⁺ can be transferred by the spheroplasts of C3B⁺ to C3B⁻, the cultures of C3B⁺ and C3B⁻ were grown in the presence of penicillin to form spheroplasts and then mixed and plated out as before. The results showed that there was not a single colony which was B⁺. This confirms that there is no transfer of bacteriocinogeny.

4. *Host range of bacteriocin of Cl.*—The activity of the bacteriocin produced by C3B⁺ against various organisms was tested. The following anaerobes were tested by the ditch-plate method :—

- (i) *Cl. tetani* (Harward strain & A471),
- (ii) *Cl. welchii*,
- (iii) *Cl. oedematiens* (strain Frankfurt),
- (iv) *Cl. septicum* (N. I. C. 282).

Among these, only *Cl. welchii* was found to be insensitive to the bacteriocin action, the remaining three were susceptible. Besides the above, clostridia isolated from soil in our laboratory were also screened. Out of 11 isolates, three *Clostridia*, namely, C4, C8 and C9 were susceptible to its action.

The following anaerobes were tested by streak method :—

- (i) *E. coli*,
- (ii) *Staph. aureus*,
- (iii) *V. cholerae*, and
- (iv) *S. typhi* O 901, H 901.

None of these were sensitive to bacteriocin action.

5. *Nutritional requirements of Cl. tetani (Harward strain)*.—*Clostridium tetani*, which produces the tetanus toxin, is unable to grow or produce toxin in a medium composed of acid hydrolysed casein. It has to be supplemented with tryptic digest of casein or beef heart extract and vitamins. It was, therefore, decided to take up the study of the nutritional requirements of this organism on a synthetic or semi-synthetic medium. The results might help to improve toxin production using a simple well-defined medium.

The basal medium was acid hydrolysed casein (casamino acid). To this medium, various supplements of amino-acids, vitamins, etc. were added or taken out to see growth and toxin production. Preliminary results using all minus one technique showed that *Cl. tetani* (Harward strain) could grow and produce fair amounts of toxin if acid hydrolysed casein was supplemented with cystine, tryptophane, 7 vitamins and salts.

The optimum requirements were then worked out. The results are as follows :—

- (i) Casamino acid 2.5 per cent. (deferrated),
 - (ii) Glucose 0.4 per cent,
 - (iii) NaCl 0.25 per cent,
 - (iv) MgSO₄ 0.1 per cent,
 - (v) FeCl₃ 6H₂O 3.2 mg. per cent,
 - (vi) Cystine 10 mg. per cent,
 - (vii) Tryptophane 3 mg. per cent.
 - (viii) Calcium pantothenate 0.3 mg. per cent,
 - (ix) Nicotinic acid 0.05 mg. per cent,
 - (x) Pyridoxine 0.025 mg. per cent,
 - (xi) Riboflavine 0.025 mg. per cent,
 - (xii) Vitamin B₁₂ 0.005 ug per cent,
 - (xiii) Biotin 0.5 ug per cent,
 - (xiv) PABA 0.1 mg. per cent.
- pH 7.3 to 7.6

6. *Immunoglobulins in horse sera*.—Mammals exhibit a complex spectrum of immunoglobulins. Antibody activity against a single antigen is found to be associated with a number of immunoglobulins. It is well-known that when horses are repeatedly injected with a protein antigen like diphtheria or tetanus toxin, in the beginning of immunization, the antibody activity is present in the IgG immunoglobulin class but in later stages of immunization, most of the activity is found in the IgA (T-component). The number of immunoglobulins in horse serum was investigated using two methods.

One method consisted of preparation of anti-immunoglobulin serum by injecting washed floccules of diphtheria toxin and horse antitoxin into rabbits. This anti-immunoglobulin serum was then used to determine the number of immunoglobulins in the horse serum by gel diffusion and immunoelectrophoresis technique.

In the second method, tetanus toxoid was polymerized with ethyl chloroformate and then used to absorb the specific anti-bodies from the horse anti-tetanus serum. The anti-bodies were then dissociated from the antigen-anti-body complex by means of potassium iodide. This anti-body preparation was then analyzed for the number of immunoglobulins by gel diffusion and immunoelectrophoresis techniques.

Eight immunoglobulins were detected by means of these methods. IgG and IgA (T) comprised the major portion of the immunoglobulins. These have been named IgGa, b and c, IgA(T), IgM, α -2 macroglobulin and 2 unidentified lines.

7. *Antigenic cross-relation of IgG and IgA(T).*—IgA(T) is a distinct class of immunoglobulin present in large amounts in a horse repeatedly immunized with tetanus or diphtheria toxoid and it is the main source of commercial anti-toxins. It was of interest to determine its antigenic relationship with the IgG immunoglobulin which also possesses antibody activity in the early stages of hyperimmunization with tetanus or diphtheria toxoids.

Gel diffusion test and the immunoelectrophoretic techniques were employed in this work. The pseudoglobulin fraction obtained by ammonium sulphate precipitation of horse anti-diphtheria sera was subjected to preparative electrophoresis in agar gel. The fractions were tested for homogeneity and fractions showing single line of IgG and IgA were separated and concentrated by hanging cellophane bags containing solution to be concentrated in front of a fan.

IgG and IgA were analyzed against rabbit anti-immunoglobulin serum (floccule serum mentioned above) in gel diffusion test. IgA showed a spur over IgG, which suggested that IgG was deficient in some determinants with respect to IgA.

IgG and IgA were digested with papain. Both showed two lines, each corresponding to Fab and Fc fragments in gel diffusion and immunoelectrophoresis analysis. The papain digests were subjected to preparative electrophoresis and the Fab and Fc fragments were separated.

On gel diffusion analysis, Fab fragments of IgG and IgA merged to show a reaction of identity, while the Fc fragment of IgA showed a distinct spur over the Fc fragment of IgG. These results suggested that the IgG and IgA differed in their Fc fragments.

8. *Anaphylactic reactivity of horse serum.*—Horse anti-sera are used for the prophylactic or therapeutic treatment of humans against diphtheria or tetanus. But, besides offering protection, horse anti-sera also render a person sensitive to an anaphylactic shock. We attempted to investigate this anaphylactic reactivity by separating the different components of the horse serum and studying their reactivity before and after degrading them with various reagents. Ovary's passive cutaneous anaphylactic test in guinea-pigs was employed for determination of anaphylactic reactivity. Electrophoretically homogeneous preparations of albumin, IgG and IgA were obtained by preparative electrophoresis method.

The comparison of the reactivity of the different fractions and the whole sera was made in terms of ug/ml. It was found that whole serum, albumin, IgG and IgA were equally effective on a protein weight basis. PCA activity of IgG and IgA was decreased ten-fold on digestion with pepsin and seventeen-fold on digestion with papain. IgG, on reduction with mercaptoethanol, was comparable in its activity to pepsin digestion products.

The difference in the activity of the papain and pepsin digests of IgG and IgA was assumed to be due to the difference in the molecular weight of the fragments obtained on digestion with these two enzymes.

(7) DEPARTMENT OF NUTRITION & BIOCHEMISTRY

Nutrition Section

1. *Studies in lipid metabolism.*—(A) *Effect of dietary fat on tissue lipids.*—Two dietary fats, viz. safflower seed oil and lard were fed to growing albino rats at 10, 20, 30 and 50 per cent levels (caloric intake) for two months. The tissue lipids were studied :—

(i) *Serum.*—(a) The serum cholesterol level was higher in the lard group, (b) as the dietary fat was increased, the serum cholesterol also increased and this rise was also more marked in the lard group, (c) there was practically no difference in the free-cholesterol and phospholipid levels.

(ii) The serum lipids were saponified and the fatty acid patterns were studied by gas chromatography.

In both the groups, the saturated fatty acids ($C_{16} \div C_{18}$) decreased, while the PUFA (Poly Unsaturated Fatty Acids) $C_{18:2} \div C_{20:4}$ increased as the fat intake was increased.

The saturated fatty acids ($C_{16} \div C_{18}$) were lower and PUFA ($C_{18:2} \div C_{20:4}$) were higher in the safflower oil fed groups while it was just the reverse in the lard groups. Oleic acid level remained fairly constant in the safflower oil groups, but in the lard group there was a 30 per cent increase when the fat intake was 30 and 50 per cent.

(iii) *Liver.*—The liver-fat was higher in the 10 per cent and 50 per cent fat groups (both safflowers and lard). Cholesterol and phospholipids followed the same pattern.

The percentage of $C_{18:2}$ and $C_{20:4}$ were much higher in safflower oil fed rats than in the lard group.

(B) *Cholesterol esterase activity in the intestines and pancreas.*—The esterase activity was studied *in vitro* using palmitic, stearic, oleic, linoleic and arachidonic acids. The results are summarised as follows :—

(i) *Intestinal esterase activity.*—This was more with unsaturated acids as to the saturated ones in all the groups. The

activity was almost the same for both palmitic and stearic acids. However, in the case of unsaturated fatty acids a progressive increase was noticed, i.e. the more the double bond the greater the esterase activity.

(ii) *Pancreas*.—Here again, the esterase activity was much more with unsaturated fatty acids. The esterification was slightly more with stearic acid as compared to palmitic acid. The maximum activity was seen in the case of oleic acid in all the groups. The pancreatic esterase activity was very much higher than the intestinal esterase activity.

2. *Microbiological evaluation of protein quality*.—(A) *Relative nutritive value of foodstuffs*.—The work on microbiological evaluation of protein quality with *T. pyriformis* W was continued further.

The studies on growth response of the microorganism to varying levels of test foods were carried out. The purpose of the experiment was to determine the relative nutritive values of test proteins on the basis of regression coefficients of growth-curve and to test the validity of the results obtained at a fixed level of 0.3 mg. N/ml. of the test food.

The regression coefficients were calculated from the growth response curves in the range of 0.1-1.5 mg. N/ml. for each test protein. The linear regression coefficients in the range of 0.1-0.5 mg. N/ml. were maximum in case of all the test proteins. The relative nutritive value computed from the regression coefficients in this range (0.1-0.5) gave a close correlation with the values of net protein utilization in young and old rats. These observations proved statistically the validity of the results obtained earlier at a level of 0.3 mg. N/ml. of the test foods.

(B) *Amino acid requirements of T. pyriformis* W.—The studies on the pattern of amino acid requirements of *T. pyriformis* W were carried out using the improved basal medium of Stott *et al.* (1963).

A mixture of 16 L-amino acids stimulating the amino acid composition of cow's milk was prepared and used as the base line of the experiments. It gave the maximum growth at a concentration of 1 mg. N/ml. of the final medium. By omitting each amino acid from the mixture in turn, it was found that the protozoan also required the 10 essential amino acids. In addition, serine was required for optimum growth. In the absence of glycine, serine was found to be essential for the growth of the protozoan.

The quantitative requirements of 10 essential amino acids were determined from the standard curves for each amino acid added to the basal medium at a series of concentrations. The quantitative pattern of amino

acid requirements obtained in this way was in close agreement with of higher animals.

3. *Nutritive value of "Gawar" meal (Cyamopsis tetragonoloba different protein fractions isolated from it*: The major problem developing nations including India is the impending protein crisis. order to meet this situation, a search for new and cheap sources is needed, besides increasing the protein production from tional plant and livestock sources.

With this view in mind, "Gawar" meal, a protein rich (48 per protein) by-product of a gum-producing industry was investigated its nutritive value and usefulness.

Feeding experiments on rats showed that the unautoclaved "Gawar meal produced growth inhibition, mild anaemia with 50 per cent mortality within 4 weeks. By autoclaving the meal for 1/2 hr. at 15 pressure, the growth inhibitors were destroyed. This fact could observed from the increase in growth-rate with the increase in the level of protein in the diet from 10 to 20 per cent.

The microbiological assay of amino acids from the hydrolysed showed that "Gawar" meal protein was deficient in tryptophane, nine and threonine. The deficiencies of these amino acids were per cent as compared to corresponding amounts in egg-protein.

Study of the biological value and "net protein utilization" *T. pyriformis* W also showed that the "Gawar" meal was a poor protein. But, supplementation with tryptophane and methionine its nutritive value to a considerable extent.

Fractionation of the proteins of the defatted meal was carried out the usual solubility method. The main fractions were albumin, lins A and B and glutelins. Albumin, globulins A and B 10 and 44 per cent of the total nitrogen, respectively. Glutelins non-protein nitrogen were present at 36 per cent and 6 per cent vely. The amino acid composition indicated that all the protein fractions were mostly deficient in both tryptophane and methionine.

The relative nutritive values of different fractions tested with *T. formis* W showed that the globulin B fraction had a higher value than other fractions.

The nutritive value of all the fractions could be enhanced by mentation with both tryptophane and methionine that of globulin twice its original value.

4. *Protein metabolism with special reference to vitamin B₁₂ and pantothenic acid—Pantothenic acid sparing action of vitamin B₁₂.*—This was investigated in pantothenic acid deficient rats. Pantothenic acid sparing action of vitamin B₁₂ was manifested by an increase in the time taken for the production of typical symptoms of pantothenic acid deficiency, reduction in the death rate and severity of anaemia due to pantothenic acid deficiency. Vitamin B₁₂ supplementation delayed the production of typical symptoms of pantothenic acid deficiency, viz. bloody whiskers and reddening of paws. Vitamin B₁₂ supplementation also reduced the mortality in pantothenic acid deficient rats. The death rate during the 10 weeks in the pantothenic acid deficient rats receiving vitamin B₁₂ was 31 per cent and that in rats receiving no vitamin was 58 per cent. The levels of various blood constituents, viz. haemoglobin red cells, leucocytes and total plasma protein in pantothenic deficient rats were also significantly increased by giving vitamin B₁₂ supplements.

5. *Studies in E. coli metabolism—(A) Cystine metabolism.*—Although cystine is an essential constituent of protein and glutathione in the cells of *E. coli* cystine, when added to the culture medium of *E. coli*, inhibited its growth under aerobic conditions. In the semi-aerobic condition, cystine is less inhibitory. The mechanism of this reaction is not yet known. Hence, studies on the utilization of cystine-S in *E. coli* 113/3 in the medium deficient in inorganic sulphur ($\text{SO}_4\text{-S}$) were carried out.

In the aerobic condition, *E. coli* 113/3 did not utilize cystine for growth, but the addition of aspartic acid to the medium helped the utilization of cystine-S and optimum growth was obtained. Experiments are in progress to study the effect of Krebs cycle intermediary compounds on the utilization of cystine for growth.

In the semi-aerobic condition (stationary phase) *E. coli* 113/3 responded favourably to the graded amounts of cystine. The optimum growth was obtained at a concentration of 10 ug. cystine/ml. of the final basal medium. Addition of aspartic acid did not enhance the growth.

(B) *Vitamin B₁₂ requirement of E. coli 113/3 in the presence of cystine as a sole source of sulphur.*—The requirement of vitamin B₁₂ in the presence of cystine in the medium, as a sole source of sulphur, was determined by test dose response. The requirement (1.5 mug./ml.) under this condition was more than in the presence of inorganic-S (0.1 mug./ml.).

6. *The effect of high doses of sodium cyclamate on the growth and reproductive system of albino rats.*—Three groups of pregnant female

rats were given 0, 10 and 50 mg. of sodium cyclamate/rat/day respectively. Sodium cyclamate was found to have no adverse effect on fertility rate, litter size, birth weight and survival of young ones.

These litters were weaned after 21 days from the mothers and the same feeding tests were continued for 90 days. Sodium cyclamate had no adverse effect on the growth of rats.

Biochemistry Section

1. (A) *Metabolic studies using antimetabolites—vibriostatic action of berberine.*—Earlier studies had indicated that this 'antidiarrheal' drug inhibits partially the growth of *V. cholerae* (Inaba) and El-Tor (Inaba) in synthetic medium and casein hydrolysate. A 40-50 ug. concentration of berberine per ml. culture completely inhibited the growth of *V. cholerae* for the 1st 8 hours whereas the same concentration could inhibit the El-Tor strain only to 50 per cent, i.e. El-Tor seemed to be more resistant to the action of the drug.

(B) *Effect of berberine on the biochemical processes—Protein synthesis.*—Effect of berberine on the incorporation of certain precursors of proteins—phenylalanine, leucine and glycine—was studied at intervals of log phase. About 75 per cent inhibition was observed in the incorporation of these three amino acids by the two strains as indicated by specific activity and total radioactive count in the total cells.

Nucleic acid synthesis.—Similarly the effect of berberine on the incorporation of nucleic acid precursors—adenine and guanine—was studied. About 50 per cent inhibition was observed as studied by specific activity and total radioactivity.

Since the inhibition observed was more in the case of protein synthesis, further confirmatory experiments using the common precursors of protein and nucleic acids, viz. glycine and aspartic acids, have been taken up.

2. *Comparison of V. cholerae and El-Tor with reference to their synthetic ability.*—The incorporation of phenylalanine, leucine, glycine, guanine and adenine by both the strains was compared. The phenylalanine, leucine, glycine and adenine were used more by *V. cholerae*. The El-Tor strain could incorporate only guanine to a greater extent. This difference in utilisation pattern will be studied in greater detail by further analyses of the 4 bases namely adenylic, guanylic, uridylic and cytidylic acids from the RNA fraction.

3. *Metabolism of salmonella, Metabolic studies using antimetabolites-chloromycetin.*—Studies on the growth kinetics of organisms (*S. typhosa*, *S. paratyphi A* and *S. paratyphi B*) in the presence of chloromycetin showed that *para B* is inhibited twice as much as the other two. A quantity of 2.5 ug. of this antimetabolite produced about 80 per cent inhibition in the case of *para B*.

Incorporation of phenylalanine.—This was studied at log phase in the presence of chloromycetin. Incorporation of this amino acid was inhibited more by *para B* as indicated by total and specific activities.

Incorporation of adenine and guanine.—A preliminary study was carried out at intervals of 0, 3, 6, 9 and 12 hours of their growth using a concentration of 2.5 ug./ml. of chloromycetin (both stationary, and shaken culture). The treated cells showed higher incorporation of these nucleic acid precursors during the early stages of the growth. No difference was observed when the cultures were shaken.

Effect of chloromycetin on the synthesis of sub-fractions of RNA.—A study of guanine incorporation into soluble RNA and ribosomal RNA (16S and 23S) was carried out. The treated cells showed higher specific activity in all the subfraction, i.e. the RNA synthesis, was enhanced by chloromycetin action. Further, it was observed that this enhanced activity was much more in the ribosomal RNA fraction. In all these studies, *S. paratyphi B* showed maximum activity.

Effect of chloromycetin on the incorporation of common precursors of nucleic acids and proteins—glycine and aspartic acid.—Good inhibition was observed in the case of glycine incorporation, whereas the same amount of chloromycetin showed comparatively less inhibition with aspartic acid. The specific activity of the fractions—acid—soluble fraction, RNA, DNA and proteins—was compared in normal and treated cells. Only in the RNA fraction, the treated cells showed higher specific activity whereas in all the other fractions, it was lower. Again, the protein, synthesis was inhibited much more than the other fractions. Again, this effect was more prominent in the case of *S. paratyphi B*.

Further fractionation of the RNA of aspartic acid-incorporated cells into its four nucleotides showed that adenine nucleotide had maximum activity in all the three organisms. It is interesting to note that nucleotide synthesis is not affected by chloromycetin, i.e. the site of action of this drug seemed to be after the nucleotide stage in the RNA synthesis

(8) DEPARTMENT OF PHARMACOLOGY

1. *Studies on anti-diabetic drugs.*—Investigation on the hypoglycemic activities of different plant extracts and synthetic drugs was continued. The plant extracts were prepared by extracting the dried plant with suitable solvents and concentrating under reduced pressure. The residues were suspended in distilled water and administered orally to fasting healthy rabbits.

During the year under review, different extracts of the following 7 plants were screened.

IDR 22 (*Babul gum*), IDR 23 (*Mayurshikha bark*), IDR 24 (*Hinganel fruits*), IDR 25 (*Palach flowers*), IDR 26 (*Vakermul*), IDR 28 (*Shegat bark*) and IDR 2 (*Banakadali*). Significant reduction in blood sugar was obtained with water extract of IDR 22. The other extracts had practically no hypoglycemic activity. A few of them, on the other hand, (IDR 23, IDR 25), showed hyperglycemic activity.

About 15 synthetic compounds were tested for antidiabetic activity. Out of these, 7 compounds were found to be mildly or moderately active when administered orally in a dose of 25 mg/kg. However, none of the drugs tested was found to be superior to tolbutamide, the well-known anti-diabetic drug.

2. *Studies on experimental amoebiasis.*—The screening of extracts of different medicinal plants was continued. The following plants were studied :—

(i) IDR-14 *Holostemma annulare* K. scehum (alcohol, chloroform, ether, petroleum-ether and water extracts).

(ii) IDR-16 *Phyllanthus niruri* Linn. (Alcohol, chloroform, ether, petroleum-ether and water extracts).

(iii) IDR-19 *Cedrus deodara* Roxb Loud. (Alcohol extract).

(iv) *Nyctanthes arborescens* Linn. (CDR-20) : (alcohol, chloroform, petroleum-ether and acetone extracts).

(v) IDR-21 *Woodfordia floribunda* (alcohol, chloroform, petroleum-ether, ether and water extracts).

(vi) *Asparagus racemosus* Willd (Res-1367) : (alcohol, extract).

All the different extracts mentioned above were tested against *E. histolytica* *in vitro* and a few *in vivo*. Water extracts of different plants mentioned above were tested *in vitro* at a concentration representing 100 mg. of crude drug per ml. of medium and other extracts at a concen-

tration representing 1 g. of crude drug per ml. of the medium, none showed any activity.

The alcoholic extract of *Asparagus racemosus Willd* at a concentration of 1 mg/ml were found to inhibit the growth of *E. histolytica*.

In vivo experiments were carried out with golden hamsters. Water extracts of IDR-14 and IDR-16, when administered at a dose level representing 10 g. of crude drug per kg. of body weight, showed no activity.

3. *Studies on indigenous plants.*—(a) *Berberine.*—Berberine hydrochloride has been found to be useful in the treatment of cholera and acute diarrhoea in clinical trials undertaken in 1967. As an anti-amoebicidal drug, berberine reduced the infection in the hamster by 80 per cent in a dose of 150 mg/kg and 82 per cent in amoebiasis of the white rat in dose of 100 mg/kg given orally. *In vitro* study has shown marked anti-amoebic effect in a concentration of 10 mc/ml.

(b) The mode of action of berberine in the prevention of diarrhoeal symptoms is being investigated. The antagonistic action of berberine hydrochloride against certain biogenic substances was examined. It was found that it reduced the effects of histamine, 5 H. T. and bradykinin on isolated tissues (small intestine of guinea pig and rat uterus), thereby suggesting interaction of these substances in reducing diarrhoeal symptoms.

(c) *Cynodon dactylon (Durva) (IDR-7).*—The crude water extract of *Cynodon dactylon* was tested for different pharmacological actions. In atropinised cat, it showed a slight rise of blood pressure in doses of 0.1 ml to 0.3 ml/kg (1 ml equivalent to 1 g. of drug) intravenously and on isolated toad's heart, marked reduction in force of contraction without affecting the rate; larger doses showed inhibition but revived to normal within a short period. In atropinised toad's heart, the drug showed the same effect. Adrenaline effect on toad's heart was checked by the drug. Topical application caused local vaso-constriction in mesenteric capillaries of albino rats and mice which lasted for a few minutes. The extract was found to produce mild dilatation of bronchial musculature in guinea-pig, (0.5 ml intravenously). Contraction of isolated tracheal chain of guinea-pigs *in vitro* was observed as well as potentiating effect in pentobarbitone induced sleep (albino rats and mice). Lep'tazol induced convulsion in rats could not be checked, but tendency towards prolongation of the survival time was indicated. It did not have any sedative effect in rabbits, rats and mice. No change could be detected in general behaviour in rabbits and mice when injected intravenously and in rats intraperitoneally. Clotting

and bleeding times were slightly increased. Slight diuretic effect was observed in rats. It did not have any inhibitory action on the growth of *E. histolytica* *in vitro*. On isolated organ experiments like guinea-pig's ileum, the extract showed relaxation (5 mg/ml) but no effect on rat uterus (up to 40 mg/ml.).

(d) *Nyctanthes arbortristis*, Linn. (*Parijita*) (CDR-20).—Chemical Study.—A preliminary chemical analysis of the alcoholic extracts of the leaves of *Parijita* showed presence of fatty substances, essential oil, sugars, glycosides and alkaloids. One kg. of powdered leaves of *Parijita* was extracted with the rectified spirit. Total alcoholic extract was then concentrated under reduced pressure at 50°C. The alcoholic extract was extracted with 5 per cent hydrochloric acid till the last acid extract did not give test for alkaloids as tested with Mayer's reagent. Total acidic solution was filtered and extracted with chloroform to remove non-alkaloidal acid soluble substances. The acid solution was then made alkaline with 10 per cent NaOH solution. The alkaline solution was then filtered and extracted with chloroform till last chloroform extract did not give test for alkaloids. The total chloroform extract was dried over anhydrous sodium sulphate and chloroform removed on a water bath. The yellowish coloured residue gave test for alkaloids.

(e) *Iris florentina*, (*Pushkarmula*) (IDR-5).—A steroid (m.p. 111°C) was isolated from *Pushkarmula* and the molecular formula was suggested to be $C_{16}H_{18}O_2$.

The viscous brown liquid remaining after removal of the steroid and the essential oil was considered to be a fixed oil. It was established by chemical methods that this fixed oil contains tridecanoic acid.

Alkaloids.—The following alkaloids were isolated from defatted extract of *Pushkarmula*.

Either insoluble alkaloids: Two alkaloids were isolated by chromatography (Al_2O_3).

Alkaloid (Liquid) m.p. of picrate 135-37°C.

Alkaloid (solid)—m.p. 176-78°C.

m.p. of picrate 155-56°C (decom).

Glycosides.—A glycoside was obtained from defatted extract of *Pushkarmula*. The melting point was 178-80°C.

(f) *Indigenous drug in the prevention and treatment of small-pox and chicken-pox.*—In India, several preparations made from indigenous medicinal plants have been used from time immemorial against small-pox and chicken-pox. Amongst them, one plant is very popular.

Three constituents were isolated from the seeds of the plant (Patented) which inhibited the growth of small-pox and chicken-pox viruses on the chick chorioallantoic membrane. In mice, they prevented variola infec-

tion and cured them even when the drug treatment had begun at a late stage. These drugs were relatively non-toxic when injected into the mice. Experimental evidences suggest that these active constituents will be useful in the prevention and cure of small-pox cases.

4. *Anti-bacterial activity of medicinal plants.*—Of the extracts of six plants species (IDR-24, 25 and 28-31) tested for their antibacterial activity against *Staphylococcus aureus*, *Salmonella typhosa*, *Vibrio cholerae*, *Escherichia coli*, *Shigella dysenteriae*, the water and alcohol extracts of IDR-28 *Moringa oleifera* inhibited all the five test bacteria. The water and alcohol extracts of IDR-30, *Picrorhiza kuroa* inhibited the growth of four of and five test species (*E. coli* was not inhibited) and the water alcohol extracts of IDR-31 *Santaloides minus* inhibited *Shigella Dysenteriae* only.

5. *Studies on the stability of panthenol.*—The effect of pH, different buffers, vitamin B₁ and C on the stability of panthenol was studied under accelerated condition. Panthenol was found to be stable after 30 weeks at pH 5 and its stability decreased as it changed to either acidic or basic side. Then tested at pH 6, it was found that phthalate buffer had more stabilising effect on panthenol than citric acid-sodium phosphate buffer or phosphate-sodium hydroxide buffer. It was found that B₁ and C had deteriorating effects on panthenol.

(9) DEPARTMENT OF VIROLOGY

1. *Rabies.*—(a) *Tissue culture studies.*—(i) Cultivation of rabies virus. As stated earlier, freshly trypsinized hamster kidney tissue cultures (HKTC) grown in a medium of Hank's balanced salt solution (HBSS) with 10 per cent. normal horse serum and 0.5 per cent. lactalbumin hydrolysate, were used. About the 5th–7th day, when monolayer sheets were fully formed, the concentration of serum in the medium was reduced to 5 per cent. and the tubes were inoculated with virus material (0.1 per cent. inoculum in 1 ml. medium per tube).

The first attempts were made to cultivate Paris strain material, previously adapted to HKTC and stored at the 10th passage, at -20°C . This material caused irregular degeneration of cells which could not be related to any viral contaminant and was, therefore, considered to be toxic.

A fresh attempt was made to cultivate CVS rabies material. A 10 per cent. suspension of mouse brain containing $10^{6.8}$ mouse LD₅₀ of the virus per 0.03 ml. was used as the initial inoculum. No CPE was seen upto 20 days after inoculation. On the 5th day, the fluid was harvested and fresh medium added. The 5th day material was inoculated in a fresh batch of tubes. Five such passages, using 5th day harvest in each case, were made.

At the 3rd HKTC passage, the harvested fluid was also inoculated into mice by the I.C. route. No paralysis or death was seen in either adult or infant inoculated mice. Such results might be obtained before the virus adapts to the new host system. Its significance will be evident after further passages.

(ii) *Indicator virus*.—Four virus strains were taken for adaptation. Vaccinia virus, derived from USSR freeze-dried vaccine; Coxsackie A₉, B₂, and B₃ viruses derived from monkey kidney T. C. (MKTC). The vaccinia virus adapted rapidly showing marked CPE and destruction of the cell sheet by 24 hours in the 2nd passage onwards. The enterovirus strains showed doubtful CPE on the first two passages. By the third passage Coxsackie B₂ and B₃ showed well marked CPE which was accelerated on subsequent passage. Coxsackie A₉ was slower to adapt, but showed marked CPE by the 4th passage. At the 5th passage in MKTC, all the three strains showed 2+ to 3+ CPE by the 3rd day after inoculation. The work is being continued.

(b) *Pathogenesis of rabies*.—(i) Studies on prolongation of incubation period are progressing. (See previous report).

A study of the brown fat as a reservoir of rabies virus during the latent period ("incubation period") is undertaken in mice. On the basis of previous studies, the brown fat appeared to be a site for virus multiplication though the virus titres obtained were low. Even before detectable virus appeared in the brown fat, histological changes in this tissue were apparent. These consisted of reduction in cell size, increase in granularity of cytoplasm, together with congestion and extravasation of red cells. In an effort to determine whether these changes were specific or not in nature, the following experiment was undertaken: A batch of mice was inoculated with approximately 100 LD₅₀ of CVS fixed virus (mouse brain suspension) into the muscle of the thigh of the left limb. At 24-hour intervals, groups of two mice were sacrificed and the tissues harvested. Half of each tissue, from each mouse harvested, was ground in a sterile mortar to make a 10 per cent. suspension. Each suspension was inoculated by the intracerebral route into a batch of 6 mice for virus isolation. The other half of each tissue was fixed in formalin saline for paraffin sections. The tissues taken were: brain, salivary gland, brown fat (the interscapular dorsal pad of brown fat) and muscle tissue from the site of inoculation—(about 3.5 cu. mm. of the vastus muscle from the anteromedial aspect of the thigh). Two sets of control mice were also sacrificed daily and the tissues harvested and processed in an identical manner. The controls were:—

(a) normal uninoculated mice of the same age to see histological changes which might occur in the brown fat spontaneously or as a result of and

(b) mice similarly inoculated with A. R. Vaccine instead of virus, to study the histological changes in the brown fat as a result of injury and the introduction of foreign material similar in antigenic character to the virus suspension, i.e. brain tissue.

While the study is mainly undertaken to assess the role of brown fat as a reservoir of rabies virus in a non-hibernating animal (its reservoir role in hibernating bats has already been established), it may also give some information on virus content and histological changes, if any, in the other tissues under study.

2. *Influenza*.—The number of patients who come forward for throat gargles are very few. During the year 5 throat gargles and swabs and 6 blood samples were collected.

One paired blood sample was available. Most of the patients were either from the institute staff or were referred by practitioners in the vicinity. One new strain of influenza virus (Type A2) was isolated during the year.

3. *Measles*.—Studies on antibody titres of mother's and cord's blood, and among children of different age groups are being continued. It is hoped, when the study is complete, the antibody pattern of different age groups will give an indication of the probable age of exposure to measles in children in Bombay. The summarized results in the following table show the number of blood samples tested and the numbers having antibody titres which may be indicative of recent infection, in each age group.

Age group			Total samples	HAI titres above 1 : 20	CF titres above 1 : 8	NT titres—above 1 : 8
Sc (cord)	...	..	50	1	Nil	1
Sm (mother)	..	..	50	4	Nil	1
0 to 6 months	..	..	28	7	1	4
6 months to 1 year	..	..	17	3	Nil	1
1 to 3 years	..	..	17	10	4	6
3 to 5 years	..	..	3	1	Nil	Nil
Above 5 years	..	..	8	7	4	5
Adults	...	..	50	6	Nil	Nil

4. *Studies on enteroviruses.*—During the period under report, materials for virological studies were either referred here on collected by the enterovirus laboratories from 238 clinical cases. All these cases showed involvement of the central nervous system viz. paralytic and non-paralytic poliomyelitis, aseptic meningitis, encephalitis and other miscellaneous unspecified cases. Materials for these studies include those collected from local hospitals in Bombay, those received from Ceylon and Andaman Islands and those referred by private practitioners. The studies carried out so far are presented below :—

(i) samples from hospitals in Bombay (LTMG, KEM, BJW BJ & Kasturba).

These Samples were from cases of polio, encephalitis, aseptic meningitis, gastroenteritis.

Total serum, stool, C.S.F. and throat swabs, etc.	872
No. of samples for isolation	... 753
No. +ve in TC	... 234
No. +ve in mice	... 58
Identity—Polio type 1	... 18
Polio type 2	... 37
Polio type 3	... 13
Cox. B ₁	... 9
Cox. B ₄	... 9
Cox. B ₆	... 17
NP	... 84

N.B.—Identification of remaining samples is being pursued.

(ii) Material received from private practitioners (Serum, stool, C.S.F., throat swabs etc.)

Total	... 35
No. —ve both in TC and in mice	... 13
No. +ve either in TC or in mice	... 13
Identity—Polio type 1	... 1
Polio type 3	... 1
Cox. B ₄	... 2
Cox. B ₅	... 1
NP	... 4

N.B.—Identification of remaining samples is being pursued.

(iii) Samples received from Ceylon.

(i) Batch received in November 1967.

Total	...	16
No. —ve in both in TC and in mice	...	6
No. ve either in TC or in mice	...	10
Identity—Polio type 2	...	1
Cox B ₄	...	2
NP	...	7

(ii) Batch received in February 1968.

Total	...	65
No. +ve in TC	...	44

N.B.—Identification of remaining samples is being pursued.

5. Experiments on storage of fully grown monkey kidney cell sheets at refrigeration temperature :—

In the previous annual reports, the results of the viability and susceptibility of primary monkey kidney cell suspensions at different temperatures for varying periods of time were presented. The results may be summarized as follows :—

Monkey kidney cell suspensions could be successfully stored at 0°C to 4°C up to 15 days; at room temperature (28°C to 34°C) up to 2 days and at 37°C up to 7 days without any detectable loss of susceptibility to any of the three types of poliovirus.

In the present report, storage of fully-grown monkey kidney cell sheets at 0°C to 4°C was studied.

Monkey kidney cultures showing complete monolayer cell sheets (obtained after incubation at 37°C for 7 days after seeding) were changed with M+Hanks Half the cultures were changed in medium containing 2 per cent. horse serum (A) and the remaining half with 4 per cent. horse serum (B). The changed cultures were kept in a refrigerator (0°C to 4°C) for 7, 15, 21 and 30 days in stationary positions at an incline of 5°. After appropriate periods of storage, cultures were transferred to 37°C and also kept in stationary positions at an incline of 5°, cell sheets being observed microscopically daily for 7 days for extent and quality of the growth, both before and after, transferring to 37°C. No difference was noted in cultures which were changed with M+Hanks containing 2 per cent. horse serum and those changed with M+Hanks containing 4 per cent. horse serum.

In all the categories of cultures, it was noted that the cell sheets started showing deterioration after 7 days' storage at 0°C to 4°C and were

practically in the form of single cells (resembling 2+ to 3+cytopathic effect caused by polio-viruses) after 15 days storage at this temperature. However, when the same cells were transferred to 37°C, they showed a revival or rejuvenation. Cell sheets stored upto 30 days at 0°C to 4°C showed clean, healthy and monolayer sheet formation within 3 days of reincubation at 37°C.

The same experiment was done with two more batches of monkey kidney cultures with similar findings. However, in these experiments cultures were changed only with M+Hank's medium containing 2 per cent. horse serum prior to refrigeration.

The susceptibility for poliovirus type 1 was studied in cell sheets from two monkey kidney batches and stored at 0°C to 4°C for (a) 7 days (b) 15 days and (c) 21 days.

Parallel titrations were carried out using fresh (unstored) cell sheets as controls. Cultures from all categories were changed with serum free media before titrating the virus. Six cultures were employed per dilution and 0.1 ml. of the inoculum was used per culture.

It was found that there was no significant difference in the titre of poliovirus Type 1 in all the categories of tissue cultures. This finding suggests that there was no difference in the susceptibility of stored cell sheets for poliovirus Type 1.

The present finding would be confirmed using more batches of monkey kidney cultures. Storage at 0°C to 4°C should be extended for periods over 30 days.

6. *Studies on rats as carriers of human virus infections.*—Rats have been known to be the reservoirs of plague, leptospirosis and other bacterial or parasitic diseases. They have been included, among other rodents, in field studies on the epidemiology of some of the Arbor viruses, but no systematic study has been made of the possible role of rats in the spread of human virus disease. The rat is closely associated with man. It lives in or around human dwellings, it raids the larder or food store, often contaminating the food with its droppings, and it is susceptible to diseases which affect man. It was, therefore, felt that a study should be made to find out any virus infections which a rat population suffers from and to see whether any of these viruses are related to human pathogenic viruses.

A programme for rat destruction and trapping in four villages of Panvel Taluka was undertaken by the Department of Entomology of this Institute. Rats were trapped in and around the houses during the period, following the first rains (June & July, 1967) and this seemed to be a good opportunity for undertaking such a study.

Blood samples were drawn from the heart of each rat prior to sacrifice. The rat was then killed by chloroforming and then dissected. The brain,

lung, spleen, dorsal pad of brown fat and intestinal (large) washing were collected under sterile precautions. Each tissue constituted one sample. Samples from 2 rats were mixed together, five such tissue pools being prepared from each pair of rats. (See table). A part of each of the first four tissues was removed and fixed in formalin for histological study. The rest of each tissue pool was ground up to make a 10 per cent. suspension, treated with antibiotics and stored for investigation.

Later, it was decided to study also urban rats, trapped in Bombay city which were supplied to us by the Bombay Municipal Corporation, and several pools of tissues from urban rats have also been similarly collected.

The work of trapping of rats in Panvel commenced in July 1967 and the dissection, inoculation of pooled specimens etc. has been going on for the past six months. Some viral agents have been detected but no identifications have yet been made. The strains are being studied for ascertaining their characters, and for preparation of antiserum in rabbits. The progress of work made so far is shown in the Table below :—

	Rats from rural area June-Aug. 1967	Rats from urban area (Kamathi- pura area) Sept.-Dec. 1967.
No. of tissue specimens ...	325 (65×5)*	200 (40×5)*
No. inoculated in mice (infant and adult).	60	10
in MKTC ...	8	5
in eggs ...	2	...
No. showing no pathogen for mice.	21	...
No. showing pathogen to infant mice.	10	5
to infant & adult mice ...	5	2
to infant mice & MKTC ...	1	Not done
to infant and adult mice and MKTC	1	Not done
No. under study	22	0

*5 tissue pools from each pair of rats (refer to text).

7. *Tissue Culture Section*.—Preparation and maintenance of new cell lines in the department and efforts to grow them in continuous cultures.

Cell cultures processed in the department for continuous cultures included chick embryo liver and heart, human embryonic kidney, liver, lung and muscle. Each of these could be subcultured 2-3 times.

PART III

ROUTINE ACTIVITIES

ROUTINE ACTIVITIES

(1) ANALYTICAL AND CONTROL DEPARTMENT

General

This is the fifth year of the existence of the Analytical and Control Department. During the major part of the year, work was mainly conducted in the two rooms on the second floor. This space is totally inadequate for the multifarious activities of the Department. Due to acute shortage of space, the Department could undertake only the standardisation of the finished products. As and when newer products were introduced in the market, their control for quality, purity and strength was undertaken by the Department.

Dr. M. K. Habbu retired during the year. Dr. R. B. M. Colah was appointed as Assistant Director incharge of the Department. The Department lost the services of Shri R. G. Tamhane who was transferred back to the Department of Pharmacology. The following Senior Technical Assistants joined the Department :—

Kum. K. S. Dalvi, Kum. A. T. Dadlani, Kum. M. R. Chedda,

Kum. S. D. Kasbekar, and Kum. A. K. Shah.

The following Senior Technical Assistants left the department :—

Shri A. D. Kulkarni, Shri J. N. Gadre,

Kum. B. D'Souza, and Kum. M. R. Chedda.

The following new procedures were established by the Department : —

Estimation of aluminium content in alum precipitated toxoids, standardization of 'Diphtheria and Tetanus Vaccine, B.P.', estimation of sodium in pasteurised human plasma.

During the later part of the year, the space in the old penicillin building, vacated by the Polio Unit, was occupied by the Department. This building is being fitted up and equipped with a view to expand the activities of the Department.

Routine

The number of different tests conducted by the Department during the year are tabulated below :—

TABLE

Products	Test	No. of sample tested
<i>Sterility</i>		
Bacterial Vaccines	...	11,064
Antirabic Vaccine	...	11,365
Parenteral bulk solutions	...	7,820
Antitoxins and Sera	...	12,056
Miscellaneous	...	630
<i>Pyrogen</i>		
Parenteral bulk solutions and blood products etc.		1,384
<i>Chemical and physical examination</i>		
Parenteral bulk solutions	...	1,415
Vitamin preparations	...	1,171
Sulpha drugs	...	234
Miscellaneous	...	47
<i>Toxicity</i>		
Antitoxins, toxoids, vaccines etc.	...	747
<i>Absence of infective virus</i>		
Viral Vaccines	...	383
<i>Potency</i>		
Antitoxins	...	84
Antivenine	...	37
Toxoids	...	19
Triple antigen	...	4
<i>Preservatives</i>		
Vaccines, Antitoxins	...	727
<i>Free formaldehyde</i>		
Bacterial vaccines, toxoids	...	200
<i>Microbiological assays</i>		
'Pemon'	...	70
Total		49,457

(2) DEPARTMENT OF BACTERIOLOGY

Routine

During the period under review, the production of Cholera—El-Tor and T. A. B. vaccines was continued. No plague vaccine was produced, as the Department had sufficient stocks to meet reasonable demands.

The quantities of vaccines produced and issued are given below :—

Vaccine	Quantity produced	Quantity issued
Plague ...	Nil	109,160 ml.
Cholera ...	Nil	108,249 ml.
El Tor ...	Nil	642,240 ml.
Cholera-El Tor ..	12,242,180 ml.	9,793,902 ml.
T. A. B. ...	670,780 ml.	228,737 ml.

The above vaccines were also issued to States other than Maharashtra.

In addition to production of the vaccines, samples of frog legs and some marine products were taken up for bacteriological examination at the request of some exporters. The following table shows the number of sample examined :—

Product	Number examined	Examination for absence of pathogen or bacteria
Frog legs ...	77	Total bacterial count and Salmonella.
Lobster tails ...	2	Do.
Fish meals ...	2	Salmonella.
Bone meals ...	2	Salmonella and other pathogenic organisms.
Meat meal ...	2	Do.

None of the samples of frog legs and lobster tails was found to be contaminated with Salmonella organisms. However, most of these samples failed to meet the requirements as regards total count, which clearly indicated the necessity for adoption of stricter sanitary procedures in handling the material during processing.

Media section

The Media Section continued the manufacture of varieties of media, stains and reagents, required for diagnosis of diseases and for testing of drugs. The manufacture of parenteral solutions also continued in full swing.

In addition to the above, the department provided general facilities for sterilisation and cleaning of glassware, etc. The Ampoule Section supplied cut, washed and sterilized ampoules to the Departments of Bacteriology, Immunology and Virology. The production figures of different items are presented below :—

Production of parenteral solution

Products	Litres
Injection of Dextrose and Sodium Chloride.	58,248
5 per cent. Dextrose Solution ...	74,831
25 per cent. Dextrose Solution ...	1,707
Normal saline	27,037
Sodium lactate	2,273
A.C.D. Solution	16
Water for injection	7,158

Production of media

Media	Litres
Casein hydrolyste for Cholera Vaccine ...	22,765
Sterility test media (1) Aerobic ...	6,448
(2) Anaerobic ...	8,744
Agar for Cholera research ...	156
Nutrient agar	828
Nutrient broth	215
Glucose phosphate	3
Lowenstein Jensen	15
Löffler's	34
Glucose broth	45

Products			Litres
Mac-conkey agar	...	...	60
Mac-conkey broth	...	...	9
Ox bile	...	...	20
Sabouraud broth	...	...	34
Sabouraud agar	...	...	122
Hartley's broth	...	...	14
Dysentery Enteric Cholera	...	...	76
Peptone water	...	...	35
Glucose	...	...	35
Sucrose	...	...	35
Mannite	...	...	35
Lactose	...	..	37
Dnlcitol	...	...	29
Arabinose	...	...	1.5
Xylose	...	...	1.5
Maltose	...	...	1.5

Production of stains and diagnostic reagents

Stains and diagnostic reagents			Litres
Albert 1	...	...	4
Albert 2	...	...	6
J. S. B. 1	...	...	21
J. S. B. 2	...	...	18
Carbol Fuchsin	...	...	54
Gram stain 1	...	...	21
Gram stain 2	...	...	21
Diluting fluid for Red Blood Cells	...	...	15
Methylene blue	...	...	42
Benedict solution	...	...	404
Löffler's Methylene blue	...	...	15
Methyl violet	...	...	15

(3) DEPARTMENT OF BLOOD BANK

General

As the demand for the products has been on the increase, attempts are being made to step up collection of blood both at the Institute and through the mobile unit. In this connection, the Government has created a post of Public Relations Officer in accordance with the recommendations of the State Advisory Board. The post has been filled up recently and the progress has to be watched. The mobile blood collecting unit visited various prisons in the State for collecting blood every month.

The department took part in the International Tourist Fair and put up a blood collection stall conjointly with the Tata Blood Bank during the months of December 1967 and January 1968. The response of the donors was encouraging.

The blood collection room has been converted into an air-condition room, so that more satisfactory conditions are maintained during blood collection.

Antweiler Rapid micro-electrophoresis apparatus Zeiss refractometer and refrigerated blood centrifuge are new additions to the department.

It has been possible to start plasma phoresis on donors. In this, way, the plasma yield was increased and the donors were bled at less frequent intervals.

The mobile blood collecting unit extended its voluntary blood donation drive to the colleges in Poona and achieved a good response. It is proposed to extend such visits to other cities in the State.

Staff

Shri C. J. Karkhanis has been promoted as Senior Scientific Officer, Class II and is in charge of the Blood Plasma Processing Unit. Shri A. S. Quershi has been promoted to Junior Scientific Officer.

Routine

Statement showing production and sale of blood products for the year ending 31st March 1968

(1) Blood donors—

Voluntary	...	...	741
Paid	...	...	4,778
Prisoners	...	...	3,692
Hospitals	...	...	2,986
Total	...	...	12,197

(2) *Blood plasma—*

Produced		Supplied	
Dried Plasma	... 737.50 litres	Dried Plasma	... 790.50 litres
Plasma supplied for Defence Forces,	... 208.00 litres	Plasma supplied by Army,	... 205.00 litres
Total		Total	
... 945.50		995.50	

(3) *Human gamma globulin—*

1 ml. × 5708	...	1 ml. × 5654
2 ml. × 126	...	2 ml. × 126

(4) *Normal human fibrinogen—*

150 ml. × 99	...	150 ml. × 88
10 ml. × 50	...	10 ml. × 40

(5) *Rh tests done—* 321

(6) *Pack cells supplied—* 433

(7) *Blood grouping reagents—*

Produced		Supplied
24770 × 1 ml.	Anti—A	25894 + 1 ml.
23819 × 1 ml.	Anti—B	25655 × 1 ml.
1170 × 1 ml.	Anti A+B	1041 × 1 ml.
982 × 1 ml.	Coomb's Reagent	827 × 1 ml.
1016 × 1 ml.	Bov. Albumin	995 × 1 ml.
668 × 1 ml.	Anti-C+D	649 × 1 ml.
1486 × 1 ml.	Anti-D	1146 × 1 ml.
134 × 1 ml.	Anti-A ₁	58 × 1 ml.
142 × 1 ml.	Anti-A ₁ Lectin	29 × 1 ml.
150 × 1 ml.	Anti-H	112 × 1 ml.
54 × 1 ml.	Anti-M	46 × 1 ml.
53 × 1 ml.	Anti-N	44 × 1 ml.

(4) DEPARTMENT OF CHEMOTHERAPY

General

All the research problems taken up in the department were pursued during the period under report. In the field of anti-tumor studies, 17 new compounds were sent for screening to the Cancer Chemotherapy Centre of U. S. Public Health Department. Two of the compounds sent earlier showed encouraging results. Preliminary screening reports on two Central Nervous System (CNS) depressants synthesised earlier by us were quite encouraging. These compounds were prepared in larger quantities and the detailed pharmacological evaluations have been completed. At present, subacute and chronic toxicity studies are in progress. Three patents, which were under preparation when the last report was submitted, have been filed.

In the antimicrobial screening unit, the support from C. S. I. R. has been useful in extending the screening of compounds to additional pathogenic organisms, viz. : *E. histolytica* and for *antifilarial* activity. Over 300 compounds were screened for antimicrobial activity. The broad screening programme revealed unexpected anti-tubercular and anti-fungal activity in a new class of compounds primarily synthesised as CNS depressants. This new lead is being taken up for further synthetic preparation of compounds for the possible anti-fungal and anti-tubercular activity.

Post-graduate research activity was maintained at full level. Nine students have been working for Ph.D. degree and five for M.Sc. degree in the department under different guides.

Two Ph.D. and one M.Sc. these tueses were accepted by the University of Bombay and six research papers from the department were published in Indian and Foreign journals during this period.

Manufacture of routine clinical products was maintained and all the demands from Government hospitals, dispensaries and E.S.I.S. were met. In view of the newer formulations demanded by E.S.I.S. for their new schedule beginning April 1968, developmental work on these new items was taken up and we hope to supply these newer formulations. The pilot plant was utilised for the preparation of R-1230 and R-1361 (CNS depressants required for detailed investigations and clinical trials) as well as for the production of tolbutamide, pemon etc. Microanalytical section of this department carried out 480 analyses for nitrogen and 124 for carbon and hydrogen during the period under review.

*Statement showing the production during the period
April 1967 to March 1968*

Products			No. of tablets manufactured.
Vitamin A.D. (Ped)	...	...	9,903,500
Vitamin A forte	...	...	96,500
Vitamin A.D. (C)	...	...	1,421,200
Vitamin B.C. tablets	...	...	27,205,620
Vitamin B. C. No. 3	...	...	1,503,300
Vitamin B ₁	...	...	2,313,718
Vitamin B ₂	...	...	266,064
Vitamin C	...	...	5,854,600
N. Acid	...	...	1,166,400
Vitamin K tablets	...	...	306,700
Calcium with D	...	...	232,320
Multivitamin tablets	...	...	34,507,380
Tolbutamide tablets	...	...	2,153,040
Sulphadiazine tablets	...	...	7,201,000
Suphaguanidine tablets	...	...	9,239,500
Sulphadimidine tablets	...	...	16,564,500
Thiacetazone and Isoniazide	...	...	1,332,600
Iodochlor tablets	...	...	780,100
Chlorpropamide (100 mg.)	...	...	15,400
Chlorpropamide (250 mg).	...	...	10,300
Total		...	121,973,742
Products			Quantity.
Pemon	...	...	299,700
Sulphathiazole paste	...	...	70 kgs.
Solapsone strong B.P.C.	...	...	713 × 50 ml.
Tolubutamide powder	...	...	888.55 kgs.

(5) DEPARTMENT OF CLINICAL PATHOLOGY AND DIAGNOSTIC REAGENTS

General

The Department continued its research, production, testing and teaching activities. Dr. M. V. Sant, Assistant Director In-charge of the Department, was deputed abroad on a W.H.O. Fellowship in November 1966. He returned after a study tour of about one year. In Prague, he successfully completed a six-month training course in Epidemiology and Biostatistics, for which he received a post-graduate Diploma in Epidemiology. He was also trained in zoonoses which he studied at various National, Veterinary and University laboratories in Czechoslovakia. At the National Communicable Disease Centre (N.D.D.C.), U.S.A., he completed a course for the "Epidemic Intelligence Service". He visited specialized laboratories to see the manufacture and preparation of the various biological reagents for laboratory use.

Shri K. N. Joishy, Government of Maharashtra Scholar, was awarded the Degree of M.Sc. in Microbiology by the University of Bombay for his thesis entitled "Studies on Anonymous Mycobacteria". Shri R. K. Bhatnagar, Indian Council of Medical Research Scholar, was awarded the degree of M.Sc. in Microbiology by the University of Bombay for his thesis entitled "Studies on Leptospirosis in Bombay." Kum. M. S. Kulkarni of the Department of Immunology was awarded the Degree of M.Sc. in Microbiology by the University of Bombay for her thesis "Comparative study of Typhoid Vaccines prepared from standard strains and locally isolated strains of *S. typhi* with special reference to O, H, Vi and protective antibodies".

The yearly course of D. M. L. T. was conducted. Twelve students attended the course. Short-term course of training were given to three persons.

Routine

From this year, the Department has taken up the additional work of screening Haj pilgrims for evidence of any cholera infection. About 5,000 stool samples were examined for *V. cholerae*. No positive evidence of cholera infection was detected.

The total number of routine tests carried out was 17,772 inclusive of paid and free tests.

Production of diagnostic reagents.—The Diagnostic Reagent Section continued its very useful activity of production and supply of various reagents. The demands were steady. In order to make this section more useful, the Indian Council of Medical Research has provided a Fellow from 1st November 1967 and more items of reagents are being added to the existing list.

		Production (ml.)	Supply (ml.)
Dead Emulsion	...	4,46,145	3,77,970
High titre sera	...	3,086	3,479
Khan Antigen	...	11,980	12,420
W. R. Antigen	...	1,580	1,270
Casoni's Antigen	...	252	304
Cobra Venom solution	...	2,653	2,422
R. V. Venom solution	...	1,203	888
Biological stain	...	7,62,000	7,14,100
Total	...	12,28,889	11,12,853

(6) DEPARTMENT OF ENTOMOLOGY

General

The meeting of the Indian Standards Institution on the "Transport of live Animals Committee" was attended by Dr. P. J. Deoras, Assistant Director. He also attended the International Symposium on Endocrinology at Delhi, Terminological Seminar at Ahmedabad and Indian Science Congress at Benaras.

Kum. K. M. Karnikar was awarded Ph.D. degree of the Bombay University for her thesis on "Studies on Muscid flies of Bombay".

Shri G. C. Chaturvedi and Shri N. E. Vad submitted their synopses for Ph.D. degrees. Shri D. M. Renapurkar and Shri T. G. Tare continued their work for Ph.D. and M.Sc. degree respectively.

Three samples of foreign bodies in milk bottle and one in an *Ayurvedic* drug were examined. Approximately 96 inquiries were attended to in connection with identification and control of snakes, rats and insects of public health importance. Cockroach "Cock ill pill" was tested for efficacy.

Laboratory colonies of fleas, *X. cheopis*, *X. astia*; flies, *M. domestica* *nebulosa* and cockroaches (*P. americana*) were maintained. Samples of different rodenticides were tested. Forty dead snakes, 12 skins of rats and 9 cast skins of snakes, 2 gms. of cobra fat, and slides of biological material were supplied to different educational institutions.

Production

The total quantities of snake venoms extracted and supplied during the year were 170.45 gms. and 306.99 gms. respectively. Number of snakes of different varieties purchased were 872. The total number of scorpions purchased was 2085. The quantity of scorpion venom extracted was 1.12 gms.

(7) DEPARTMENT OF IMMUNOLOGY

General

All the sera manufactured during the year were of the enzyme-refined type. No complaints of any serum reaction were received during the period. At Pimpri, construction of a hay shed and one quarantine stable was taken up during the year.

The following two new products were marketed :—

- (i) Adsorbed Tetanus Toxoid Tet/Vac/PTAP.
- (ii) Diphtheria Tetanus Vaccines DT/VAC/PTAP.

Dr. S. S. Rao, Assistant Director in charge of the Department went abroad on a W. H. O. Fellowship to U. S. A. and Canada for a period of 3 months from September to December 1967. There, he had an opportunity of visiting laboratories concerned with manufacture of sera and vaccines and also some laboratories carrying out researches in the field of immunology. The experience he gained abroad is being utilised for making some improvements in the working of the Department.

The demands for anti-tetanus sera were fully met during the year. The capacity for production of Tetanus Toxoid was increased during the year, but as the sales did not go up, we have accumulated stocks of this item. There was also a drop in the sales of Antivenin during the year. It was not possible to meet all the demands of anti-diphtheria and anti-gas gangrene sera.

Two experienced officers of the department left our services during the year for better emoluments elsewhere. Such loss of trained and experienced personnel hampers the working of the department.

Production

Litres.

Tetanus toxin—

(a) Peptic-digest medium	...	...	...	300
(b) Protein-free medium	...	...	...	1300

Tetanus toxoid—

(a) Peptic-digest medium	...	...	...	100
(b) Protein-free medium	...	...	...	1100

Diphtheria toxin—

(a) Papain-digest medium	...	...	...	100
(b) Protein-free medium	...	...	...	200

Diphtheria toxoid—

(a) Papain-digest medium	...	...	...	30
(b) Protein-free medium	...	...	...	155

Gas-gangrene (Welchii) toxin	...	...	...	200
------------------------------	-----	-----	-----	-----

Pertussis vaccine	...	...	...	33
-------------------	-----	-----	-----	----

Triple antigen	...	...	...	25 (bul)
----------------	-----	-----	-----	----------

Tetanus anti-sera	...	...	...	468
-------------------	-----	-----	-----	-----

Diphtheria anti-sera	...	...	...	26
----------------------	-----	-----	-----	----

Gas-gangrene anti-sera	...	...	...	72
------------------------	-----	-----	-----	----

DEPARTMENT OF NUTRITION & BIOCHEMISTRY

General

During the year under review, the Department continued the research, routine and field activities. The training of the various functionaries, under the Applied Nutrition Programme of the Maharashtra State, was resumed in November at the Public Health Institute, Nagpur. Our staff members visited the Gram Sevak Centres at Shindewahi and Amraoti as guest lecturers. The Field Unit continued to carry out the activities in Public Health Applied Nutrition, such as education, services, teaching and training in practical nutrition at various levels. The services of two members of the staff were spared to the FAO on special assignment to two African countries. Smt. A. George was promoted to officiate as the Factory Canteen Adviser in place of Kum. M. S. Kotnis, while 3 fresh Home Science graduates were recruited to fill the vacancies in the Field Unit. Shri A. J. Modak joined the Nutrition Section as a Junior Research Fellow.

The Biochemistry Section continued to give effective service to the hospitals and attended to some special work whenever required. Shri U. J. Dumaswala, Kum. L. M. Modak and Kum. S. V. Kotnis were recruited as Senior Technical Assistants.

Kum. Shantha Nair, Shri M. J. Modak and Shri P. Divakaran have registered for the Ph.D. degree, while Shri U. J. Dumaswala, Smt. H. V. Phadnis, Shri V. G. Rao and Shri A. J. Modak have registered for the M.Sc. degree. Shri J. K. Kamath has submitted his thesis for the M.Sc. degree. Shri A. M. Bijur and Shri V. M. Patankar are ready to submit their thesis.

Two students from the D.M.L.T. Course underwent training in advanced chemical pathology for four months.

Nutrition—Field Unit

The salient features of field work are summarised below :—

1. *Diet-cum-nutrition survey.*—The field staff had trained the non-technical personnel of selected Applied Nutrition Project Areas in the technique of conducting diet surveys by initiating the work in 6 A. N. P. Blocks. It was carried out as follow :—

- (i) Any two (Lady) Nutritionists visited a given survey area (or Block).
- (ii) In each block, two villages were selected.
- (iii) In each village, 25 families were covered.

Data had been received from the periphery for five villages representing three areas. Analysis of the dietary data revealed the following :

(i) Most of the communities could ill-afford to consume 'protective' foods and were perforce vegetarians. (ii) While the caloric yield of the diet was satisfactory, viz. between 2,800 to 3,000, it was derived mostly from one or the other cereals supplemented by pulses and starchy roots. These are the energy-rich staple foods grown by the community and, hence, available free to the population. (iii) The total protein intake varied from 75 to 90 g. per c.u. This range of average consumption was much higher than the recommended allowances of 55 to 60 g. (iv) On a detailed fractionation of the caloric content of the average diet, it was observed that while the percentage of calories derived from protein was adequate, the figure for fats was rather low. (v) The vitamin content appeared to be adequate. This was also true, to some extent, as far as minerals, such as calcium and iron, were concerned.

Recently, a blue-print of how such surveys could be conducted by the trained village-level workers has been prepared and submitted to the Rural Development Department. This is expected to serve as a guideline for the peripheral participants of A. N. P.

2. *Social dietetics*.—The dietary conditions in the various units of the Indian Institute of Technology—an All-India Institute of Higher Studies at Powai (Bombay)—were studied at length. The main recommendations were as follows :—

(A) Increase of protein-rich foods for the vegetarians—pulses to be 85 g., milk to be 300ml. and one egg.

(B) To maintain the intake of leafy vegetables at 100 g. per head per day.

3. *Industrial canteens*.—During the year 1967, over 25 industrial canteens were visited in Greater Bombay, Thana and Poona Districts to evaluate the nutritional merits of the meal/snacks supplied to the employees.

4. *Square-meal canteen at Sachivalaya*.—This is a Government Canteen providing a balanced mid-day meal to the Government employees at a subsidized rate. Regular visits were paid to the canteen to ensure that the proper standard and variety in food preparations are maintained. In spite of difficulties of obtaining acceptable quality of foodgrains and a regular supply of "protective" foods such as milk, fresh vegetables, etc., the canteen continued to be popular with the diners. About 1,200 of them took part daily in the meal.

5. *Hospital diets*.—Hospitals in the State were visited routinely. Although most of the hospitals were trying to follow the recommended dietary scales, still there was lot of scope for improvement, particularly in kitchen hygiene. The general complaint from most of the patients was with regard to the monotony of food as served. Financial limitations came in the way of many of our ideas of improvement.

6. *Experimental cookery*.—While the information on the nutritive value of individual foods, as purchased, was readily available, there was paucity of information on the caloric and nutrient content of food combinations as prepared, served and consumed by the different communities. Our attempt to provide the necessary information was continued by standardising further a number of recipes.

NDPCal per cent (Net Dietary Proteins *vis-a-vis* total calories) of some of these recipes was also worked out (details published).

7. *Education and publicity*.—(A) The popular brochure 'Road to Good Nutrition' (first published in 1951) has been thoroughly revised and enlarged in scope and published by the State Directorate of Publicity under the more appropriate name 'TOWARDS BETTER NUTRITION'. It was released for sale in October, 1967. Proposal has been made to Government in the Rural Development Department to get it translated into Marathi as part of A.N.P. activity.

This Department had been constantly designing posters and charts and other materials for educational purposes. Some of the important charts were got printed. Recently an additional set of five posters has been selected for multi-colour print. They are in the press at the moment.

(B) *Cookery demonstrations*.—With the rising spiral of food prices, the population of low-cost, nutritious and palatable recipes from locally available foodstuffs was restricted to a few organisations only. A series of five cooking demonstrations were arranged at the welfare centre of the industrial canteen.

(C) *Exhibitions*.—Nutrition education stalls were arranged in different exhibitions in Greater Bombay. Depending upon the theme of the exhibition, suitable low-cost nutritious recipes, along with inexpensive foodstuffs such as ragi, the leafy greens, sprouted pulse, amla, carrot, papaya, etc., were invariably displayed.

(D) *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 groups, such as students of social sciences, social workers, the middle class

intelligentia, pursuing various vocations, etc. These talks had proved effective since even the so-called non-labouring middle-class found it difficult to provide adequate and satisfactory diets to their families.

8. *Consumers' acceptability tests with Dhaan Ata.*—Paddy is usually de-husked and the rice grain milled/polished, unlike other food-grains, most of which are either eaten whole or ground to flour. While it is agreed that husk alone is not a human food, it is not known how the body reacts to superfine flour of paddy or whole rice (called Dhaan Ata), when served with other usual preparations. In this connection, the Khadi and Village Industries Commission, Bombay, had planned a Human Feeding Experiment to ascertain the effect of this Dhaan Ata admixed with pulses such as *Udid dal* (black gram) in the conventional *Chapati* and served as part of the usual meal, on health, particularly the digestibility and the feeling of well-being of adult volunteers. The medical nutritionist and a lady dietitian provided the technical know-how in this study. Over 50 volunteers participated in this feeding trial, which lasted for about 3 months. Almost all the volunteers were happy with the meals and menus which provided over 2,200 calories (between two meals) and 70 g. of proteins. While the feeling of well-being and increase in weight could be attributed to the well-balanced and highly satiative meals, the experimental study showed that the 'high-roughage' *Dhaan Ata* was not harmful and that it did not hamper digestibility.

9. *Training in practical nutrition.*—(A) Apart from undertaking training of various functionaries under A.N.P. in practical nutrition and applied dietetics, the field staff was actively participating in the academic courses for paramedical personnel, such as the trainees of post-certificate course in Pediatric Nursing at Bombay.

(B) *Medical interns.*—During the stay of 6 weeks or more at one or the other of the three Rural Health Training Centres of the State, medical graduates were acquainted by the medical nutritionist with techniques of evaluating the role of nutrients and dietary defects/deficiencies in the causation of ill-health and nutritional disturbances in respective rural communities. At the same time, baseline data was obtained regarding the levels of dietary intake and the incidence of nutrition for each health unit for ultimate use by its paramedical staff.

During the year, a preliminary visit was paid to the Palghar Health Unit (District Thana) whereas the Sirur Health Unit (District Poona) was effectively covered.

10. *Child nutrition workshop.*—Sir V. T. College of Home Science, S. N. D. T. Women's University, Bombay, organised a Balwadi

Workshop in Child Nutrition during May for over 30 Mukhya Sevikas representing A.N.P. areas of the States in the southern region. The field staff actively participated by acquainting the Teacher-Trainees in the applied aspects of nutrition and social dietetics.

11. *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, S. N. D. T. University and the like. Technical advice was given (by correspondence) to institutions and individuals regarding their queries on nutritional merits of foods, problems in practical dietetics, etc.

Routine

Three hundred and six tests were carried out on 130 samples of food, serum and others as shown below :—

Item	No.	No. of tests.
Jowar	... 77	154
Reanut kernels	... 2	16
Skim milk powder	... 12	36
Atta	... 1	10
Cotton seed oil	... 1	6
Milk samples	... 11	33
Butter	... 2	8
B ₁₂ samples	... 2	2
Serum from hospitals	... 4	8
Sheep and cattle serum	... 17	32
Sheep liver	... 1	1
Total	... 130	306

The following analytical tests were carried out in the Biochemistry section :—

Blood	...	2,735
Urine & stools	...	146
Body fluids	...	109
Special tests	...	640
Total	...	3,630

(9) DEPARTMENT OF PHARMACOLOGY

General

During the year under review, the different functions of the Department namely, Research, standardization of drugs and training of personnel etc. continued to be pursued vigorously.

Dr. N. K. Dutta, D.Sc. (Oxon.), D.Phil. (Oxon.), M.B.B.S., (Cal.), F.N.I., Head of the Department, has been awarded the Shanti Sarup Bhatnagar Memorial Award for 1965 for his distinguished contributions in the field of medical sciences.

Dr. M. B. Bhide, M.B.B.S., M.D., joined this department as Senior Scientific Officer Class I. Dr. R. N. Roy, M.Sc., M.B.B.S., D.Phil., was appointed as Research Officer, Indian Council of Medical Research. Kum. S. H. Ravani M.Sc. and P. K. Chatterjee, M.Sc., were appointed as Unichem Research Fellow and Research Assistant, Indian Council of Medical Research respectively. Kum. Usha M. Shah, B.Sc., Shri S. S. Karkhanis, B.Sc. and Shri P. T. Khedekar, B.Sc., were recruited as Senior Technical Assistants.

Shri B. C. Mehta, M. Pharm., S.S.O. II, was transferred to the Analytical and Control Department and Shri R. G. Tamhane, B.Sc. (Hons.), B.Sc. (Tech.), rejoined this Department as Senior Pharmaceutical Chemist.

Dr. A. N. Dohadwala, M.Sc., Ph. D., Unichem Research Fellow, was awarded the Doctorate degree in Applied Microbiology. He left for United Kingdom to continue further researches. Kum. S. W. Babrekar, M.Sc., Research Assistant, Indian Council of Medical Research, resigned and left for higher studies abroad, Kum. R. P. Motwani, M.Sc., Junior Scientific Officer, resigned and left for England.

Training of personnel in standardisation of drugs was continued. In addition to the six graduate trainees, several scientists from commercial firms were given training in specialised fields for varying periods. Amongst them, mention may be made of Shri B. D. Pandit (Mercury Pharmaceuticals), Shri P. Gopinath (Trinity Pharmaceuticals, Kerala) and Shri K. K. Patkar (Arpi Chemicals), Kasgang, U. P., Shri V. J. Thomas and Shri George Varghese of Drug Control Administration, Kerala State, completed their training during this year.

Analytical Services

This Department continued to maintain the tempo on the analysis of drugs. Different departments of our Government, such as Drugs Control Administration, Prohibition and Excise and Police, Government Hospitals etc. continued to send samples belonging to pharmacopoeial and non-pharmacopoeial preparations. The total number of samples that were tested was 2,864. The percentage of non-standard quality was found to be 20.2 which, as compared to the previous year (15 per cent. in 1966-67), is slightly more.

This is probably due to the selective sampling adopted by the Drugs Control Administration on special information. Nine hundred and seventy-five samples were examined under the Drugs and Cosmetics Act, 1940, of which 211, i.e. 21.7 per cent, were declared of non-standard quality.

The number of biological and special products examined during the year was 841. This forms about 30 per cent of the total number of samples examined. Analysis of this type of samples usually needs more time, standard animals and skill. Good advantage of the special facilities available at this Institute was taken by different commercial firms. As many as 591 samples were tested on behalf of commercial firms.

Two collaborative assays for the establishment of National Reference Standards of Penicillin G Potassium and Neomycin sulphate, were undertaken and reported to the Central Drugs Laboratory.

Biological Section

It appears from the samples sent to this laboratory by the manufacturers of pharmaceutical concerns, that the trend for putting new drugs in the market is gaining ground. Drugs such as alkylating agents, dichlorohydrate derivatives, radiometric agents, radio-opaque agents, liver extracts with added vitamins, phenyl derivatives of benzene sulphonates etc., were a few among the drugs sent for toxicity studies.

Out of 41 samples of Insulin preparations, 3 samples failed to comply with the claim, as the potency was found to be less. As many as 16 samples of intravenous infusions failed to comply with the test for clarity, as they contained suspended matter. In most cases, the suspended matter proved to be fungus-like growth. Two hundred and forty-nine samples of antibiotics were tested for toxicity tests. These samples included streptomycin sulphate, chloramphenicols, tetracyclines, penicillins etc. Two hundred and thirty-four samples were examined for pyrogens. Five samples failed to comply with the test. One hundred and six samples were tested for adrenaline content and they were all found to be of good quality. A few preparations, containing adrenaline with extracts of some Ayurvedic drugs, were sent for carrying out the assay for adrenaline content. In all the three samples, the hormone content was up to the claim made. One hundred and twenty-eight samples were examined for depressor substances test. They were usually antibiotics or liver extracts. All the samples complied with the test. Four herbal medicines were received for anti-snake bite test. None of the samples was found to have any anti-venom activity. Thirty-seven samples of mechanical contraceptives (condoms) were tested. Only 6 samples have complied with all the tests. It has been observed that most of the samples failed in length, weight and air-inflation tests. A few samples were not made of good elastic rubber, as some of the samples could not hold enough water to pass the water leakage test.

Microbiological and Biochemical Section

A total number of 563 samples were analysed and reported from the section. The samples consisted mainly of vitamins, antibiotic preparations, carminatives containing digestive enzymes fortified with vitamins surgical dressings, surgical sutures and antitoxic sera and vaccines. Chemical, microbiological and biochemical procedures were used for the testing of these drugs.

Out of one hundred and seventy-four samples of antibiotic preparations tested, 26 (or 15 per cent) samples were found to be of non-standard quality. This percentage is higher when compared with the last year's figure of 3 per cent. This is due to the fact that a good number of tetracycline capsules I. P. and tetracycline hydrochloride injections in a non-aqueous base were found to be of non-standard quality. One sample of chloramphenicol palmitate oral suspension did not comply with the identification test of chloramphenicol palmitate. Two samples of tetracycline ointments were found to be gritty and non-homogenous. Three samples of procaine benzyl penicillin in oil with aluminium monosterate (P.A.M.) were tested for blood level duration test in rabbits according to the W.H.O. specifications. Two of the samples complied, while one sample failed to comply with the standards laid down.

Fourteen samples of antitoxin and nine samples of vaccines were analysed. All the samples of antitoxins complied with the pharmacopoeial requirements. Two samples of cholera vaccines did not comply with the test for sterility.

In the case of vitamin preparations the percentage of samples found to be of non-standard quality was about 18, as 36 out of 202 samples tested were found to be of non-standard quality. A large number of thiamine hydrochloride injections and vitamin B-Complex injections were found to be turbid and contained suspended matter.

Six out of sixty-nine samples, i.e. nearly 8 per cent of the samples assayed microbiologically for vitamin B₁₂ activity, did not conform to the claim made on the label.

The percentage of non-standard samples in carminative preparations containing pepsin, diastase, papain etc. is still very high. About 33 per cent of the samples tested, did not conform to the claim made for proteolytic or diastatic activity.

Five samples of calciferol (Vitamin D₂ tables were assayed for the content of calciferol. In all the samples, the estimated values of calciferol were found to be below 10 per cent of the labelled amounts.

Ten samples of absorbable gelatine sponge were tested according to pharmacopoeial specifications; one sample did not comply with the tests for sterility.

Chemical Section

This section, as in the previous years, had to tackle with samples of different varieties. The various categories included fine chemicals, anti-pyretics, narcotics tranquilizers, respiratory stimulants, disinfectants and insecticides, Ayurvedic preparations, "ganja", "bhang" etc. Six samples of tablets were found failing in the pharmacopoeial requirements for uniformity of tablets. Two of the tablet preparations did not comply with the test for disintegration. Fifteen samples of cosmetic powders were tested and 8 were found to be failing in one ingredient or the other. A few preparations of anti-inflammatory drugs were examined. In 7 out of 8 prednisolone tablets, prednisolone was found to be absent. A few P.A.S. samples were found not to comply with the limit for 3-aminophenol. In a mixture of Ayurvedic preparation and aspirin the latter drug could not be detected.

One hundred and eighty-seven samples of Ayurvedic formulations were referred to this Department by the Director of Ayurveda, Maharashtra State. The samples were analysed for the presence of costly ingredients like gold, silver, opium, mercury and others like sulphur, copper, borates, volatile oils etc. A sensitive method for the detection of gold in Ayurvedic preparation has been standardised. Forty-three samples, i.e. 23 per cent, did not give positive test for the presence of expected ingredients.

The Government Medical Stores continued to send samples to this Department for test and analysis during the year under review.

The percentage of non-standard samples tested on behalf of the Police Department appears to be the highest as, out of 630 samples reported, 362, i.e. about 55 per cent samples, were found to be of nonstandard quality. Out of 411 samples of cosmetics and other alcoholic preparations, 340 did not comply with the claim for alcohol content. About 15 per cent samples of galenicals failed either in the alcohol content or in the content of medicinal ingredients.

The Prohibition and Excise Department referred 80 samples, amongst which 30 samples were of toddy. The alcohol content of toddy at various stages of fermentation was found to vary from 0.3 to 0.7 per cent and the acidity between pH 5.0 to 3.5.

(10) DEPARTMENT OF VIROLOGY

General

Dr. Samuel, Diamond Jubilee Scholar, left the department on 31st October 1967.

Kumari P. D. Patel, a post-graduate student in the department, was appointed as Sr. Technical Assistant from 1st December 1967.

Routine

The products manufactured during the period from 1st April 1967 to 31st March 1968 are as follows :—

Product	Quality (ml)
Carbolised Rabies Vaccine, I.P. ...	4,232,022
Canine Rabies Vaccine ...	32,790
Oral Polio Vaccine (Processed) ...	706,150 doses.

(a) *Laboratory diagnosis of viral diseases.*—Diagnostic studies on virus infections were made on cases referred from various hospitals. The main clinical conditions covered during the period under report were as follows :—

Small-pox ...	1
Polio and enterovirus infections and encephalitis ...	753
Progressive measles encephalitis ...	3

On a reference from Dr. Dastur, Professor, Neuropathology Unit, Grant Medical College, it was decided to study cases of progressive encephalitis for a possible measles aetiology. Sera from 3 cases of progressive were received. Two of these showed such high HI titres against that would suggest a recent or active infection. One of these cases had no history of measles, the other had a history of measles more than 4 years previously. The high titres in relation to the history of the case were suggestive of slow or latent infection with measles virus which could be the cause of encephalitis. The interpretation of these results be possible when C. F. and neutralization tests on these are completed.

(b) *Tissue Culture Section.*—The work during the year was directed

(a) preparing routine cultures for use in the department.

Cultures were made from hamster kidney, amnion and chick for use in the department ;

(b) maintenance and storage studies on standard cell lines.

Some established cell lines (e.g. Hela, HEP2 human liver) were brought from the tissue bank at the Cancer Research Institute and kept in continuous cultures in this department without much difficulty. However, the adaptation of these cell lines to a simple medium (like M+Hanks) has not yet proved successful. Efforts will be continued to achieve this so as to permit workers to use them without complex media presently required grow them.

(c) *Chromosome studies.*—Techniques for chromosome studies of various cell lines have been established. These include processing and staining suit individual cultures in order to obtain clear, well-spread out in the cells.

(11) ANIMAL FARM

The breeding and supply of all types of laboratory animals were quite satisfactory during the year under report. Rabbit and guinea-pig colonies were increased during the year.

The students of the Medical Laboratory Technicians' Training and D.M.L.T. Courses were trained in handling, breeding and rearing of the animals. Shri Makkiat Singh of Christian Medical College and Hospital, Ludhiana, Punjab, and Shri George Verghese of the Department of Drugs Control Administration, Kerala State, also received training.

The following figures show the number of animals of various species bred in the animal farm of this Institute during the year :—

White mice	...	1,82,420	Guineapigs	...	5,026
Albino rats	...	11,313	Hamsters	...	1,370
Rabbits	...	3,183	Cotton rats	...	95

Besides the breeding of above species of animals, sheep, monkeys, dogs, cats and poultry were maintained in the Animal Farm for the requirements of the departments of the Institute.

(12) LIBRARY SECTION

The Library caters to the needs of ours as well as of research workers of other scientific and technical institutions. It also participated in the Inter-library Loan System, which has been in vogue in other neighbouring Libraries.

The following figures showed the work attended to by this Library during the year under report :—

				Number
A. <i>Readers—</i>				
Visitors for reference work	...	...	...	958
B. <i>Loan of books and periodicals—</i>				
Books and periodicals issued to our staff	...	...	...	368
Books and periodicals issued under Inter-library Loan to the other Libraries	...	...	...	350
Books and periodicals obtained for our staff from other Libraries	...	...	...	72
C. <i>Additions of the library materials during the year—</i>				
Books (Acc. Nos. 11016 to 11119)	...	...	...	103
Bound volumes of periodicals	...	...	...	395
Current periodicals subscribed	...	...	...	151
Current periodicals exchanged	...	...	...	25
Current periodicals received free of charge	...	...	...	138
Reports of scientific institutions received	...	...	...	60

*List of research papers published from the Haffkine Institute
during the year 1967-68*

1. Advaney, Maya and Venkataraman, A. :
A study of the diet pattern of the Sindhi Community in Bombay (1967). *Journal of Nutrition and Dietetics*, 4, 110-117.
2. Ambegaokar, S. D., Shinde, V. P. and Kamath, J. K. :
Groundnut oil meal—a substitute for skim milk powder in low cost stock diet for laboratory animals (1967). *Journal of Nutrition and Dietetics*, 4, 281-284.
3. Athavale, V. B., Aiyar, P. R., Khot, N. S., Das, K. K., Santt, M. V. and Kandoth, W. K. :
Drugs in the management of acute gastroenteritis in children. *Journal of J. J. Group of Hospitals and Grant Medical College* (1967), 12, 175-180.
4. Bañani, S. G., Umarji, G. M., Bellare, R. A. and Merchant, H. C. :
Chronic manganese poisoning—a case report (1967). *Journal of Post-Graduate Medicine*, 13, 116-122.
5. Bhamaria, R. P., Bellare, R. A. and Deliwala, C. V. :
In vitro effect of 1-Acyl-4-Alkyl (or Aryl)-Thiosemi-carbazides 1-(S-Cioresalicylidine)-4-Alkyl (or Aryl)—Thiosemicarbazones and some Hydrazones of 5-Chloresalicylaldehyde against pathogenic bacteria including *M. tuberculosis* (H37 Rv) (1968). *Indian Journal of Experimental Biology*, 6, 62-63.
6. Bhat, A. K., Bhamaria, R. P., Bellare, R. A. and Deliwala, C. V. :
Chemotherapy of fungus infections : Part I. 1-Acyl-4-substituted Thiosemicarbazides, 3-Aryl-4-substituted-5-mercapto-1, 2, 4, 4H-triazoles and related compounds (1967). *Indian Journal of Chemistry*, 5, 397-401.
7. Bhat, A. K., Bhamaria, R. P., Bellare, R. A. and Deliwala, C. V. :
Chemotherapy of fungus infections : Part II. Aliphatic and aromatic acid hydrazones and alkyl or aryl Thiosemicarbazones of 5-chlorosalicylaldehyde (1967). *Indian Journal of Chemistry*, 5, 616-618.
8. Deoras, P. J. :
Tolerance status of some rats to an anticoagulant rat poison (1967). *Current Science*, 36, 207-208.
9. Deoras, P. J. :
Laboratory breeding of two rats (1967). *Current Science*, 36, 406-407.
10. Deoras, P. J. :
Rat Problem in India (1967). *Pesticides*, 1, 67-70.
11. Deoras, P. J. :
rat control ? 1 19-20.

12. Deoras, P. J. and Bhaskaran, G. :
Studies on Neuroendocrine System in the Housefly (*Musca Nebulo*, Fabr.) V—The probable origin and development of the Ring Gland (1967). *Indian Journal of Entomology*, 29, 385-388.
13. Deoras, P. J. and Karnikar, Karuna :
Studies of house flies (1967). *Current Science*, 36, 491-493.
14. Deoras, P. J. and Prasad, R. S. :
Feeding Mechanism of Indian Fleas *X. cheopis* (Roths) and *X. astia* (Roths), (1967). *Indian Journal of Medical Research*, 55, 1041-1050.
15. Deoras, P. J. and Prasad, R. S. :
A note on the feeding mechanism of two fleas (1967). *Current Science*, 36, 518-519.
16. Deoras, P. J. and Vad, N. E. :
Studies on Snakes of Maharashtra.
1. Some observations on the ecology of *Echis carinatus* (1967). *Bombay University Journal*, 34, 41-56.
17. Deoras, P. J. and Vad, N. E. :
Studies on Snakes of Maharashtra.
2. Observation on the morphology of *Echis carinatus* (1967). *Bombay University Journal*, 34, 57-93.
18. Dhaplapur, M. G., Sabnis, S. S. and Deliwala, C. V. :
Potential anticancer agents I :—Synthesis of some Nitrogen Mustard containing Bensylidene Hydrazides (1968). *Journal of Medicinal Chemistry*, 11, 154-156.
19. Dimitrov, G. D. and Kankonkar, R. C. :
Fractionation of *Vipera russelli* Venom by gelfiltration, I (1968). *Toxicon*, 5, 213-221.
20. Divakaran, P. and Venkataraman, Alamela :
Effect of Dietary Fats on cholesterol esterase activity in the albino rats. *Indian Journal of Experimental Biology*, 6, 15-18, January 1968.
21. Dutta, N. K. and Dohadwalla, A. N. :
Laboratory Assay of Field-tested Cholera Vaccines by the Infant Rabbit Test (1967). *Journal of Pathology and Bacteriology*, 94, 469-476.
22. Gadgil, M. D., Nimbkar, Y. S. and Jhala, H. I. :
Temperature induced growth imbalance of *P. pestis* in relation to RNA, DNA and protein synthesis (1967). *Indian Journal of Pathology and Bacteriology*, 10, 127-137.
23. Gadgil, M. D., Nimbkar, Y. S. and Jhala, H. I. :
The Role of nutrients and temperature in the growth of *P. pestis* for vaccine production, I (1967). *Indian Journal of Pathology and Bacteriology*, 10, 235-244.

24. Gadgil, M. D., Nimbkar, Y. S. and Jhala, H. I. :
The Role of nutrients and temperature in the growth of *P. pestis* for vaccine production, II (1967). *Indian Journal of Pathology and Bacteriology*, 10, 245-254.
25. Jai, V. N. :
Applied Nutrition programme in Maharashtra. *The Bombay Civic Journal* (April 1967), 1-4.
26. Jhala, H. I. :
Haffkine Institute (1967). *Pulse*, 3, 10-27.
27. Khurana, Usha, Anantanarayan, K. G. and Panse, M. V. :
Pharmacological studies on Hypoglycemic sulphonylureas (1967). *Indian Journal of Medical Research*, 55, 1084-1091.
28. Kulkarni, K. V. :
Blood, its components and their clinical use, Part I (1967). *Maharashtra Medical Journal*, 14, 23-27.
29. Kulkarni, K. V. and Karkhanis, C. J. :
Blood, blood components and their clinical uses, Part II (1968). *Maharashtra Medical Journal*, 14, 28-32.
30. Modak, M. J., Nair, Shantakumari and Venkataraman, A. :
Biochemistry of the *Salmonella*, Part IV (1968). *Indian Journal of Pathology and Bacteriology*, 11, 24-32.
31. Modak, M. J., Nair, Shantakumari and Venkataraman, A. :
Biochemistry of the *Salmonella*, Part I (1967). *Indian Journal of Pathology and Bacteriology*, 10, 312-317.
32. Mukherjee, Amita, Chavan, S. R. and Bhagwager, Silloo :
Preliminary pharmacological screening of the total oil, essential oil and the glycosidal fraction from *Carum copticum* Benth and Hook (Umbelliferae) (1967). *Indian Journal of Medical Research*, 55, 1003-1006.
33. Mukherjee, Amita, Chavan, S. R. and Dutta, N. K. :
Pharmacological actions of an alkaloidal fraction isolated from *Hedyotis suricularia*, Linn, and *H. hispida*, Retz. N. O.—(Rubiaceae)—preliminary study (1967). *Indian Journal of Medical Research*, 55, 1103-1106.
34. Nair, Shantakumari, Modak, M. J. and Venkataraman, A. :
Biochemistry of the *Salmonella*, Part II (1967). *Indian Journal of Pathology and Bacteriology*, 10, 318-323.
35. Nair, Shantakumari, Modak, M. J. and Venkataraman, A. :
Vibriostatic action of berberine (1967). *Indian Journal of Pathology and Bacteriology*, 10, 389-391.

36. Nair, Shantakumari, Modak, M. J. and Venkataraman, A. :
Biochemistry of the Salmonella : Part III (1968). *Indian Journal of Pathology and Bacteriology* 11, 19-23.
37. Nair, Shantakumari, Modak, M. J. and Venkataraman, A. :
Cellular constituents of the Salmonella (1967). *Indian Journal of Pathology and Bacteriology*, 10, 150-154.
38. Nanavati, A. N. D. :
Complications of therapy with anti-rabic vaccine (1967), *Maharashtra Medical Journal*, 14, 493-498.
39. Oza, N. B. and Dutta, N. K. :
Characterization of the antidiuretic factor in the lysate of *V. cholerae* (1967). *Indian Journal of Physiology and Pharmacology*, 11, 101-106.
40. Sabnis, S. S. :
Synthesis of potential anti-cancer agents derived from Benzaldehyde Nitrogen Mustard and Aminophenylthiazoles (1967). *Indian Journal of Chemistry*, 5, 611-621.
41. Sabnis, S. S. :
Synthesis of potential anti-cancer agents derived from Benzaldehyde Nitrogen Mustard and Aminophenylthiazoles (1967). *Indian Journal of Chemistry*, 5, 619-621.
42. Shah, M. H., Mhasalkar, M. Y., Varaya, N. A., Bellare, R. A. and Deliwala, C. V. :
3, 4-Distributed—5-carboxy, methylmercapto-1, 2, 4, (E) Triazoles as possible antiviral agents (1967). *Indian Journal of Chemistry*, 5, 391-393.
43. Shinde, V. P. and Ambegaokar, S. D. :
Effect of pantothenic acid deficiency on blood constituents (1967). *Journal of Nutrition and Dietetics*, 4, 189-192.
44. Vaughan Williams and Dohadwalla, A. N. :
Absorption of cholera toxin into blood from a separated jejunal segment (1967). *Nature*, 215, 552-553.

(13) MAINTENANCE ENGINEERING SECTION

This year, both the desivac plants were constantly used. The number of harges were 43. This section is looking after the maintenance of cold ooms, centrifuge machines, deep-freezes, ovens, vacuum pumps, air-conditioners, air compressors, refrigerators etc. A new condenser of airconditioning nachine of Chemotherapy Tablet Section was installed. The carpenter shop prepared varieties of crates and small furniture for all the departments. A complete paddock for Stables Section was prepared by our carpenters.

**Maharashtra Government Publications
can be obtained from—**

THE DIRECTOR

Government Printing and Stationery,
Publications Branch, Charni Road Gardens,
Netaji Subhash Road . . . BOMBAY-4

THE SUPERVISOR

Government Book Depot,
(for Central Government Publications)
Charni Road Gardens,
Netaji Subhash Road . . . BOMBAY-4
(Sale on counter only)

THE MANAGER

Government Photozinc Press
and Book Depot POONA-1

THE MANAGER

Government Press and Book Depot
Civil Lines NAGPUR-1

THE ASSISTANT DIRECTOR

Government Stationery Stores and
Book Depot AURANGABAD

THE HIGH COMMISSIONER FOR INDIA
India House

Aldwych LONDON, W.C.-2

AND

THE RECOGNISED BOOK SELLERS
