



सत्यमेव जयते

GOVERNMENT OF MAHARASHTRA

**HAFFKINE INSTITUTE
BOMBAY**

**ANNUAL REPORT
1965-66**

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THE HAFKINE INSTITUTE, BOMBAY

MEMBERS OF THE STAFF

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

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r. P. J. Deoras, M.Sc. (Nagpur), Ph.D. (Durham), LL.B., in-charge, Department of Entomology.

Dr. C. V. Deliwala, M.Sc. (Bom.), Ph.D. (Bom.), in-charge, Department of Chemotherapy.

r. M. V. Sant, M.D. (Bom.), F.C.P.S. (Bom.), in-charge, Department of Clinical Pathology and Diagnostic Reagents.

r. A. N. D. Nanavati, M.D., in-charge, Department of Virology (up to 12th April 1965).

r. K. H. Dave, M.D., in-charge, Department of Virology, (from 15th April 1965).

Dr. (Smt.) Alamela Venkataraman, B.A. (Madras), M.Sc. (Madras), Ph.D. (Madras), in-charge, Department of Biochemistry.

r. S. S. Rao, M.Sc. (Banaras), Ph.D. (Toronto), in-charge, Department of Immunology.

r. K. V. Kulkarni, M.B.B.S. (Bom.), in-charge, Department of Blood Bank.

hri Y. S. Nimbkar, M.Sc. (Bom.), Dip. Bact. (Manchester), in-charge, Department of Bacteriology.

r. M. K. Habbu, B.Sc., Ph.D. (Bom.), in-charge, Department of Analytical and Control.

Heads of Sections.

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

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Shri M. T. Jagtiani, B.A., Special Officer (Old Recoveries).

Shri B. A. Sakhardande, Labour Welfare Officer.

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

Shri S. J. Haridas, Assistant Engineer (Maintenance).

Kum. K. A. Ghare, B.Sc. (Poona), Dip. Lib. (Bom), Librarian (Up to 31st January 1966, afternoon).

PART I

GENERAL REVIEW

REPORT OF THE HAFKINE INSTITUTE FOR THE YEAR 1965-66

GENERAL REVIEW

The year 1965-66 was a very critical year for the country in general and it made no exception in regard to the work of this Institute. The border skirmishes starting in August and resulting in Indo-Pakistan conflict in September, put a heavy strain on the Institute. The Institute having been declared as a National key Industry, had to observe regulations in regard to security as enforced by the Ministry of Home Affairs. Some of the activities, demonstrations and visits had to be curtailed. Production of salines, sera, caccines and particularly blood plasma, had to be considerably augmented and oriented towards defence needs. The Institute rendered yeomen service in relation to organisation of blood plasma production for the country. The response of the public to the voluntary blood donation drive was spontaneous and magnificent and it was exploited to the fullest extent. The staff worked with *esprit de corps* and with full discipline of days together without any rest. The challenge was met in regard to supplies to be made. Owing to defence-oriented economy, there was retrenchment of staff and curtailment of expenditure as per instructions of the Government from time to time. The raw material shortage and absence of import licence resulted in regulation of supplies and the production programme towards immediate needs. Indigenous materials were used wherever possible e.g. casein, peptone etc. With great strain the year came to an end. The prices of the drugs could not be revised in pursuance of the general policy of the Government. The new construction activity came almost to a stand-still, resulting in a greater backlog in construction work. There was considerable tight-rope walking in the management of the Institute.

During the year, the D. M. L. T. Course was started at the Institute. Twelve students were admitted to the University Diploma Course. Nine teachers were recognised for the teaching in 3 different specialities. The Technicians' Training Course was also continued as before. The other training programmes continued as hitherto.

The K. F. D. Vaccine Scheme was also closed down on completion of the work and a laboratory for virological testing of oral polio vaccine was set up in its place at the request of Government of India. The research assistance from the National Councils continued. The World Health Organisation gave a grant-in-aid in support of work on cholera and E1 Tor Vibrios, at this Institute, under Dr. N. K. Dutta. Dr. N. K. Dutta was awarded Wattumal Foundation Award for the best medical research India for the year 1965. Dr. (Kum.) B. A. Ver was awarded Khan

Prize for best published research for the year by the Indian Association of Pathologists. The Indian Medical Association awarded C. L. Zaveri award to Dr. K. V. Kulkarni for best presentation at the annual conference. Dr. H. I. Jhala was invited by the Calcutta Medical Club to deliver Sir Nilratan Sircar Memorial Oration at Calcutta. The officers of the Institute continued to serve on expert committees of the National Research Councils and on the committees of the World Health Organisation.

Dr. V. N. Jai was deputed abroad for undergoing a course in diploma in nutrition in the School of Hygiene and Tropical Medicine along with field studies at Ibandan, for a period of 13 months. Dr. K. V. Kulkarni was deputed, under Indo-Soviet Cultural Exchange Programme, for visiting the blood transfusion facilities in the U.S.S.R. for a fortnight, at the invitation of the Soviet Government. Dr. A. N. D. Nanavati was appointed Associate Professor in Virology at the New York University for a year and was granted study leave to take up the assignment. Dr. (Kum.) Shanti Aurora was offered an assignment as Short-term Consultant by the Food and Agricultural Organisation at Rome. She was allowed to take up this assignment by the Government for a period of four months. Kum. Shanta Kumari Nair was admitted to a course in microbiology organised by Czechoslovakian Academy of Medical Sciences at Prague. She has been permitted to proceed on study leave for one year.

The progress of the IIIrd Five-year plan under every scheme was satisfactory, except for the backlog of construction, which has resulted in spill-over. Out of the total allotment of Rs. 5.80 lakhs, Rs. 3.41 lakhs have been spent, the major difficulty being completion of buildings which were postponed due to various economic reasons during the plan. The draft for the 4th five-year plan was discussed but only the annual plan for the next year has so far been finalised.

The Haffkine Institute Reorganisation Committee submitted its report in September 1965. The detailed recommendations are being scrutinized by the Government for implementation. As the recommendations involve recasting of some of the functions and set-up of the Institute involving financial commitments and expenditure, the implementation of major recommendations has been delayed.

During the year, the Institute's cold temperature laboratories for plasma fractionation went into production. A number of new products like gamma globulins, pasteurised plasma proteins and blood grouping reagents have been marketed. Supplies of antirabic vaccine to the Gujarat State continued during the year. The E1 Tor Cholera Vaccine and the quadrivalent E1 Tor and Cholera Vibro Vaccine have been marketed at the request of the Central Government. The construction of the Diamond Jubilee Hostel has been completed. The first phase of construction of the Polio Vaccine

building is nearing completion. The difficulties of water supply, cold storage space and warehouse facilities continued. Newly constructed Stables at Pimpri have been occupied. One stable at Bombay has been released by exchange. An attempt has been made to scale up the physical standards of production laboratories, but not much advance could be made during the year owing to reasons of economy.

The Diamond Jubilee Endowments of the Research Units and Scholarships have been functioning and yielding good results. The Research Fellows were deputed for taking part in symposia on Cell Biology and Regulatory Mechanism at Delhi and as Observer at Indian Science Congress held at Chandigarh. The Haffkine Institute Research and Development Fund Committee met twice during the year and appreciated the work carried out by the Fellows. There were no meetings of the State Advisory Board for the year, as the Board was not yet reconstituted. Dr. N. K. Dutta, delivered lectures for a fortnight at the Institute of Science, Bangalore. Dr. P. J. Deoras published a book on Snakes of India. Dr. M. K. Habbu, Assistant Director, has contributed a chapter to a notable German Handbook on Preventive Vaccination edited by Dr. A. Herrlich, published from Germany.

During the year under review, research work of the Institute was reoriented on projectwise basis at the instance of the State Advisory Board. Four projects on studies on plague, cholera, enteroviruses and diphtheria were planned for joint inter-disciplinary participation. The project committees met thrice during the year and discussed the details of working of the projects. The format of this report has, therefore, been changed. The research report has been separated from the routine and general matters. Research report deals with report of the projects and other departmental researches and the routine and miscellaneous sections have been added to cover other aspects of the administration.

The studies on acid soluble nucleotide composition of the *Vibrio cholera* Inaba and *El Tor* strains were undertaken using radioactive labelled isotopes. The Inaba strains showed presence of thymine monophosphate in small quantity along with other nucleotides common with others. The question of lysis of cholera vibrios that occurs after production of vaccine has been taken up for study. While the results of the various experiments are useful, it has not so far been possible to localize the enzyme system responsible for lysis. An attempt was made to establish quantitative relationship between frog skin reaction and choleragenicity of the cholera toxin. It appears that these activities are related to differentiable substances, neither of which is identical with mouse lethal activity. The toxic moiety responsible for choleraic effect is not identical with cholera endotoxin. It appears that there is close clinical, pathological and biochemical resemblance between experimental cholera in rabbits and the human cases. Under

suitable laboratory conditions, it has been possible to show that *El Tor* epidemic strains are capable of producing disease in infant rabbits. A strains of cholera vibrio has been developed by repeated passages in the McConkey's broth. The resulting change in the vibrio is the loss of virulence. The attenuated strain has passed through 30 passages. In the mouse protection test, an experimental live vaccine from the strain gave by Sokhey and Habbu methods, degree of protection which can be compared with agar-grown vaccine. Large number of drugs have been screened *in vitro* for anti-cholera activity. Nineteen compounds have shown some activity, though this is less than that antibiotics. The experimental approach using chloramphenicol and berberine in experimental cholera has proved successful.

The plague project concerns itself with the nutritional requirements of *P. pastis*. A systematic analysis of casein hydrolysate vaccine has been undertaken. There was practically no difference in the composition of the cells grown at 27° and 37°C. *P. pestis* is remarkably sensitive to temperature variation between 36° and 38°C and the growth is remarkably affected with the amount of calcium, magnesium and vitamins in the medium. There is morphological variation in this organism when cultures are grown under different conditions. By addition of calcium in requisite quantity, it may be possible to cut down the incubation period of vaccine from 14 days to 6 days in stationary culture.

An important contribution has been the resistance of rats to plague infection. The rate of susceptibility from rural and near forest areas was 70 to 90 per cent., while in the urban areas or near urban areas, the rate was 50 per cent. These results indicate that rural and near forest areas seem to be virgin fields for plague infection. A new technique of using habit boxes to lower the flea index has been reported. Plastic bags for finding out flea index of individual rats have been devised. Studies on population of rats and use of rodenticides have been undertaken.

In the diphtheria project, it has been shown that the phage plays the role of transducing the toxigenic factor. The published work has aroused considerable discussion in the literature and several other laboratories in the world have taken up work to confirm these findings. The toxinogenic factor, like some of the colicinogenic factors, could be liberated by ultra-violet irradiation *in vitro* and then it was possible to infect the non-toxinogenic strains. The kinetics of production of toxin have been studied in relation to iron. A strain of *C. diptheria* has been isolated which utilises succrose in place of maltose.

The enterovirus project aims at establishment of aetiology of gastro-enterities in infants and children from viral and bacterial angles. Ninety-nine cases have been studied so far and a number of isolation have been

made. Identification studies are still in progress, but it appears that the children between 9 months to 12 months are more susceptible and the cases seem to occur in crops. The work on aseptic meningitis on similar lines is also in progress.

The Department of Bacteriology concerns itself with the preparation of typhoid vaccine from local strains and the standardisation of the same. No definite conclusions have so far been reached. The department is also trying to study the possibility of producing acetone-dried typhoid vaccine. The W. H. O. is actively interested in this aspect. The Department of Blood Bank has studied the genetic distribution of some of the characters of blood in certain endogamous groups. The results require completion and further statistical analysis. Coomb's reagent, Anti-Rh Sera and Bovine Albumin have been processed for release. The plasma fractionation is now well on the way and the tissue drying plant has been installed. The Department of Chemotherapy has been studying anti-tubercular, psychotropic, cytotoxic and anti-viral compounds. The work on anti-fungal substances is also in progress. A number of newly synthesised compounds have been screened on these lines. The Department of Clinical Pathology has taken up the study of chorionic gonadotrophic hormones in cases with bad obstetric history. They have also studied some strains of a typical *Mycobacteria* from clinical cases. The problem of leptospirosis, from the point of view of ecology and epidemiology, has been under investigation. A rapid fluorescent antibody technique for the diagnosis of diphtheria has been adopted. It is possible to differentiate toxinogenic strains by this test.

The Entomology Department has carried out studies on host specificity of fleas. Four insecticides have been studied. Some rodenticides have also been compared against the conventional ones. The Department of Immunology studied the mechanism of action of tetanus toxin at the molecular level in the brains of rats and pigeons. The general paralysis is initiated by low A. T. P. content of the brain. The local tetanus is due to the decrease of A. T. P. level in the injected muscles. The adsorption of toxin was highest on mitochondrial fraction prepared from brain of rats as well as pigeons. The tetanus toxin was not chemically bound with gangliosides but is only loosely attached to it. The study of clinical tetanus showed increase in aldolase in serum in patients having rigidity. The investigations on galactose cataract from lens, have shown that the development of capacity is delayed by feeding rats concurrently on urea. The Department of Biochemistry and Nutrition has standardized a microbiological method of evaluating protein quality. Studies on dietetics of communities with reference to serum cholesterol and other lipids have been undertaken. The Department of Pharmacology studied the anti-diabetic.

anti-bacterial and anti-bronchial drugs. Experimental amebiasis is being used for screening of drugs. The Department of Virology studied the sewage samples for their role in transmission of enteroviruses. Studies on the kinetics of growth of rabies in tissue culture have been completed and similar studies with pox virus are in progress. The epidemic of poliomyelitis in Ceylon was investigated at the request of the W. H. O. The K. F. D. vaccines prepared from mouse brain, chick embryos and tissue culture have been compared in laboratory and arrangements are in progress for volunteer studies with tissue culture vaccines in collaboration with the Virus Research Centre, Poona.

Supplies made by the Institute through its Finished Products Section have been listed in the report. It is important to point out here that substantial amounts of sera sold during the year were all purified by enzyme digestion technique.

The Government Analysts at this Institute tested 1,942 samples of drugs. These samples were examined under the Drugs and Cosmetics Act. Only 12 per cent. of them were of non-standard quality. The Clinical Pathology and Biochemistry Sections continued to offer diagnostic services at advanced level. Besides other tests, 2,848 samples of urine for pregnancy test, 1,133 samples of blood cultures for typhoid and 4,023 samples for histology were referred to this Department. There was increased demand for supply of Diagnostic Reagents.

The newly started Analytical and Control Department carried out over 44,000 tests on different samples of products manufactured at the Institute.

During the year under review, two Pool Officers were posted at the Institute by C. S. I. R. Twenty-six short-term trainees, 8 Technicians Training Course Trainees, 12 D. M. L. T. students were trained for their courses. There were 31 graduates under different fellowships and scholarships at the Institute.

The animal colony has expanded. The Critics Club Library, Dispensary Welfare Activities and Sports Club continued with vigour. Twenty-six important visitors visited this Institute. Fifteen Science Graduates obtained their M.Sc. and 16 were admitted for M.Sc. and Ph.D. Degrees.

Director is grateful to all members of staff and to the members of the various committees for their continued assistance and guidance. Director also wishes to thank the granting bodies for their generous grants. Without the co-operation of the Employees' Unions, the present task of emergency could not have been met. Thanks are, therefore, due to every one for his co-operation and hard work.

PART II
RESEARCH

A. Projects

PROJECTS ON CHOLERA

This is a multi-disciplined project to attack the problem of cholera from several angles. Many of the aspects have some practical importance but, by and large, it is also a fundamental study. The several aspects are reported below :—

Biochemistry of *Vibrio cholerae*

(Diamond Jubilee Scholar)

The incorporation of C^{14} labelled purines and pyrimidines in the growing cells of Ogawa strain of *V. cholerae* and the acid soluble uncleotide composition to the same strain have already been studied. During this investigation, it was observed that the nutritional requirements of the different strains of *V. cholerae* viz. Ogawa, Inaba and *El Tor* Ogawa vary. In order to study the metabolic differences in these strains, similar studies with Inaba and *El Tor* strains were undertaken.

C^{14} labelled adenine, guanine, uracil, sytosin and thymine were added to the basal synthetic medium and cholera organisms of both sub-types mentioned above were grown for 6 hours at 37°C in shaken conditions. The cell mass was collected and extracted with different solvents. The radio-activity incorporated in total cell mass and that in each extract was measured.

From the 24 hours growth of cholera organisms on Roux agar bottles at 37°C , the nucleotides were extracted with cold TCA (trichloroacetic acid). The extract was made free from TCA and was fractionated on Dowex-1-X₂ format column using linear gradient system. The fractions were further subjected to sugar, nucleic acid base and phosphorus analysis. The paper chromatography was also carried out and the nucleotides in each fraction were identified.

A striking observation was made with nucleotides of Inaba strain. Along with all ribonucleotides, only one deoxyribonucleotide viz. thymine monophosphate was identified in small quantities.

This nucleotide might either be the only deoxyribonucleotide that was detected, while others might have escaped detection due to their very low concentrations, or this might be a nucleotide which was a part of short-lived messenger (RNA) which had base composition quite similar to that of DNA.

To investigate the later possibility, the DNA, ribosomal messenger and S-RNAs of cholera organisms (all the 3 strains) are being isolated.

Lysis of *V. cholerae* organisms

(DEPARTMENT OF BIOCHEMISTRY AND NUTRITION)

V. cholerae when suspended in a fluid menstruum showed considerable lysis. The exact mechanism of this process is not known but the phenomenon is of importance from the points of view of both fundamental as well as practical aspects.

As a preliminary survey, the effect of heat, different concentrations of cations and varying osmotic tensions on the lysis of *Vibrio cholerae* (Ogawa) organisms was studied.

It was observed that the organisms were killed by heating at 55°C for half-an-hour and that there was no lysis when they were stored. The different storing media, such as solutions of various concentrations of sodium chloride (0.425 per cent. to 3.40 per cent.) and buffered (phosphate) salines of different molarities (M/15, M/5) did not prevent the lysis. With cations (0.00001 to 0.1 M), it was observed that Ca^{++} and Al^{+++} in appropriate concentrations inhibited the lysis to an appreciable extent. As a further step Ca^{++} was incorporated into the growth medium (broth) for cholera organisms and it was observed that though the growth rate was slightly less in the initial stages as compared to the one without Ca^{++} , the lysis was very much less. This experiment is further extended at present to find out the optimum Ca^{++} concentration to enable maximum growth and and minimum lysis. Studies on the effect of storing the organisms in solutions of various concentrations of sucrose (0.1 to 1.0 M) showed that these media have some effect on the lysis.

Addition of formalin to the organisms immediately after the harvest did prevent lysis to an appreciable extent.

The saline washed culture was allowed to lyse and the lysate was used as an enzyme source. The cells heated at 55°C for half-an-hour were used as substrate. The results were rather erratic. Further, the saline washed cells were disintegrated in an ultrasonic disintegrator and the clear supernatant was used as an enzyme source. Similarly, the clear supernatant obtained on repeated freezing and thawing of the saline harvested cells was used as an enzyme source. Freshly harvested cells were used as substrate. These experiments also failed to localise the enzyme system.

Cholera toxins : Quantitation of the frog skin reaction and its relation to experimental enteric toxicity.

(DEPARTMENT OF PHARMACOLOGY)

The inhibitory effect of soluble toxic substances derived from the cholera vibrio on the membrane potential and on short circuit potential and

current of the frog skin, taken to represent an inhibitory effect on the active transport of sodium, has been confirmed and quantitated. The per cent inhibition of potential was linear with respect to the logarithm of the toxicity concentration, allowing the interpolation of a 50 per cent, inhibitory dose of toxic activity. The membrane potential appeared to be about twice as sensitive to the inhibitory effect as the short circuit potential. The activity occurred in both intracellular and cell wall substance as well as in whole cell lysate of the vibrios, from all of which it was freely dialyzable. It was also found in the supernatants of peptone water cultures. The activity producing fatal enteric toxemia in the infant rabbit was found to be present in whole cell lysates, as described earlier, and in the intracellular but not the cell wall substance of the vibrios. It too was present in peptone water culture supernatants, but it was not dialysable. On the basis of differences in the distribution and dialysability of the 2 kinds of toxic activity, it is concluded that these are differentiable substances, neither of which is identical with mouse lethal activity.

Intestinal manifestations of cholera in infant rabbits

(DEPARTMENT OF PHARMACOLOGY)

The infant rabbit is a valid model for the study of experimental cholera. Its tissue responses and the copious diarrhoea with the resulting dehydration and fluid and electrolyte imbalance are comparable to cholera gravis in man.

The clinical effect and the morphologic response of the intestinal tract to an experimental, intestinal *Vibrio cholerae* infection can be reproduced by oral administration of sterile filtrates of ultrasonically disrupted vibrios and of young broth cultures in which vibrios have been grown. These findings indicate that the choleraic effect is due to a vibrio toxin which is present in each of these agents. The choleraic effect does not depend on tissue invasion, which characterizes infection by most enteric pathogens, such as *Salmonella*, *Shigella*, *Pasteurella* organisms and others.

The toxic moiety responsible for the choleraic effect is not identical with cholera endotoxin. In fact, potent endotoxin given alone does not cause diarrhoea in our model. The choleraic toxin does not affect the continuity and anatomical integrity of the intestinal epithelial lining, it has little effect on the histologic appearance of individual intestinal epithelial cells which does not exclude a physiologic alteration affecting the transport mechanism of fluid and electrolytes. However, the choleraic moiety does affect the microcirculation of the intestinal mucosa. These are hyperemia, stasis and interstitial edema, conditions which could materially contribute to the fluid loss into the gut lumen.

Serum protein changes by paper electrophoresis in rabbit and human cholera.

(DEPARTMENT OF PHARMACOLOGY).

Serum protein changes by paper electrophoresis were studied quantitatively in human cases of cholera and in normal subjects. These results were compared with rabbits suffering from cholera and normal rabbits. Significant increase in the alpha two and gamma components of globulin was observed in both cases, while a decrease to a significant level was noted with the albumin in the rabbits of experimental cholera and the beta globulin in human cholera. The present study offers an additional support to the suggestion that close clinicopathological and biochemical resemblance exists between experimental cholera in rabbits and in human cases of cholera.

Choleraenic activity in infant rabbits to *El Tor* vibrios of South-East Asian origin.

(DEPARTMENT OF PHARMACOLOGY).

Several strains of *El Tor* isolated from South-East Asian countries were studied on infant rabbits in relation to classical cholera vibrios, Hikojima and *E. coli*.

All the *El Tor* epidemic strains are choleraenic in nature and under suitable laboratory conditions are capable of producing the characteristic disease in the infant rabbits like the classical vibrios.

The Middle East Strains of *El Tors* or those isolated from water sources from non-epidemic (*El Tor*) areas are potentially choleraenic and may give rise to an epidemic under favourable condition.

Factors influencing the mouse protection test of cholera vaccine

(DEPARTMENT OF BACTERIOLOGY).

The mouse protection test as laid down in I. P. for the assay of cholera vaccine prepared in casein hydrolysate medium has been in use at the Institute over a number of years. The test gave reproducible results apparently under certain rigid experimental conditions but, lately, it was difficult to get reproducible results. It was observed that some of the mice of the control group survived the challenge dose of live vibrios which was ten times the certain lethal dose as obtained earlier. Increasing this challenge dose 100 times did not materially alter the response. Secondly, the dose response relationship was found often to be irregular thus making the assay of doubtful value. It was, therefore, obvious that some of the experimental factors were responsible for these irregularities.

It was apparent at the outset from the design of the assay that one of the factors either singly or in combination may affect the outcome of the result :—

- (i) The quality of mucin used for preparing the challenge dose.
- (ii) The virulence of the organism used as challenge strains.
- (iii) The susceptibility of the mouse.

It was found that the batch of mucin in use in the laboratory, though carefully stored at refrigeration temperature, had deteriorated. A fresh batch improved the results to a great extent.

The challenge strains in use have also fallen in their virulence to some extent and by passage through mice a highly virulent mutant was selected.

The strain of mice in use had also undergone a change in its characters and did not always yield the progeny of a very susceptible type. Few other local strains of mice which were tried for this purpose but it did not show encouraging results. Therefore, a strain known to be very susceptible to *V. cholerae* was imported and bred at the Institute.

When these changes were introduced in the mouse protection test, the precision of the assay greatly improved and the results were reproducible.

Attenuation of *V. cholerae* and production of live cholera vaccine

(DEPARTMENT OF BACTERIOLOGY)

Earlier work of Dutta and his colleagues at this Institute has shown that better protection could be given by live *Vibrio cholera* vaccines when administered parenterally to rabbits. A study of attenuation of a virulent vaccine production strain of *V. cholerae* was undertaken. It was observed that the strains on repeated sub-culture in MacConkey's broth lost their virulence. The virulence of the strains was estimated by determining LD50 for mice. Starting with a strain having the LD50 of 1000-2000 organisms, 30 passages through MacConkey's broth were carried out. The biochemical and serological characters were tested at intervals along with the virulence test, the other characters remaining constant. The LD50 of the passaged strains rose from 1000-2000 organisms to 10^6 organisms. An experimental live vaccine lot was prepared with the 30th passaged strain and was tested by the mouse protection test (Sokhey and Habbu method) and compared to the agar-grown vaccine. No significant difference was observed between the two vaccines.

Screening of anticholera drugs in vitro

(DEPARTMENT OF CHEMOTHERAPY)

Although considerable progress has been made in the chemotherapy of bacterial infections with synthetic drugs and antibiotics, no potent drugs

have yet become available for the treatment of cholera. Except for the antibiotics chloromycetin and neomycin which have shown *in vitro* activity and a limited protection afforded by them in experimental cholera of infant rabbits as shown by Dutta and co-workers, this field of study has remained unexplored. In order to find out possible leads as to the types of organic compounds of structural configuration that might show anticholera action, a large number of synthetic compounds (nearly 180) of different series (hydrazides, hydrazones, thiosemicarbazides, thiosemicarbazones, thiazones, thiazoles etc.) were screened for *in vitro* anticholera activity. Only 19 compounds showed anticholera action at a minimum concentration of 0.01 µg/ml. and these were all thiosemicarbazides and thiosemicarbazones from halogenated salicylic acid and salicylaldehyde respectively.

A new approach to the treatment of cholera based on experimental evidence.

(DEPARTMENT OF PHARMACOLOGY)

The maximum benefit of drug therapy, besides fluid and electrolyte replacement, in clinical cases of cholera lies in combating further growth of the vibrios and neutralizing the cholera toxin derived from the organisms. This was achieved by a combined therapy of chloramphenicol and phosphorylase in experimental cholera of the rabbit. It is suggested that this mode of treatment would be of benefit in clinical cases.

PROJECT ON PLAGUE

This project mainly concerns the epidemiology of plague in retrospect. It also concerns the study of fleas and rats. The original studies on *P. pestis* are also being carried out from several angles. Work on these aspects is reported below :—

Composition of casein hydrolysate used for vaccine preparation

(DEPARTMENT OF BIOCHEMISTRY AND NUTRITION)

A systematic analysis of the casein hydrolysate medium has been undertaken. The essential amino acids arginine, threonine, lysine, histidine, methionine, phenylalanine, leucine, isoleucine, and valine have been estimated by microbiological procedures. Composition of the medium was as follows :—

Total nitrogen, 2.13 per cent. ; Total solid 2.91 per cent. ;

Ash, 0.538 per cent. ; Amino nitrogen, 138 mg. per cent. ;

Amino acids : arginine 97, Threonine 92 ;

lysine, 187 ; Histidine 69 ;

methionine 79.6 ;

Phenylalanine 92 ; leucine 220 ;

isoleucine 187 ; valine 152 ;

total to balance, Nil.

Cellular constituent of *P. pestis*

(DEPARTMENT OF BIOCHEMISTRY AND NUTRITION)

The cellular composition of *P. pestis* was studied by growing the organism for 24 hours and 48 hours at 27° and 37°C in acid digested casein hydrolysate under stationary and shaking conditions. The washed cells after drying were quantitatively processed for total nitrogen, protein, RNA, DNA, total sugars and lipid phosphorous. The cells contain 12-14.5 per cent. DNA, 9.1-14.5 per cent. total sugars, 0.4-0.7 per cent. lipid phosphorous. There was practically no difference in the composition of the cells grown at 27°C and 37°C. The RNA content diminished by 15 per cent. when the cultures were allowed to grow for 48 hours, while there was no appreciable change in protein content, DNA and lipid phosphorous. A definite increase was observed in the cellular constituents particularly RNA (15.1), DNA (10 per cent.), total sugars (30 per cent.) and lipid P (20-40 per cent). under shaken conditions.

Nutritional requirements of *P. pestis* in relation to vaccine production

(Diamond Jubilee Scholar)

It has been accepted so far that there is a better development of the protective antigens of *P. pestis* at temperatures nearing 37°C than at 27°C. However, the major difficulty in the manufacture of plague vaccine at 37°C has been the poor growth of *P. pestis* in liquid media. Systematic shaken culture studies were, therefore, undertaken to find out the nutritional requirements of *P. pestis* at 27°C and at 37°C. With this end in view it was first decided to confirm the original observation of Higuchi *et al* (1959) regarding the temperature dependent, calcium requirement of virulent *P. pestis* strains. During the course of these investigations the following observations were made :—

(i) *P. pestis* is remarkably sensitive to temperature variation (36° — 38°C) and to the effect of calcium, magnesium and vitamins for its growth.

(ii) There was a great deal of morphological variation in shape and size of the organisms when the cultures were grown with only calcium or magnesium or in the absence of both. The cells of *P. pestis* grown in a medium containing calcium at 37°C appeared to be distinctly enlarged and swollen as compared with those grown in the absence of calcium.

(iii) In the absence of calcium at 37°C, the strain 195/P (avirulent strain of *P. pestis*) showed a lag period of 42 to 48 hours after which the culture grew with an increased rate along with simultaneous loss of virulence. On the basis of DNA synthesis by the strain 195/P

during the abovementioned period, it appeared that the normal process of cell division of this organism was affected by a shift in the incubation temperature from 27°C to 37°C. This hypothesis is further supported by the observation of distinct chain formation during its growth following the end of its initial lag phase. On the contrary, the calcium grown culture at 37°C grew exponentially without any lag.

(iv) The plague vaccine is at present manufactured at the Institute by incubation of a stationary culture at 32°C for 14 days. By addition of calcium in requisite quantity the above period was successfully cut down to 6 days in a stationary culture, and to 48 hours in a shaken culture. The vaccine from a shaken culture at 37°C after 48 hours in a medium fortified with calcium is as potent as the plague vaccine in current use.

Virulence and immunogenicity of *P. pestis*

(Diamond Jubilee Scholar)

The shaken culture studies showed that areated cultures of a virulent strain of *P. pestis* at 37°C, had a lag period of about 24 hours, after which the cell division commenced with an increased growth rate with simultaneous loss of virulence. Surgalla has already postulated an hypothesis that *in vitro* the production of some of the virulent antigens is incompatible with the process of cell division.

An attempt was, therefore, made to correlate virulence and immunogenicity of different strains of *P. pestis* with their respective growth rates at 37°C. The growth rates of 195/6 ($F_1 + VW + P +$), TRU ($F_1 - VW - P -$); M_{15} ($F_1 + VW - P +$), M_{17} ($F_1 - VW + P +$) at 37°C were studied in the following media :—

- (1) Plain casein hydrolysate medium ;
- (2) Plain casein hydrolysate fortified with salts and vitamins (designated 37H) and
- (3) 37H medium with calcium and magnesium in various proportions.

Since 195/P strain exhibited a partial stasis for 24 hours in contrast with the strain TRU ($F_1 - VW - P -$) which completed 80 per cent. of its growth at 37°C within the same period, it had indicated that 37°C was not a favourable temperature for the growth of a virulent strain. The increased growth rate after the lag period was probably related to the fact that the strain became avirulent as TRU ($F_1 - VW - P -$) M_{15} ($F_1 - VW - P +$) grew slightly faster than the strain M_{17} ($F_1 - VW + P +$). This had indicated that the loss of VW antigen might be responsible for the faster growth of M_{15} at 37°C. All the four strains failed to grow in the plain casein hydrolysate medium up to a period of 48 hours at 37°C.

All the cultures were microscopically observed during different stages of growth. In the case of strain TRU (F_1 -VW-P-) except when grown in the medium (3) with 0.03 M Mg^{++} the culture showed lysis within 8 hours. It was observed that at 37°C strains M_{15} (F_1 +VW-P+) and $_{17}$ (F_1 -VW+P+) exhibited in 37H medium a typical morphology specially in the sense that they appeared almost roundish in contrast with the red-like 195/P culture. With the addition of Ca^{++} and Mg^{++} (Medium 3) there was no significant change in the morphology.

Resistance of rats to plague infection

(DEPARTMENT OF BACTERIOLOGY AND DEPARTMENT OF ENTOMOLOGY)

The absence of any overt plague infection in the State of Maharashtra for the last twelve years has distracted the attention of researchers from the plague problem and no systematic data regarding the susceptibility of rats in different parts of the State except that of the city of Bombay are available. The appearance of plague infection in border districts of Mysore, Madras and Andhra Pradesh during the past few years, however, points to the necessity of keeping a track of even the latent infection and detect it at a very early stage in the infection cycle. With this end in view, a study of the susceptibility of house rats, the likely primary reservoir of plague infection in Vidarbha region of the State, was undertaken. The rats mainly *Rattus rattus* collected from near forest areas and near urban and urban areas were brought to the Institute and after observing them for more than one week, they were challenged with a challenge dose of approximately 1,000 organisms of a virulent strain (195/P) of *P. pestis*. These rats were observed for two weeks after inoculation. So far, 427 rats from different areas have been challenged. The results show that the rats from rural and near forest areas are highly susceptible to plague infection while those from urban and near urban areas are relatively resistant. The rats from rural and near forest areas showed a susceptibility of between 70 per cent. to 90 per cent. while average susceptibility in urban and near urban areas was approximately 50 per cent. These results indicate that rural and near forest areas are virgin fields for plague infection while some immunity exists in the rats of urban areas.

Studies on use of bait box, plastic bags, population of rats and control measures

(DEPARTMENT OF ENTOMOLOGY)

The frequency of population studies was done for a period of 21 days each at Amgaon in Bhandara district and Deolapur in Nagpur district. *Walleria indica* were collected from the former while *Rattus blandfordi* were collected from the latter. All these rats when challenged to a standard dose of *P. pestis* were found to be susceptible to plague.

Tatera indica that were brought to the laboratory are being bred. Three litters from the same pair have so far been obtained.

Rat flea surveys were carried out at 23 places of Vidarbha region. In all 2,772 rats were trapped out of which 2,703 were *Rattus rattus* forming 9,752 per cent., while the others were *Bondicota bengalensis*, *Mus musculus*, *Leggada nagarum*, *Tatera indica*, *Rattus blandfordi* and shrew (*Suncus caeruleus*). Nine thousand and forty-two fleas were recovered from these rats and the majority of these were *X. cheopis* while the only others species recovered was *Xenopsylla astia*. It was interesting to note that in spite of a large number of *Rattus rattus* trapped from Sironcha, a far away secluded hinterland area, not a single *X. cheopis* was recovered. Further observations suggest that *X. astia* still predominates in the rural and hinterland areas while *X. cheopis* predominates in urban and market areas.

Experiments were also done by using bait boxes dusted with 5 per cent. BHC powder at bait stations. The rodenticides used were zinc phosphide, Shoxin and Rodafarin. The reduction in flea index was noted at majority of places where dusted bait boxes were used. Zinc phosphide even at the concentration of 0.1 per cent. gave good results. Shoxin (0.005 per cent.) and Rodafarin (Warfarin 0.025 per cent.) were also tried but though effective they were not as effective as zinc phosphide the reduction in rat density due to zinc phosphide was 96.0 per cent. while the same in case of Shoxin and Rodafarin was found to be 63.6 per cent and 33.5 per cent. respectively.

DIPHTHERIA PROJECT

This project mainly concerns the study of *C. diphtheria*, its metabolism, toxin production and the role of phage. The work is of theoretical importance at this stage and the contributions made may prove to be of fundamental importance. These aspects are reported below :—

Role of phage in toxinogeny of *C. diphtheria*

(DEPARTMENT OF IMMUNOLOGY)

It is known from the work of Freeman in 1951, that certain 'phages can convert nontoxinogenic strains of *C. diphtheria* into toxinogenic strains. I was previously considered that phage carries the code for toxin production. However, certain 'phages like Y phage were known which did not induce toxin production. The work done in the Immunology Department so far has shown (*J. of Gen. Microbiol.*, 1965, 40, 421) that the role of phage is only to transduce a toxinogenic factor which we have termed T+ factor. This factor occupies a cytoplasmic position in the organism. It is possible to remove the toxinogenic factor as well as the 'phage and thus convert a toxinogenic strain into a nontoxinogenic nonlysogenic strain by treatment with small amounts of acriflavin which is known to remove cytoplasmic factors

It was also possible to treat the 'Toxinogenic' phage *in vitro* with very small amounts of acriflavine in order to convert them into nontoxinogenic types. These results indicate that we have been able to remove the toxinogenic factor from the phage. The nontoxinogenic phages can pick up the T factor again after passing through toxinogenic and nonlysogenic strains. This indicates that the phage plays the role of transducing the toxinogenic factor.

Another finding was that high toxin yielding strains contained the toxinogenic factor and no prophage. When such strains were infected with phage, they produced less toxin.

Transfer of toxinogenic factor without phage

(DEPARTMENT OF IMMUNOLOGY)

All attempts to transfer the toxinogenic factor (T+) from a nonlysogenic toxinogenic culture to a nonlysogenic, nontoxinogenic culture by cell contact failed.

Infection of toxinogenic factor (T+) after treatment with UV irradiation was then tried. A 24 hours old culture of PW₈T+ was subjected to UV irradiation for 15 minutes and then killed by heating to 60°C for 10 minutes. This killed culture was added to the 2 hour old growth of a PW₈T culture which was then incubated for 2 hours more and then plated out. On plating on antitoxin agar medium toxinogenic colonies could be observed. These results were confirmed by intracutaneous injection test in rabbits. This experiment proved that the toxinogenic factor (T+) like some of the colicinogenic factors could be liberated by UV irradiation and heating and then they are able to infect nontoxinogenic strains.

Role of iron in the control of toxin production

(DEPARTMENT OF IMMUNOLOGY)

It is known that excess iron does not inhibit growth but inhibits toxin production. The mechanism of its action is not known. Our work on the kinetics of production of toxin shows that toxin is produced after the growth of the diphtheria bacillus is over. There is a lag of 24 hours between the change in concentration of iron in the medium and its effect on toxin production.

The antigenic analysis of culture filtrate with optimum iron and excess iron was carried out using gel diffusion test. It was found that excess iron inhibits toxin production but some other nontoxic antigens were produced in larger amounts.

Isolation of cultures more suitable for research or production

(DEPARTMENT OF IMMUNOLOGY)

At present one has to import considerable amounts of pure Maltose for use in the culture medium for toxin production. It is necessary to isolate strains capable of growing on sucrose. Another advantage of using sucrose over Maltose is that it can be added to the medium and then sterilized instead of sterilizing separately and adding just before inoculation. One strain of sucrose utilizing *C. diphtheriae* has been isolated which produced about 10 Lf of toxin/ml. under shaken conditions within 96 hours. With this strain later using optimum concentrations of constituents in the medium it was possible to obtain 30 to 40 Lf/ml. of toxin in 96 hours under shaken conditions.

Study on cellular constituents of *C. diphtheriae*

(DEPARTMENT OF BIOCHEMISTRY AND NUTRITION)

A preliminary study was undertaken to study the cellular composition of the organism as grown for the production of diphtheria toxin in the Institute. The cells contain about 12 per cent. of total nitrogen, 28 per cent. protein, 31.2 per cent. RNA and 1.2 per cent. DNA. Total sugars constitute about 14 per cent. while lipid P seemed to be quite low 0.12 per cent. The iron content of the dry cells was 1,700 ug. per 100 g.

Study of Pethogenic Enteroviruses

(DEPARTMENTS OF VIROLOGY AND CLINICAL PATHOLOGY)

Aims and objectives.—Aetiology of gastroenteritis particularly in infants and children in a large number of cases is obscure. Suggestive evidence for relationship of certain Echo virus types to undifferentiated summer diarrhoeas of childhood has been put forward by some workers. It is also postulated that the clinical syndrome of undifferentiated acute gastroenteritis in children may be as a result of a symbiotic action of an enterovirus and a enterocoli.

Few workers in India have tried to investigate the problem of viral etiology of gastroenteritis but no systematic investigation has been carried out so far.

The aim of the present project is to investigate the problem of acute gastroenteritis in children from viral-cum-bacterial etiology.

Selection of cases.—Cases were selected by a paediatrician. All cases of dysentery (Bacterial or protozoal) were excluded from the present study. After this preliminary screening material for virological, bacteriological and serological studies were collected from the undifferentiated diarrhoea cases.

From September 1965 to February 1966, materials from 99 cases have been collected for this study.

Methodology.—For virus isolation, stool suspension or rectal swab suspension are inoculated in Rhesus monkey kidney tissue cultures and infant mice. All materials are carried for two blind passages before being labelled as negative. For viral identification, a preliminary screening against pooled and individual polio antisera is carried out. Those failing to be neutralised with polio antisera are put up against groups of Cocksackie and Echo antisera. Further, these isolates are studied for hæmagglutination and HAI activity.

Serological studies.—To determine the specific role of a viral isolate towards the clinical syndrome paired samples of sera are collected at an interval of two to three weeks and these are to be tested against the virus isolate for rise of specific antibodies.

Bacteriological studies.—Separate rectal swabs are collected for isolation of enterocoli from cases of gastroenteritis. These are inoculated on routine bacteriological media and the enterocoli thus isolated are typed against the available group antisera. Results of virus and enterocoli isolation studies are shown in table 2.

Results.—Of the 99 cases studied for virological studies, the results obtained are as follows :—

Virologically positive		52 cases
1. Virus isolation only in Rhesus MKTC	...	27 cases
2. Virus isolation only in infant mice	...	10 cases
3. Virus isolation in both 1 and 2	...	15 cases

Identification of viral isolates.—Of the 83 viral isolates obtained from 52 cases of gastroenteritis, 59 strains have been taken up for typing. Of these 9 strains have proved to be polioviruses and the remaining 50 have been non-polio enteroviruses. Further identification of these strains with Cocksackie and Echo antisera is in progress.

Hæmagglutination studies are in progress.

TABLE 1
Results of virus studies.
Agewise distribution

Age group	Total No. tested	Positive cases
Less than 1 month	1	1
1 to 2 months	1	0
2 to 3 months	2	1
3 to 6 months	9	3
6 to 9 months	13	6
9 months to 12 months	27	16
1 to 2 years	16	5
2 to 5 years	19	11
Above 5 years	6	2
Total	94	45

TABLE 2
Analysis of E. Coli serotypes isolated from Cases of Gastroenteritis

E. Coli type.	No. of cases Virus+	No. of cases Virus—
0 20	3	2
0 26	10	9
0 44	3	1
0 55	3	1
0 86	6	3
0 88	1	0
0 111	4	1
0 114	6	2
0 119	3	2
0 124	1	2
0 125	10	4
0 126	2	1
0 127	5	1
0 128	4	2
Untypable	20	16

TABLE 3

Analysis of cases showing number of E. coli serotypes present in cases of Gastroenteritis.

No. of serotypes of E. Coli.			Virus+	Virus—
One type	...	...	16	12
Two types	...	...	10	8
Three types	...	...	7	5
Four types	...	...	2	1
Five types	...	...	2	0
Six types	...	...	1	0

PART II

RESEARCH

B. Other Departmental Research

DEPARTMENT OF BACTERIOLOGY

This department is concerned with the production of vaccines. It is engaged in research connected therewith. Following is the brief report of studies carried out by the department :—

Preparation of typhoid vaccine from local strains and standardization of typhoid vaccine.—Typhoid vaccine is prepared by different laboratories mainly by using standard strains such as Ty2 of Rawlins etc. The role of strains isolated in a particular area is not fully studied. This is mainly because of the fact that there is no reliable laboratory method for assessing the potency of the vaccine as observed in the controlled field trials carried out by W.H.O. in Yugoslavia. It has been reported that virulent typhoid organisms mixed with mucin produce a reaction in the rabbit skin when injected intradermally. It was therefore, decided to explore the possibility of this reaction for standardization of typhoid vaccines prepared by different methods using standard and local strains. With this end in view, the following work was carried out :—

(1) Three strains of *S. typhi* isolated in the Clinical Pathology Department of the Institute were checked for their identity, agglutinability and other characters and selected for vaccine production.

(2) Four sets of vaccines were prepared with the selected strains as shown below :—

(a) Vaccine prepared by killing the organism with formalin and preserved with p.m.n. using standard Ty2 strain.

(b) Vaccine prepared by killing with heat and preserved with phenol using standard Ty2 strains.

Sets (c) and (d) were the same as (a) and (b) but were made by using one of the local strains.

(1) Agglutinin response using recommended immunization procedure was used for standardisation of different vaccines. No significant difference between Vi and O agglutinins was observed with different vaccines. H agglutinin response was little lower with heat-killed vaccines, but vaccines could not be graded by using this test.

(2) Active mouse protection tests were carried out.

(3) The skin response of rabbits to *S. typhi* Ty2 strain was found to be regularly graded varying from just red flush with smaller number of organisms increasing to redness and induration to finally necrosis with highest

number of organisms used. The M.R.D. giving a red flush of about 10 m.m. diameter was found to be approximately 2×10^5 organisms. The same result was obtained either by use of agar washing or broth culture.

(4) Rabbits immunized with different vaccines were bled and sera were collected. The same rabbits then were injected with graded doses of mucinized *S. typhi* organisms. The preliminary results indicate that rabbits immunized with formalized vaccines produce a positive reaction with less number of M.R.Ds. than those immunized with heat-killed vaccine. The exact quantitative assessment is in progress. Similar results are also obtained in passive protection tests carried out by mixing sera of immunized rabbits with mucinized *S. typhi* suspensions.

These results indicate that either of these tests may reflect the potency of vaccines in the laboratory.

DEPARTMENT OF BLOOD BANK

This department is concerned with the study of blood groups, preparation of blood grouping reagents, preparation of plasma and plasma fractions and tissue banking. Most of the research is concerned with the applied aspects of these problems. Some of these are reported below :—

1. *Study of Genetical Characters in some Endogamous Groups in Indian Population.*—This problem was undertaken to study distribution of blood group factors and other Genetical Characters in the different Endogamous Groups. Three groups on the Western Coast, viz. Syrian Christians from Malabar—Mahars from Konkan and four groups from Gujarat were taken up for study. The following characters are being studied :—

- (i) Blood Groups : ABO, Rh, MNS, Duffy and Kell Systems.
- (ii) Glucose-six—phosphate dehydrogenase test.
- (iii) Phenyl — thio-carbamide test.
- (iv) Secretor and non-secretor status.
- (v) Haemoglobin and sickling.
- (vi) Hepataglobin.
- (vii) Colour blindness.

Unrelated 225 Syrian Christians, 146 OSWAL — VISA Kuttchi Jains, 23 Mahars and 48 Audichya, Sahasra Brahmins are tested for all of the above tests. In Syrian Christians, among the ABO system the O Group is predominant while distribution of B group is little less than that of A Group.

2. *Study of Bacterial contamination of Banked Blood and Evaluation of use of sulpha drugs in its prevention.*—The study was undertaken with a view to study the chance contamination as encountered in blood processing and the steps to be taken to reduce the same. The study was planned with use of 3 sulpha group of drugs. The results did not show great advantage for the use of these drugs.

3. *Standardisation of Coomb's Reagent.*—The production of anti-human serum was standardized by immunising a bigger animal in order to increase the production. The manual and protocol of production were prepared and the product was put for regular issue to the laboratories.

4. *Preparation of Anti-Rh Sera.*—This work was taken up with full vigour from December 1965. This work was carried out in two ways :—

- (i) Preparation of Anti-Rh from iso-immunised (recently delivered) from maternity hospital.
- (ii) Preparation of Anti-Rh from purposely iso-immunised donors.

(i) *Preparation of Anti-Rh from iso-immunised women from hospitals.* About a dozen hospitals were requested for their co-operation to supply us with iso-immunised cases.

In all, 5 iso-immunised women have donated blood for this purpose.

(ii) *Preparation of Anti-Rh by purposely immunising Rh negative blood donors.*—Two new Rh negative 'O' Group Donors were taken up for immunisation. Two to three injections of Rh positive (Ror) blood (5 ml. each) were given to them; but they failed to turn up for further injections. Five Rh negative blood donors who had already been immunised were tested. All of them showed high titre Anti-Rh antibodies but they contained multiple anti-bodies. They have been bled and 400 ml. of Sera have been collected. As this is an initial work, samples of some of these sera have been referred to Dr. Goldsmith, Director, B. G. R. L., London, for confirmation of our results. These sera will be processed for final issue on getting the results from that laboratory.

5. *Bovine Albumin.*—Preparation of bovine albumin was taken up on regular basis (the previous experiments of using equine albumin did not prove successful). Bovine albumin was prepared from the bovine blood collected from slaughter houses. The plasma was separated and albumin fraction was obtained using Howe's Ammonium Sulphate Fractionation Method. The final product was freeze-dried and dissolved in buffer at pH 7.5 at 22 per cent. strength and this was used as blood grouping reagent. Nine batches have been processed so far.

The product was tried in this laboratory and the neighbouring laboratories and was found to be quite satisfactory.

The N. I. H. of U. S. A. and the B. G. R. L., London, were consulted regarding the standardisation of these products. There are no existing standards. The manual of production and testing were prepared. The product is now issued to all the laboratories for regular use.

6. *Plasma Fractionation Project.*—This project, which was started at the end of the last year, was continued and the following fractions were prepared; gamma globulin, albumin and pasteurised plasma protein solution. The results of laboratory tests on samples of these fractions sent with Dr. Krijnen to our collaborating laboratory (Central Laboratory of the Dutch B. T. S.) were received and these were declared satisfactory and suitable for clinical trials.

Limited clinical trials were conducted with the co-operation of the neighbouring hospitals. Gamma globulin was used in cases of infective hepatitis, cirrhosis of liver and measles.

The albumin solution (25 per cent.) was tried in cases of nephrosis and cirrhosis of liver. Pasteurised Plasma Protein Solution was tried in case of burns as Plasma Volume Expander. The reports received so far have been satisfactory and encouraging.

Fibrinogen and Antihæmophilic globulin were prepared in small batches from the fresh blood and these were issued for clinical trials. The results here also have been encouraging. The assay methods are being standardised in collaboration with other laboratories.

7. *Tissue Banking*.—The tissue drying plant was installed. Bone pieces were taken up for preservation. The ribs removed at thoracoplasty operations were obtained from R. P. T. B. Hospital and preserved in antibiotic solution. They were further processed and cut into match stick size and then freeze-dried for 6 days. The moisture content was determined and was found to be 2.5 per cent. which is satisfactory. The sterility tests were also done at each step. Some of the pieces were used clinically by an Orthopædic Surgeon and the results are satisfactory so far. The pieces have been taken and there is no sepsis.

DEPARTMENT OF CHEMOTHERAPY

This department is concerned with syntheses of new compounds and screening of compounds for several activities. It also interests itself in extracts of plant products. The studies carried out fall under the following sub-heads :—

1. *Chemotherapy of Tuberculosis*—(i) *Symmetrical Thiocarbanilides*.—A series of 4,4'-dialkoxy-3,3'-dibromo thiocarbanilides have been prepared to study the effect of the introduction of bromine in the phenyl rings of the thiocarbanilides on their antitubercular activity. All the compounds have been tested for *in vitro* activity and most of them were found to be active around 40 μ /g/ml.

(ii) *Unsymmetrical thiocarbanilides*.—Another series of mono and dibromoalkoxy-thiocarbanilides have been prepared by reacting halogenated alkoxy-anilines with mono or dihalogenated phenyl isothiocyanates. These were found to inhibit *M. tuberculosis* (H37Rv) *in vitro* in the range of 40-100 μ /g/ml.

(iii) *Halogenated derivative of (2-thiazolyl) alkoxy thiocarbanilides*.—A series 4(2-thiazolyl)-4'-alkoxy-3' bromothiocarbanilides has been prepared to study the effect of introducing the bromine in the phenyl ring. The *in vitro* study indicated that bromine had adverse effect and reduced the antitubercular activity.

(iv) *Thiazolyl thiocarbanilides*.—The earlier findings from this department that the attachment of alkoxy thiocarbanilide in the position 4 of thiazole afforded a high order of antitubercular action (*in vitro*) prompted the synthesis of related thiocarbanilides attached to other positions (2 and 5) of substituted thiazoles. About thirty p-(2-thiazolyl)-p'-alkoxy and p-(5-thiazolyl)-p'-alkoxy-thiocarbanilides have been synthesised and tested. The order of activity has been found to be 4-thiazolyl 2-thiazolyl 5-thiazolyl thiocarbanilides.

2. *Psychotropic and CNS Active Substances*—(i) *Synthetic psychotropic substances*.—Synthesis of substituted amino aryl (including 3, 4, 5-trimethoxy phenyl) acetophenones, propiophenones and their corresponding alcohols was continued. The amino group of all these compounds forms either a part of various nitrogen containing heterocyclic ring systems viz. N-substituted piperazine, morpholine, piperidine or it is 2 : 6 dimethylaniline, cyclohexylamine, 2-amino-pyridine, 2-aminopyrimidine, etc. Another series of unsymmetrical ethylenediamine derivatives have been synthesised. About 60 new compounds in all have been prepared and further preparation is in progress. Preliminary screening of the compounds carried out

by C. S. I. R. Unit at the Department of Pharmacology, Seth G. S. Medical College, showed that most of the compounds induced the same general pattern of activity of Central Origin. Smaller doses resulted in sedation and tranquilising effect while with increasing higher doses, CNS stimulant effects such as increased spontaneous movements, convulsions, death etc. were observed. Some of the compounds are being taken up for secondary screening.

(ii) *Celastrus paniculatus*.—The work on purification and characterization of fractions obtained from *Celastrus paniculatus* was continued. Two polyesters were obtained in pure forms and were hydrolysed to obtain polyalcohols and acids.

(iii) *Paspalum scrobiculatum*.—The stable fraction isolated from the toxic variety of *Paspalum scrobiculatum* (Harik) seeds after biological standardisation and toxicity studies was taken up for trials in several excited psychotic patients in the K. E. M. Hospital. Majority of patients receiving this fraction in doses ranging from 24-36 mg/day quietened down in 2-4 days and showed progressively increasing improvement. Haemogram, liver function and kidney function tests did not show any appreciable changes. The only side effects observed in a few cases were tremors, parkinsonism, vomiting and anorexia.

3. *Cytotoxic Substances*.—In view of the low order of toxicity and good activity against Dunning Leukemia and Walker Carinosarcoma shown by Schiff bases from aminophenyl thiazole and benzaldehyde nitrogen mustard, synthesised in the department and got screened through the courtesy of Cancer Chemotherapy National Service Centre, Bethesda (USA), further work on this type and related compounds including nitrogen mustards of aminophenyl thiazoles was continued. Also a series of p-[N, N-bis (2-chloethyl) amino]-benzylidene hydrazides containing different ring systems such as pyridine, thiazole, phenylthiazole, phenylpyridine etc. have been prepared for the study of their anti-tumor activity. Nearly 60 compounds have been synthesised and characterised. Of these, 31 have been prepared in sufficiently large quantities (7-10 g. each) and sent to Cancer Chemotherapy National Service Centre (USA), for screening purposes. During the year under review, screening reports of 16 compounds were received. Most of the compounds were found to be relatively nontoxic. One of the benzylidene-hydrazide mustards was reported to effect tumor weight reduction of 98 per cent. compared to control.

4. *Chemotherapy of Viral Infections*.—2-Carboxymethyl mercapto-4-amino-5 (p-Chlorophenyl) pyrimidine (C. A. C. P.) has been reported by Czechoslovak workers (Rada *et al*) to be active against viruses. It was decided to synthesise triazole analogues carrying carboxymethyl-mercapto and substituted phenyl groups as possible antiviral substances.

Nearly 20 compounds have been prepared for testing against different class of viruses. Preliminary screening against vaccinia virus in cell culture is in progress.

5. *Chemotherapy of Fungus Infections*—(i) *1-Acyl-4-substituted Thiosemicarbazides, 3-Aryl-4-substituted-5-Mercapto 1, 2, 4-4 H Triazoles and related compounds*.—Thiosemicarbazides having in position 1 a p or m-nitrobenzoyl or a 5-bromosalicyloyl group and the position 4 either unsubstituted or carrying an alkyl or aryl group, have been synthesised. They have also been cyclised into corresponding mercapto-triazole derivatives and some of these have been further converted into thiocyanato derivatives. On testing for *in vitro* antifungal activity none showed any action against *C. albicans* but the compounds having a 5-bromosalicyloyl group in position 1 exhibited activity against *T. rubrum* and *T. mentagrophytes*. This activity increased with the length of alkyl chain in position 4 but was lost when this position was occupied by a cyclohexyl or unsubstituted phenyl group. There was no change in the activity on cyclisation of the substituted thiosemicarbazides to corresponding mercapto triazoles. Conversion of the mercapto group into thiocyanato reduced the activity.

(ii) *Aliphatic and aromatic acid hydrazones and alkyl or aryl thiosemicarbazones of 5-chlorosalicylaldehyde*.—Aliphatic and aromatic acid hydrazones and alkyl, aryl or substituted aryl thiosemicarbazones of 5-chlorosalicylaldehyde have been synthesised and screened for *in vitro* antifungal activity against *C. albicans*, *T. rubrum* and *T. mentagrophytes*. Although one of the compounds exhibited significant activity against *C. albicans*, varying degrees of activity were observed against the two strains of dermatophytes by majority of the compounds. Among the hydrazones, the compound derived from o-hydroxybenzoic acid showed maximum activity (10 $\mu\text{g/ml.}$) and among the thiosemicarbazones, 1-(5-chlorosalicylidene)-(3, 4-dichlorophenyl) thiosemicarbazone was the most active (20 $\mu\text{g/ml.}$).

6. *Toxicological Studies*.—Studies on chronic manganese poisoning were continued. It was shown that the intake of additional manganese could be easily detected by the determination of hair manganese levels. The effect of 'EDTA' chelating agent on hair manganese levels of experimental animals receiving excessive manganese in the diet is in progress. Investigations on the hair manganese levels of underground manganese-mine workers as a functions of their period of service has been taken up.

7. *Drug Screening*.—During the year under review, an additional activity, viz. Antiviral screening (against vaccinia virus in cell culture) was taken up :—

(i) Nearly 270 compounds belonging to the classes of Thiosemicarbazides, Thiocarbanilides, Thiosemicarbazones, Hydrazides, Isoxazolones,

Pyrazolones, Triazoles, Triazine, Thiazoles etc. synthesised in the department and a few received from outside, were screened for their antimicrobial action.

Nearly 35 compounds exhibited antitubercular activity (*in vitro*) against H37Rv strain in the range of 20/ μ g/ml. over 100 showed activity at 40 μ g/ml.

(ii) The same 270 compounds were also screened against gram positive and gram negative strains of bacteria. In general, it was found that none of series showed significant action against these organisms.

(iii) A large number of compounds (over 100) prepared as possible antifungal substances were screened *in vitro* against two dermatophytes (*T. rubrum* and *T. mentagrophytes*) and one systemic organism (*C. albicans*). No activity was observed against *C. albicans* by any of the compounds tested. The highest activity against the dermatophytes was in the range of 10 μ g/ml.

(iv) *Antitubercular action of vakeri roots.*—Roots of vakeri (*Caesalpinia digyna*, Rott 1.), used in the native system of medicine for long in the treatment of tuberculosis affections, have been extracted with water, ethanol and aqueous ethanol. The extracts were inactive against gram positive and gram negative micro-organisms but showed activity against *M. tuberculosis* by *in vitro* tests, the aqueous ethanolic extract being the more active one. Incorporation of this extract in the diets of guineapigs at a level of 2 per cent. resulted in the sera being tuberculostatic after continuous feeding of the medicated diet for 24 days and longer.

DEPARTMENT OF CLINICAL PATHOLOGY

This department is concerned with offering the diagnostic service to the various hospitals. Its main studies are related to the development of diagnostic tests and the study of pathogens. The work is mainly of applied nature and contributes towards the knowledge about the disease. Some of these studies are reported below :—

1. *Skin allergen testing in cases of chronic Bronchial Asthma.*—Diagnosis of the offending allergens in cases of *asthma* was undertaken in collaboration with the Chest Institute, K. E. M. Hospital. Extracts of the following allergens — Wheat, Dal, Cotton-dust, House Dust, Brinjal and Lady's finger were prepared in the department and tested in selected group of 35 patients. None of the cases showed a positive reaction to any of the above antigens. It has been reported by Shivpuri *et al* that sensitivity to pollen and moulds constitute almost 85 per cent and hence further work is contemplated including pollen and moulds in the list of allergens for skin testing.

2. *Quantitative estimation of C.G.H. in cases with bad obstetric histology (BOH).*—Cases with history of repeated abortions (B.O.H.) were investigated in collaboration with Gynaec and obstetric unit of K. E. M. Hospital. The unit undertook detailed investigations including blood sugar, K.T., Vaginal cytology, blood grouping, Listereosis, chorionic Gonadotrophin Hormonal Level etc. About 75 cases were followed up both by immunological (Prepuerin) and Biological (Rat) tests. Quantitative C.G.H. estimations were done in a total of 250 urine samples. C.G.H. levels helped in substitution therapy and thus a fair number of fetal salvation was obtained.

3. *Comparative study of Biological and Immunological Tests in abnormal pregnancies.*—Hormonal levels (C.G.H.) in cases of abnormal pregnancies such as vesicular mole, choriocarcinomas and multiple pregnancies were investigated both by biological (Rat) and immunological (Prepuerin) tests. About 100 cases were investigated. Certain cases of choriocarcinomas showed marked variations in the excretion of the two components of the hormone — biological and immunological.

4. *Studies on Atypical Mycobacteria.*—"The comparative studies of Atypical Mycobacteria and Typical Tubercle Bacilli" has been selected as the problem for the research work. This problem was undertaken with a view to find out the exact relationship of so-called Atypical Mycobacteria with typical tubercle bacilli, of all the three varieties, viz human, bovine and avian strains. So far, thorough investigations of

about 500 cases of tuberculosis of different types have been carried out and from them 65 total strains were successfully isolated. Out of these, about 47 strains turned out to be atypical types and the rest are typical ones. These 47 strains were also classified into 4 different groups according to Runyon's classification for atypical mycobacteria. The studies on morphological, cultural, cytochemical and drug sensitivity patterns of these atypical strains in comparison with typical tubercle bacilli were completed. Side by side, a beginning is made about the pathogenicity studies of these bacilli on laboratory animals. The antigens of standard strains from abroad are prepared and also immunization of rabbits for obtaining high titre sera is under progress. These sera will then be used in serological classification. The work is in progress.

5. *An attempt to evolve a rapid serological test for the Enteric Infections.*—In the interest of the patients and Medical Practitioners, it is always desirable to get a quick laboratory report of suspected enteric infection. Routine Widal Test has only a presumptive value and it takes a long time. Hence, a test which is rapid and reliable will prove very useful.

With this end in view, studies have been initiated to find out whether a rapid serological test could be evolved.

The following tests are comparatively studied :—

- (i) Routine Widal Test.
- (ii) Cultural Test.
- (iii) Vi-agglutination.
- (iv) Vi-Hamagglutination Test.
- (v) Fluorescent Antibody Test.

In all, 129 sera of culturally positive cases of enteric infection along with sera from continuous fever cases, blood bank samples, immunised cases have also been studied.

The work is in progress.

6. *Some Biochemical and Immunochemical Studies on Enteric Group of Micro-organisms.*—The work was undertaken with a view to find out the constancies or variations in their cellular constituents like :

- (i) Proteins in the form of amino acids.
- (ii) Polysaccharides.
- (iii) Nucleo-proteins in the form of nucleic acids.
- (iv) Electrophoretic studies of the cell-wall free materials.

The studies revealed that the free organisms contain relatively higher proportions of the two amino-acids, viz. Aspartic-acid and Glutamic Acid. All other amino-acids were present in very small amount. In all the strains, total polysaccharides did not vary much in their contents. Chromatographic examinations showed galactose, glucose, mannose, xylose, rhamnose glucosamine along with two unidentified spots which might be due to incomplete hydrolysis.

Ratios of the RNA to DNA were practically the same. However, *S. schottmuelleri* contained both these nucleoproteins in more amount than other two.

Antigenic analysis.—*S. typhosa*, *S. paratyphi* and *S. schottmuelleri* showed the presence of 11 to 13 precipitation lines when tested against the homologous sera. With the heterologous sera, number of lines varied.

From the growth curve studies, it may be stated that *S. paratyphi* is more fastidious in its nutritional requirements and the growth rates have no apparent correlation with the nucleic acid contents.

7. *The problem of Leptospirosis in Bombay.*—Leptospirosis appears to have received very little attention in India even though it is one of the major problems of zoonosis. After the original work of Ayyar (1932), Wagle (1933), Lahiri (1941), Ball and Shaikh (1956), very little further work has been carried out on this subject.

It was felt desirable to study the extent of leptospiral infections and the nature of serotypes involved in man and animals like the rodents, the dog, the pig, the cat and the cattle. Greater interest is aroused to study this problem from the point of view of its economic importance. Work has been started. Fourteen different serotypes of *Leptospira* cultures have been obtained.

The general techniques employed are :—

- (i) Dark-field illumination.
- (ii) Silver impregnation staining technique.
- (iii) Cultural tests.
- (iv) Agglutination Reactions.
- (v) Haemolysis (HL) Test or Sensitized Erythrocytes Lysis Test, using different techniques.

Using different techniques, the following results were obtained :—

- (i) Out of 45 Bombay City rats examined, one *R. rattus* was found positive.

(ii) Out of 12 water ponds examined, non-pathogenic leptospire were isolated from 2.

(iii) Out of 71 sera collected at random from infectious hepatitis patients, one gave *HL* titre of 1/1000 and it reacted with *L. pyrogenes* giving a titre of 1/10000. It also reacted with *L. icterohaemorrhagiae*, *L. autumnalis* and *L. patoc*.

(iv) Sera of 6 aborted pigs reacted with *L. pyrogenes*, *L. pomona* and with the local strain from rat in very high dilutions some of them even up to 1/25600. The work is continued.

8. *Serological Typing of Pathogenic, Staph. aureus*.—This study was continued during the year.

Four hundred and thirty strains of coagulase positive *Staphylococcus aureus* were subjected to serotyping by the following three techniques:—

- (i) Fluorescent antibody technique.
- (ii) Slide agglutination.
- (iii) Gel diffusion.

Fourteen factors were prepared and used for slide agglutination. Seven of these factors were labelled with fluorescent isothiocyanate and used for F.A. technique. Normal human sera against staphylococcal antibodies were used for gel diffusion technique. Serological typing, agglutination, and F.A. antibody technique gave best results.

9. *Rapid Laboratory Diagnosis of C. diphtheria with Fluorescent Antibody Technique*.—Rapid diagnosis of *C. diphtheria* was tried by immuno-fluorescent methods by use of a diphtheria antibacterial reagent against *C. diphtheria* from clinical material. In the majority of cases, fluorescent *C. diphtheria* could be identified in 3-4 hours after the swab is received.

Out of 400 cases of clinically suspected to be diphtheria, 231 cases (60.75 per cent) proved positive by this technique as well as the routine test method. In addition, 11 cases were found positive by FA test but negative by the culture test. Twenty cases which were culturally positive failed to react with F.A.

By the F.A. technique, it is also possible to differentiate the toxigenic stains.

The test has the advantage that it is rapid.

DEPARTMENT OF ENTOMOLOGY

This department concerns itself with studies on plague, fleas, rats and snakes. The studies carried out by the department are reported below under the following sub-heads :—

1. *Host specificity in fleas and the study of their salivary glands.*—Reproductive potentials of *X. cheopis* and *X. astia* were worked out on *R. rattus*, *B. bengalensis* and laboratory mouse. *X. cheopis* showed a higher fecundity when fed on *R. rattus* and white mouse, whereas a considerably lesser rate of fecundity was seen with *B. bengalensis*. *X. astia* showed almost equal rate of fecundity with *R. rattus* and *B. bengalensis* but comparatively poor value with white mouse. It was also seen that frequency of feeding influences not only the fecundity but the time taken for the development of the ovaries in a freshly emerged flea.

2. *Testing of insecticides.*—Four conventional insecticides, i.e. Sevin, Malathion, B.H.C. and D.D.T. were tested to see if any resistance has been developed by *X. cheopis* and *X. astia* from Bombay in their adult and larval stages. Results indicates that the toxicity of the four insecticides varies in the following order :—

Sevin — Malathion — B.H.C. —D.D.T.

3. *Studies on Heterozygous transplant in flies.*—Life history of *Musca sorbens* has been studied in the laboratory. Work is being done to show that allacto-my prevents maturation of ovaries while transplantation of corpus allatum restores it in *Musca nebulosa*. *M. sorbens* is intended to be used for interspecific transplantation. This work so far has given negative results.

4. *The studies on snake salivary glands and venom output of snakes.* Morphological and histological studies on the salivary glands of some non-poisonous snakes are being continued. Experiments are in progress to assess the toxicity of the extract of the salivary gland secretion of non poisonous snakes by injecting them into mice intravenously. Experiment are also in progress to determine the toxicity of the supernatant and the debris collected after centrifuging a freshly collected venom.

Seasonal variations in the toxicity of venom from the snakes kept in the farm and in the room are also being studied.

5. *Plague epidemiological studies in Maharashtra State.*—These studies were carried out at 23 places in the districts of Bhandara, Nagpur and Chanda in the Vidarbha region of Maharashtra State with the help of

a van and some staff provided by the Director of Public Health, Government of Maharashtra. This work started on 9th August 1965 and terminated on 3rd January 1966.

In all, 2,772 rats were collected in these studies out of which 2,703 were *R. rattus* forming 97.2 per cent. of the total rat catch. 9,042 fleas were recovered from these rats out of which 8,067 were *X. cheopis* and 883 were *X. astia*, while the rest 92 could not be identified as they were damaged. It is interesting to note that in spite of a good number of *R. rattus* being trapped in the collection in the hinterland at Sironcha region, no *X. cheopis* was recovered from them. All the fleas recovered from the rats at Sironcha were *X. astia*; *X. cheopis* was the flea recovered from the more populated regions like Ramtek, Kondhali, Saoner etc. It was also observed that all the fleas collected on *Tatera indica* from fields in Amgaon and from *Rattus blandfordi* from forests in Deolapar were *X. astia*. It is, therefore, suggested that *X. astia* are still found in larger numbers in the hinterland and thinly populated areas, while *X. cheopis* predominates in the thickly populated market places. Rats brought to the Haffkine Institute from different places were challenged with a standard dose of *P. pestis*. The results indicate that rats collected from areas along trade routes are comparatively resistant, while those from fields and forests in the hinterlands are still susceptible to plague.

Experiments were done by using bait boxes dusted with 5 per cent BHC dust, as bait stations for rodenticides like Zinc Phosphide, Shoxin and Rodafarin to see if these cause any reduction in flea indices in addition to the reduction in rat density due to rodenticides. It was noted that barring a few exceptions, the reduction in flea indices and rat density was noticed at all places. The reduction in rat density in the case of Zinc Phosphide was found to be 96 per cent, while in Shoxin it was 33.5 per cent. and in the case of Rodafarin it was 63.67 per cent.

6. *Trial of Rodenticides*.—Laboratory trials were carried out with rodenticides like Zinc Phosphide, Shoxin and Warfarin. Zinc Phosphide was found to be effective even at a concentration of 0.1 per cent. Shoxin (a specific rodenticide) at the concentration of 0.05 per cent. gave 100 per cent. mortality among *R. norvegicus*, 60 per cent. in *R. rattus*, while *Bandicota bengalensis* were not affected at all. Warfarin at 0.05 per cent. killed the rats from 2 days onwards to 12 days depending on the species of rats.

7. *Studies on Breeding of various species of Rats.*—*Tatera indic* brought from Amgaon were bred in the laboratory. So far, 3 litter have been recorded from one pair. Attempts are being made to breed *R. rattus* in the laboratory.

DEPARTMENT OF IMMUNOLOGY

This department is concerned with production of sera in the animals. It is also interested in studies on tetanus toxin. One important line of investigation is the production of galactose cataract in the lens. The work carried out by the department is reported below :—

1. *Studies on mechanism of action of Tetanus Toxin.*—The object of this work was to study the mechanism of action of tetanus toxin at the molecular level and also to find out reasons for the susceptibility of the rat and the resistance of the pigeon. The emphasis was laid on biochemical changes at the mitochondrial and submitochondrial level.

Brain, liver and muscle mitochondria were prepared. Respiration was determined by oxygen uptake in Warburg apparatus. The results of these experiments showed that tetanus toxin was able to uncouple the oxidative phosphorylation of brain mitochondria of susceptible rat but not of resistant pigeon. The decrease in the P : O ratio of rat brain mitochondria was due to a simultaneous stimulation of oxygen uptake and a decrease in the phosphorylation process. With pigeon brain mitochondria, there was slight stimulation of oxygen uptake but no decrease in phosphorus fixation. However, when the pigeon brain mitochondria were disrupted by freezing and thawing the P : O ratio was lowered in presence of toxin. This showed that the mitochondrial membrane of pigeon brain is impermeable to tetanus toxin. This may be the reason for high resistance offered by the pigeon. The toxin had no action on P : O ratio of liver and the muscle mitochondria of rats and pigeons. Further it was found that the uncoupling of oxidative phosphorylation of rat brain mitochondria took place in DPN, requiring step only. This was demonstrated by measuring P : O ratio in presence of toxin using succinate and ascorbate as substrates. Unlike many uncouplers, toxin had no effect on swelling properties of rat brain and liver mitochondria.

Rats with localized paralysis and generalized paralysis showed decrease in ATP content of their skeletal muscle and brain tissues. The low ATP levels in brain and skeletal muscle tissues were also obtained in pigeons injected with toxin. It was also found that tetanus toxin increased ATPase activity of myofibrils. Hence, it can be concluded that general paralysis is initiated by low ATP content of brain because of failure of brain mitochondria to fix adequate amount of energyrich phosphates due

to presence of tetanus toxin. The local tetanus produced is due to decreased ATP. levels in the injected muscle due to activation of ATP. use of myofibrils.

2. *Studies on fixation of Tetanus Toxin.*—Ganglioside prepared from protagon of nervous tissue is a receptor for tetanus toxin. Gangliosides are water soluble non-dialysable glycosphingolipids present in the grey matter. It was of interest to study sub-cellular fixation of tetanus toxin and the nature of its combination with gangliosides. Fixation was first studied by incubating tetanus toxin with homogenates prepared from brain and liver tissues of susceptible rats and resistant pigeons. The amount of toxin adsorbed was calculated by injecting toxin in mice and determining MLD. and the $L+10$ values before and after adsorption. It was found that pigeon brain homogenate had only 20 per cent. less adsorption capacity than rat brain homogenate. However, homogenates prepared from rat and pigeon liver tissues did not adsorb toxin. The subcellular fixation was then studied by preparing subfractions of brain homogenates of rats and pigeons. The adsorption of toxin was highest on mitochondrial fraction prepared from brain of rats as well as pigeons.

The mitochondrial fraction from brain contains mitochondria, myelin-fragments and nerve endings. It contains 0.75 micro moles of N-acetyl neuraminic acid (which is measure of gangliosides) per g. of tissue. It was found that ganglioside content of pigeon brain was only 20 per cent less than ganglioside content of rat brain. This figure is proportional to the amount of toxin adsorbed by mitochondrial fractions of two species. The liver homogenate of these two species contained negligible amount of ganglioside. The studies on binding of tetanus toxin with ganglioside showed that soluble ganglioside-toxin mixture did not inactivate tetanus toxin and the bound toxin was dissociable *in vivo*. This indicated that Tetanus Toxin was not chemically bound with gangliosides but is only loosely attached to it.

3. *Serum Enzymes in Tetanus.*—26 cases of tetanus and 3 suspected cases of tetanus which later on turned out to be non-tetanus were studied for serum aldolase and serum CPK. activities. There was no detectable amount of CPK. in sera of 14 cases. However, aldolase rose very high in some fatal cases of tetanus. The enzyme activity closely paralleled the clinical course of the disease and was related to spasms and rigidity. There was no rise in serum aldolase in 3 non-tetanus cases.

Serum aldolase was raised in rats injected with tetanus toxin. However, serum CPK. was not altered in these rats. Aldolase and CPK. was also estimated from brain and skeletal muscle of rats injected with tetanus

toxin. It was found that only the aldolase activity of injected paralysed leg muscle decreased by 20 per cent indicating leakage of enzyme from muscle into plasma. Our results show that leakage of aldolase may be due to change in permeability of cell membrane caused by local anoxia coupled with muscular and respiratory spasms and rigidity. In patients having rigidity, increase in aldolase in serum was quite significant and may be of some diagnostic value.

4. *Studies on changes in serum proteins and Antibody proteins in horse during hyperimmunization and bleeding.*—The object of this study was to determine quantitative changes in electrophoretically separated serum proteins and antibody proteins during immunization of horse with diphtheria toxoid. Another object was to observe for a period of 10 days after one course of immunization rise and fall of antibody titre in order to determine suitable time for bleeding the horse when the antibody activity is at its maximum. It was also of interest to study regeneration of serum proteins (antibody proteins) after bleeding. As a result of hyperimmunization, marked increase in serum total proteins in spite of decrease in albumin was observed. Appearance of Pre- α_2 globulin soon after first dose of antigen and increase of gamma globulin in initial stages and beta globulin in final stages of immunization are outstanding observations in this study. In this study, alpha-globulins and beta-globulins are separated into 4 and 3 components respectively by electrophoresis. Albumin and α_0 were devoid of antibody activity. Antibody activity is associated with remaining components of alpha and beta globulins. Studies extending to 10 days during 'rest' period after one course of immunization indicated that the peak antibody level is attained between 3rd and 5th day after last dose. An average of 18 per cent. increase in antibody activity over initial level was observed during this period.

The effect of bleeding 8 litres of blood at a time after one course of immunization on fifth and seventh day indicated that decrease in antibody activity due to one bleeding and two bleedings were 15 per cent. and 20.25 per cent. respectively. The effect of successive bleeding and immunization on serum protein was studied. There was a tendency towards increase of albumin. There was, however, no significant decrease in total serum protein after successive bleedings.

5. *Investigations on galactose cataract in lens.*—The action of concentrated urea solution in converting insoluble lens into alpha crystallin-in vitro has raised the question whether urea can be used to prevent cataract. Since it is not possible to develop senile cataract in experimental animals easily, galactose cataract in rats was used for our studies. Cataracts were

developed in experimental rats weighing (60 to 70 g.) by feeding with stock diet containing 35 per cent of D-galactose in their diet. After 20 days, when cataracts were fully developed, the galactose fed rats were divided into 2 groups. One group was continued on galactose diet and a second group was fed on same galactose diet to which 10 per cent of urea was added. No reversal of cataract was observed in rats fed with galactose and urea.

In second experiment, 10 per cent. urea was added to the galactose diet of young rats from beginning and it was observed that the group fed on galactose alone developed cataract within 20 days while the other group fed on galactose and 10 per cent urea did not develop opacity up to 35 days.

In a third experiment, it was found that glycerol fed to rats in 10 per cent. of diet could not retard cataract formation like urea though both these compounds have the same effect of reducing intraocular tension in glaucoma. The effect of urea, therefore, seems to be specific and needs further confirmation.

6. *Adjuvants to stimulate Diphtheria Toxin Production.*—It is known that highly purified diphtheria toxoid is a poor antigen and for human immunization adjuvants such as Aluminium Hydroxide, Aluminium Phosphate or Calcium Phosphate are generally used. It is very difficult to get sustained diphtheria antitoxin titres in horses without the use of adjuvants. Attempts were, therefore, made to test the efficacy of adjuvants when combined with purified diphtheria toxoid. Rabbits in groups of ten were used to test the following adjuvants: Aluminium Phosphate, Calcium Phosphate, Alginate and Freund's incomplete and complete adjuvant. The studies are continuing. It is proposed to test the effect of hormones also on the production of antibodies with and without adjuvants.

7. *Selection of strains of B. pertussis for vaccine production.*—About 20 production strains of *B. pertussis* obtained from different laboratories were tested for their antigenic value for selecting the best ones for routine vaccine production. The intracerebral mouse protection test was carried out using two different challenge strains of *B. pertussis*. Only 3 strains were found satisfactory. These strains are now being used for the routine vaccine production to be incorporated into Triple Vaccine,

DEPARTMENT OF NUTRITION AND BIOCHEMISTRY

The Research activities of the Department are chiefly concerned with :

- (A) Investigations of foodstuffs commonly consumed in the State.
- (B) Animal experimental work of Public Health importance.
- (C) Certain aspects of lipid metabolism.
- (D) Studies on the metabolism of micro-organisms.
- (E) Standardisation of Biochemical techniques.

1. *Evaluation of proteins in foodstuffs.*—(a) Processed seeds of cluster beans (*Cyamopsis tetragonoloba*): The protein isolated from the above material was assayed microbiologically for amino acids. It is deficient in methionine and tryptophane and rich in histidine and arginine in comparison to egg-proteins. When protozoon *Tetrahymena pyriformis* W. was used to evaluate the nutritive quality of the above protein, surprisingly, no growth of the protozoon was obtained with this test protein. Further work on the nature of this isolated protein is in progress.

(b) Microbiological methods for evaluating protein quality: The conventional methods for the evaluation of nutritive quality of protein, using higher animals are complicated and time-consuming. Moreover, the nutritive values of the same foodstuff obtained by different workers using these conventional methods differ widely. This is due to wide variations in the experimental conditions employed by the different workers.

With a view to develop a simple and rapid method for protein evaluation, a number of workers have focussed their attention on the possible use of protozoa or bacteria for assay purposes. The nutritional requirements of the protozoon *Tetrahymena pyriformis* W. have revealed the potentialities of the organism for assaying protein quality. The organism can digest and utilize intact protein and the pattern of its amino acid requirements is similar to that of the growing rats. The organism *S. zymogenes* NCDC 592 has also been used to evaluate protein quality, as this organism is vigorously proteolytic and requires 7 essential amino acids for its growth.

The nutritive values of 8 different foodstuffs have been studied by the conventional methods, viz. biological value (BV) net protein utilization

(NPU) and protein efficiency ratio (PER) and correlated with the results obtained by the microbiological methods using *S. zymogenes* and *Tetrahymena pyriformis* W.

The following foodstuffs were assayed for protein quality : red-gram, black-gram, 'bajara', 'jwari', rajgira (*Amaranthus paniculatus*), Rajmah (*Phaseolus vulgaris*), processed seeds of cluster beans, protein isolated from the seeds of cluster beans (*Cyamopsis tetragonoloba*) and casein (reference protein).

The values of NPU and BV of the foodstuffs show a good correlation with the values obtained by the microbiological methods.

The supplementary value of proteins from different sources is now under investigation.

2. *Protein metabolism and B-vitamins—Vitamin B₁₂ and pantothenic acid*—(a) *Effect on growth and blood regeneration*.—The metabolic interrelationships between the two vitamins and their effects on the growth and blood constituents in the rat were reported previously. Further studies on the effect of pantothenic acid on growth and blood constituted by using pair-fed control rats, were carried out. These experiments were specially carried out to separate the effect of inanition from that of pantothenic acid deficiency by having pair-fed controls. The results show that pantothenic acid has the growth promoting and blood regenerating capacity in albino rats.

(b) *Nitrogen metabolism*.—The investigation was continued to study the effect of the vitamins on nitrogen metabolism. The result obtained in this experiment can be summarised briefly as follows :—

(1) Administration of vitamin B₁₂ produced no effect on the growth of the Animals during a period of 3 days.

(2) The administration of pantothenic acid resulted in the increase of the body weight of the animals by 12 g. in three days.

(3) Vitamin B₁₂ produced a significant effect on the nitrogen retention. But this effect was seen only when Vitamin B₁₂ was administered along with pantothenic acid. Administration of vitamin B₁₂ alone to the deficient animals had no significant effect on the retention of nitrogen.

(4) Pantothenic acid exerted a very marked effect on the retention of nitrogen.

(5) Both the vitamins reduced the urinary excretion of nitrogenous constituents except amino-N).

(6) Vitamin B₁₂ lowered significantly the level of free amino acid in serum and liver. Pantothenic acid had no effect on free amino acid content of both serum and liver.

(c) *Nucleic acid metabolism.*—The effect of the two vitamins on nucleic acid metabolism was studied. The results indicate that vitamin B₁₂ elevates the level of both RNA and DNA in the liver, the effect on RNA being more pronounced. The rise in RNA content was a reflection of an increase in the supernatant fraction (microsomes and soluble fractions), while the RNA content of nuclear and mitochondrial fractions remained unaltered. Pantothenic acid had negligible effect on RNA and DNA content of liver.

3. *Studies in E. coli metabolism.*—To elucidate further the nature of the metabolic interrelationship between pantothenic acid and vitamin B₁₂ experiments have been planned to study the role of pantothenic acid on the growth of the wild strain of *E. coli* (which can bring about methionine synthesis independent of vitamin B₁₂) and *E. coli* mutant 113-3 (vitamin B₁₂/methionine auxotroph).

It is observed that calcium pantothenate added in graded quantities to the basal medium inhibited the growth of *E. coli* 113-3. The growth of the wild strain of *E. coli* was uniform and calcium pantothenate had no effect.

4. *A study of cholesterol esterase activity in the tissues of rats fed various dietary fats.*—Cholesterol esterase activity in the tissues was studied in growing rats on stock diet for preliminary observations. The effects of various dietary fats, viz. safflower seed, coconut and refined groundnut oils and vanaspati on this esterase activity in serum, liver and intestines have been studied.

5. *The serum cholesterol and phospholipid levels in a typical group using coconut oil as the chief dietary fat.*—In this country, large groups of population use coconut oil as the main source of fat in the diet. Although of vegetable origin, coconut oil is rather an exception in having a high content of saturated fatty acids, higher than the fats derived from animal sources, e.g., butter and lard which have a tendency to cause a state of hypercholesterolemia. However, no published data are available on the serum cholesterol level of such a group of people. Hence, data has been collected on cholesterol and phospholipid levels of about 100 individuals from typical families consuming mainly coconut oil.

6. *Effect of adenine dinucleotide reduced (NADH) on lipoyl dehydrogenase from E. coli (in collaboration with Tata Institute of Fundamental Research).*—The inhibiting effect of NADH in the reaction (1) of the lipoyl dehydrogenase from various sources has been reported.

Two distinct types of inhibitory effects produced by NADH — one which is reversible and protected by NAD and the second which is an irreversible inhibition slowly inactivating the enzyme and which is not protected by NAD have been reported. The present work undertaken revealed a partial explanation to the type of inactivating effect by NADH in the *E. coli* system. The findings confirm the “Bi-Bi Ping Pong” mechanism in which the enzyme acts with the reduced substrate resulting in a reduced enzyme which, in turn, reacts with the oxidised substrate and thus gets itself oxidised.

It is interesting to note that this inhibitory inactivating effect of NADH on lipoyl dehydrogenase is not observed in the mammalian system or in an obligatory aerobe such as *L. mesenteroid* and *S. subtilis*. Such an effect appears to be more of an environmental condition to which the organism is exposed. NADH appears to play a regulatory role which may be needed in facultative organisms in which case the NADH is not oxidised to a greater extent by molecular oxygen. This regulation may not be needed at all in the system where plenty of oxygen is available to take care of NADH produced.

7. *Studies on RNA containing bacteriophage* (with kind facilities from T.I.F.R.).—The bacteriophage F_4 was grown on *E. coli* K₃₇ and the protein and RNA were isolated in pure form by the method of Cooper and Zinder by cesium chloride gradient centrifugation. Using “finger printing technique”, the tryptic digested protein yielded peptides. To get the mutant of F_4 the phage was treated with utagenic agents like nitrous acid and hydroxyl amino hydrochloride. However, the host specific mutant could not be detected as the treated phage produced plaques on both the strains, namely, K₃₇ and K₃₈.

8. *A study of the dietary pattern of the Sindhi Community in Bombay.*—170 Sindhi families, comprising of 972 members from every walk of life, were interviewed for their dietary histories by the oral questionnaire method.

The consumption of various foodstuffs and nutrients were adequate. However, the intake of cereals, pulses and leafy vegetables were somewhat less than the recommended dietary allowances. Consumption of

fats, percent calories derived from fat, vitamin A and milk were more than the recommended allowances. Rest of the foodstuffs and nutrients were nearer to the recommended scale.

The average consumption of foodstuffs and nutrients were found to be more or less the same among the vegetarians and non-vegetarians, except that the consumption of milk and pulses which were found to be more among the vegetarians. The daily intake of proteins and calories were found to be more among the non-vegetarians.

9. *A study of the blood constituents of 200 individuals from the Sindhi families with particular reference to serum cholesterol, lipid P in relation to dietary fat intake.*—Two hundred blood samples collected from the individuals were analysed for serum total and free cholesterol, lipid phosphorous, serum protein fractions, blood sugar and hæmoglobin.

The mean serum total cholesterol showed gradual increase in all the groups with increasing age. The correlation coefficient computed from the bivariate distribution worked out at 0.36 which was significant even at 1 per cent level, indicating strong evidence for the positive correlation between age and the serum cholesterol level.

Effect of age on serum lipid phosphorous was found to be not significant statistically.

Considering the socio-economic factors, a significantly high level of serum cholesterol was observed in the economically richer group. The upper middle class and the middle class had more or less the same levels.

The difference in the mean serum cholesterol levels in relation to actual fat intake and calories derived from fat in the 200 individuals were not statistically significant as also the total protein intake and per cent calories derived from proteins.

Considering on a population basis, the fat intake (99 g.) and the mean serum cholesterol level (272 mg. per cent) and serum lipid phosphorous (15.1 mg. per cent) among the typical homogenous community (Sindhis) of Bombay were more than the figures reported by other Indian workers for groups consisting of either rural, industrial, army personnel etc. and this was perhaps based on socio-economic conditions.

The higher values noticed may be due to the (1) higher fat intake (2) type of fat consumed and (3) to the more or less sedentary life of the members of the community.

10. (a) *Titrimetric method for the estimation of phenol in vaccines.*—Phenol added as preservative to rabies vaccine can be conveniently estimated by a microtitrimetric procedure using a saline, tungstic acid, a TCA extract or a distillate from 1 ml. of vaccine.

(b) *Colorimetric estimation of phenol in rabies vaccine.*—Folin's phenol reagent or diazotised p-nitroaniline can be conveniently used for the colorimetric estimation of phenol in rabies vaccine, provided the phenol is extracted with petroleum ether. Alcoholic, tungstic acid and trichloroacetic acid extracts contain non-specific materials from the brain tissue which interfere with the colorimetric procedure. The colorimetric procedures are rapid and simple when compared to the titrimetric method. The diazotised p-nitroaniline is preferable as it is easy to prepare and gives more intense colour.

11. *Study of Cellular Constituents of C. diphtheriæ.*—A preliminary study was undertaken to study the cellular composition of the organism as grown for the production of diphtheria toxin in the Institute. The cells contain about 12 per cent of total nitrogen, 28 per cent protein, 31·2 per cent RNA and 1·2 per cent DNA. Total sugars constitute about 14 per cent while lipid P seems to be quite low, viz., 0·12 per cent. The iron content of the dry cells is 1700 ug. per 100 g.

RESEARCH

1. *Studies on antidiabetic drugs.*—Sixteen new synthetic compounds were tested for their hypoglycemic activity after oral administration to adult fasting rabbits. The drugs tested were R-661, R-662, R-668, R-671, R-675, R-864 to R-868, R-922, DM-1 to DM-5. Amongst these, R-668, at a dose level of 75 mg./kg. was found to be effective to the extent of 23 per cent reduction in blood sugar at the end of 5 hours. DM-1 was found to produce moderate hypoglycemic activity in rabbits and rats.

2. *An alkaloid from Solanum xanthocarpum.*—The concentrated alcoholic extract of the powdered drug was acidified, filtered and the filtrate extracted with chloroform. Crude alkaloid was precipitated from the acidic filtrate after addition of alkali. The crude material was purified by repeatedly dissolving in alcohol and re-precipitating by addition of water. Melting point of the alkaloid was 230° – 240°C. Yield 0.1 per cent. Further studies are in progress.

3. *Anti-bacterial activities of extracts of medicinal plants.*—The anti-bacterial activities of nine different extracts of medicinal plants (CDR-1 to CDR-8 and IDR-5) prepared with different solvents, (petroleum ether, ether, chloroform and alcohol), were tested, against *Staphylococcus aureus*, *S. typhosa*, *E. coli*, *V. cholerae* and *Shigella dysenteriae*. Alcoholic extract of CDR-1 inhibited *V. cholerae* alcoholic extract of CDR-5 and CDR-7 inhibited the growth of all the bacteria mentioned above. Ether and alcoholic extracts of CDR-2 inhibited the growth of *V. cholerae*. An essential oil obtained from IDR-2 showed inhibition of *Staphylococcus aureus* in a dilution of 1 : 100.

4. *Studies on Heydiotis auricularia.*—A water-soluble alkaloid was isolated from this plant. This alkaloid was proved to be useful in the treatment of cholera in infant rabbits, although it did not exhibit any vibriostatic or vibriocidal property.

Preliminary pharmacological study showed that the maximum tolerated dose in white mice was 20 mg./kg. when injected intramuscularly. It has hypotensive action in cats but no significant action on the isolated guinea-pig's intestine. Further work is in progress.

5. *Study on antibronchial drugs.*—Two plants IDR-5 and IDR-7 are reported in the indigenous system of medicine to be beneficial in the

treatment of asthma and bronchitis. Chemical and pharmacological study showed that IDR-5(a) and IDR-7(c) were ineffective as a bronchodilator in guineapigs.

When the tracheal chain preparations of guineapigs were used IDR-5(a), IDR-5(b), IDR-5(c) and IDR-5(d) showed relaxation of the tissue. IDR-7(a) did not relax the tracheal chain.

6. *Indigenous drugs in experimental cholera*.—Extracts from four medicinal plants were studied for their activities against cholera infection in the infant rabbit. The extracts were administered in a dose of 100 mg. (in divided doses) per 100 g. of body weight orally to groups of 10-day old rabbits. The drugs were given every six hours, starting from one hour before the infection was given. Extracts from the seed of *Butea frondosa*, from the bark of *Ficus bengalensis* and seeds of *Focmiculum capillaceum* had no effect on the course of the disease. Alcoholic extracts of *Euphorbia helioscopia* saved two of the six animals. This appears to be a promising drug.

7. *Experimental amoebiasis*.—As a preliminary to the study of chemotherapeutic action of drugs on *Entamoeba histolytica*, it is essential to have a satisfactory method for the culture of the protozoa. Three different media were tried for the cultivation of the ameobæ (1) Dobell's medium which mainly consists of horse serum, saline buffer at pH range 7.0 to 7.2 (2) Dobell's egg medium. The solid portion consists of whole egg and the liquid portion horse serum, saline and buffer at pH 7.1 to 7.2 (3) Pavolova's medium which includes normal horse serum, saline buffer and "Marmite" extract at pH 7.0.

Repeated trials showed that the best growth was obtained with Dobell's horse serum medium. Two methods for the study of ameobicidal drugs in animals have been standardised. One of the methods envisages the use of albino rats of 20 to 35 g. weight. The second method requires the use of hamsters. Both the methods have been working satisfactorily.

8. *Stability of panthenol*.—Stability of panthenol was studied by the 'accelerated' stability method. The effect of pH, metal ions etc. on panthenol was tried. Solutions containing panthenol were stored in the dark at 45°C and the rate of decomposition was noted. The method employed was the inhibition of growth by *Leuconostoc mesenteroides*.

Panthenol solutions were prepared in buffers at different pH and were stored at $45^{\circ} \pm 1^{\circ}\text{C}$. Panthenol content was estimated at intervals. It was observed that panthenol was stable between pH 4 to 6 and the stability decreased with the shifting of pH on either side of this range.

Panthenol solutions were prepared in buffers containing different metal ions and their stability was tested. Manganese and ferric ions were found to exert a deleterious effect on the stability of panthenol. Other metal ions like copper, calcium hypophosphate had no effect on the stability of panthenol under the condition of experiment employed.

9. *Preparation of substituted phenacyl halides and certain amino compounds.*—In order to prepare some new organic compounds having probable pharmacological activity with special reference to the central nervous system activity, certain intermediate compounds have been prepared. For preparations of substituted phenacyl halides literature was surveyed and they were prepared by standard methods. For example, p-methoxy phenacyl chloride was prepared by the Freidel-Craft reaction between anisole and chloroacetyl chloride in the presence of anhy. AlCl_3 . p-phenyl phenacyl bromide was prepared from diphenyl p-phenyl acetophenone by Freidel Craft reaction. Pe-chlorophenacyl chloride was prepared from chlorobenzene and chloroacetyl chloride by the same reaction. Many other phenacyl halides were also prepared. Certain secondary amines of the heterocyclic series were also prepared. These are being used for synthesising new organic compounds.

DEPARTMENT OF VIROLOGY

This department is interested in the polio and other enteroviruses and their aetiological role in the causation of disease. It has also interested itself in the K.F.D. virus and pox viruses. Research work carried out by the department is mentioned below seriatim :—

1. *Study of Non-specific Inhibitors in Normal Animal Sera against Polio Viruses.*—Since normal animal sera are widely used as nutrient for cells in tissue cultures (TC), the presence of non-specific inhibitors, if present, may reduce the sensitivity of TC system for virus isolation.

Sera from normal animals, viz. : cattle, horses, monkeys and rabbits (25 from each animal) have been collected with a view to study the presence of non-specific inhibitors against polio virus types I, II and III — the virulent as well as the attenuated strains of each type.

So far, 25 cattle sera and 7 horse sera have been tested using 10 per cent and 3 per cent serum concentration against 10 TCD₅₀ of each poliovirus type.

Of the 7 horse sera tested, none showed the presence of an inhibitor against any of the virus type studied.

On the other hand, 22/25 (88 per cent) cattle sera showed the presence of inhibitor against one virus type or the other. The inhibition thus detected was found to be thermostable at 56°C. In general, more inhibitors were detected at 34°C than at 37°C.

Analysing the results of sera incubated at 34°C the following observations are noted :—

(a) 11/25 (44 per cent) sera had inhibitors against all six virus types.

(b) 3/25 (12 per cent) sera did not have inhibitor against any of the virus type.

(c) The remaining sera (44 per cent) had inhibitors against one polio virus type or the other.

(d) No appreciable difference was observed between the virulent and the attenuated strains as far as the presence of inhibitors is concerned.

The work is still in progress.

2. *Study of In-vitro Markers on Polio Viruses.*—Using four genetic markers, poliovirus strains isolated from clinical cases of poliomyelitis, their contacts, healthy carriers and children vaccinated with live attenuated polio vaccine, were studied. The objective of this study was to assess the difference in the genetic character of the polio viruses circulating in the community. The markers selected for this study were :

- (a) temperature marker.
- (b) bicarbonate marker.
- (c) aluminium marker and
- (d) intra-typic serodifferentiation test.

So far, 152 strains have been studied. Results obtained so far indicate that the strains circulating in the community have varied genetic makeup.

This study will be completed by the end of 1966.

3. *Studies on Sewage Samples.*—From November 1965, a preliminary study was undertaken to test sewage samples for the presence of enteroviruses. The main purpose of the study is to standardize the technique for isolation of enteroviruses from sewage and to study the role of sewage in transmission of enteric viruses. Sewage samples were collected within the Haffkine Institute compound covering two different socio-economic status of the residents.

Todate, 64 samples are collected, of which 60 have been processed and tested for the presence of viruses in monkey kidney tissue cultures (MKTC) and in infant mice. Sixteen agents producing CPE in MKTC have been isolated which turned out to be non-polio when neutralization that (NT) was carried out with polio antisera types I, II and III. Those agents which also produced paralysis in infant mice were put up for NT and MKTC using Cocksackie A9 and B1 to B6 antisera. The typing results obtained so far are :—

- Cocksackie B2 : 1
- Cocksackie B3 : 4
- Cocksackie B4 : 2

The work is still in progress.

4. *Aetiology of Aseptic Meningitis.*—The work on aseptic meningitis was undertaken from October 1965 with a view to establish its viral aetiology. CSF samples, rectal swabs and throat swabs were collected from suspected cases of aseptic meningitis. So far, samples have been collected from 29 clinical cases of aseptic meningitis. Eight samples from 6 patients were found to be positive in monkey kidney tissue

cultures (4 from CSF samples, 3 from throat swabs and 1 from rectal swabs — CSF and throat swabs were both positive in two patients). In addition, one throat swab produced paralysis in infant mice. This sample was also positive in MKTC.

Identification of these agents is yet to be done. The work is in progress.

5. *Study of Extraneous Viruses in Monkey Kidney Tissue Cultures.*—Kidney cultures from 75 Rhesus Monkeys were studied for the detection of extraneous agents. The culture samples were tested for the presence of the following extraneous agents :—

- (a) Simian agents including SV40 virus and the foamy agent.
- (b) B Virus.
- (c) LCM Virus.
- (d) Hæmadsorption virus.
- (e) Measles virus and
- (f) Coxsackie Virus.

The samples were tested in the following primary cell cultures.

- (a) MKTC to detect presence of simian and hæmadsorption agents.
- (b) Rabbit kidney tissue culture (RKTC) to detect measles viruses.

The animal tests were carried out in the following animals :—

- (a) Rabbits to detect B viruses.
- (b) Guineapigs to detect LCM viruses and Mycobacterium tuberculosis.
- (c) Adult mice to detect LCM viruses.
- (d) Infant mice to detect Coxsackie viruses.

Of the 75 MKTC specimens tested, 9 showed the presence of hæmadsorption viruses and 25 showed the presence of foamy agents.

6. *Testing of Oral Polio Vaccine.*—To standardize the vaccine testing procedure, stock of Sabin's type I Vaccine (Strain LSc 2ab) was prepared in a Roux Bottle. The seed virus was inoculated in MKTC grown in a Roux bottle and when maximum cytopathogenic effect was observed (on the 4th day) the bottle was kept in a deep-freeze cabinet for freezing the virus fluid. Subsequently, it was thawed, centrifuged and the supernatant fluid kept in aliquets of 5 ml. in vials. Each vial was utilised for various in-vitro and in-vivo studies as mentioned below :—

In-vitro tests :

- (i) MKTC.

- (a) To determine the titres of the virus using half log dilutions.

(b) To identify the virus using neat virus and 1:4 diluted polio antisera types I, II and III.

(c) To determine a genetic marker characteristic (rct/40 marker).

(ii) Rabbit Kidney Tissue Cultures to detect the presence of B Virus.

In-vivo tests :

These were performed in animals to detect the presence of extraneous agents (refer to point No. 5).

7. *Rabies In-vivo absorption of rabies street virus.*—The absorption of the virus was studied on wounds produced artificially on shaved rabbit skin. It was observed that the virus was detectable upto one hour of its application on the wounds. There was no apparent thermal inactivation of this virus for three hours of incubation at 37°C.

8. *Studies on Pox-virus Cultivation and Adaptation of Pox-viruses to Tissue Culture.*—Two strains belonging to pox group of viruses, vaccinia (Belgaum Strain) and variola (locally isolated strain) in particular have successfully undergone eight serial passages in hamster kidney tissue cultures.

9. *Replication of Pox Viruses in Tissue Culture.*—From the studies on the behaviour of these viruses in hamster kidney tissue cultures at 37°C the following conclusions could be drawn :—

(a) The latent phase of vaccinia strain was observed to be of 7 hours while that of the variola strain was of 18 hours.

(b) The absorbed virus could be detected upto 4 hours in case of vaccinia strain and upto 6 hours in case of variola strain.

(c) The cell associated virus appeared after 8 hours with vaccinia strain and after 21 hours with variola strain.

(d) The release of the virus in the medium took place after 10 hours in case of vaccinia and 24 hours in case of variola strain.

(e) The maximum virus yield was reached after 96 hours with vaccinia strain and after 144 hours with variola strain.

10. *Vaccinia.*—Neutralization test in eggs was performed on 45 paired serum samples of vaccinated human volunteers.

Hæmagglutination test and complement fixation test were performed on 102 paired serum samples of vaccinated human volunteers.

In case of revaccinated individuals, usually immune or no response was observed and antibodies response as determined by above tests showed no rise in titre in both pre and post immunization samples.

Primary vaccination in adults resulted in good dermal reaction and substantial rise in antibody level was detected in post-immunization serum samples as compared to pre-immunization sample which showed non-significant antibody level.

The standardization of a test to assess antibodies by plaque formation and inhibition in primary monkey kidney tissue culture is progressing.

11. *Kyasanur Forest Disease*.—The scheme formally came to an end in March, 1965. Some remaining work, however, was continued on volunteer studies and preparation of seven more vaccine batches for use of Virus Research Centre, Poona. Other work done was as follows :—

Vaccine Potency Tests	..	..	..	10
Neutralisation Tests on Sera of Volunteers	..		..	18
HA-I Tests	..	..	..	5

Preparation of virus stocks and hyperimmune sera was also undertaken.

PART III
ROUTINE

ANALYTICAL AND CONTROL DEPARTMENT

The department continued to work mainly in the two rooms allotted to it which is found to be wholly inadequate for its increasing activities.

Dr. M. K. Habbu took over as Assistant Director I/c of the Department from 12th April 1965.

In addition to the work undertaken in the previous years, the department took over almost completely the testing of vitamin preparations, Sulpha Tablets and other drugs manufactured by the Department of Chemotherapy. Testing of plasma and plasma fractions has also been undertaken during the year. Methods for the estimation of preservatives in sera and vaccines were standardised and estimation of preservatives in these products is being followed as a routine. It may now be said that the first stage in the activities of the department, viz. the standardisation and control of finished products, is almost complete. The control of reagents and raw materials forms the next stage in the activities of the department. Unfortunately, the department cannot embark upon this activity due to acute shortage of space. The construction of the building where space was to be provided for the department could not be undertaken due to emergency conditions in the country. The department will take up the work of control of reagents and raw materials as soon as more space is made available.

The following table gives the number of different tests carried out during the year:—

Name of the test	Name of product	No. of sample tested
1. Sterility	(i) Bacterial Vaccines	11,783
	(ii) Antirabic Vaccine	9,486
	(iii) Parenteral Bulk Solution.	4,781
	(iv) Antitoxins, Toxoids etc.	13,175
	(v) Miscellaneous	615
2. Pyrogens	Parenteral Bulk Solutions	1,045
	Plasma and Plasma Fractions	
	etc.	

Name of the test	Name of product	No. of sample tested	
3. Chemical and Physical Examination.	Parenteral Bulk Solutions	..	1,028
	Vitamin Preparations	..	128
	Sulpha Drugs	..	160
	Miscellaneous other drugs	..	44
4. Toxicity tests	Sera and Vaccines	..	775
5. Absence of Infective Virus.	Antirabic Vaccine	..	342
6. Potency	Antitoxins	..	185
	Antivenine	..	17
	Toxoids	..	13
7. Formaldehyde Content.	In Vaccines and Toxoids	..	175
8. Estimation of Preservatives.	In Vaccines and Sera	..	334

DEPARTMENT OF BACTERIOLOGY

During the year under review, Dr. M. K. Habbu left the department to take charge of the Analytical and Control Department as Assistant Director. Dr. R. B. M. Colah was appointed in this place as Sr. Scientific Officer, Class I. Shri G. C. Munshi, Sr. Technical Assistant, left the department to improve his prospects elsewhere; Shri N. V. Wanjara joined the department as Senior Technical Assistant. Training in Bacteriological Technology was afforded to Dr. Chandrashekhar and Shri F. M. Poonawala from Alembic Chemicals, Baroda. In addition, training of 12 trainees for D.M.L.T. Course and 8 trainees for L.T.T. Course was undertaken.

The routine activity of the production of different vaccines continued as before. The production of Agar-grown Cholera Vaccine was implemented on a large scale.

During the year under review, the production of quadrivalent Cholera El Tor Vaccine was taken up on hand. At the request of the Director of Public Health, Maharashtra State, a special El Tor Vaccine was prepared for experimental field trials.

Experiments were conducted to explore the possibility of production of Acetone-dried T.A.B. Vaccine. This problem has attracted the attention of the W.H.O. and the Haffkine Institute is collaborating in this work.

As in the previous year, West Bengal, Madras and Andhra Pradesh were mainly supplied with Plague Vaccine. The demands for Cholera Vaccine were received from Maharashtra, Gujarat, Mysore, Andhra Pradesh and Kerala. The supply of T.A.B. Vaccine was mainly restricted to the Maharashtra State.

The quantities of different vaccines produced and supplied are given in the following table :—

Quantity produced

Plague Vaccine	..	..	98,520 ml.
Cholera Vaccine	..	..	13,892,402 ml.
T. A. B. Vaccine	..	..	256,919 ml.

MEDIA SECTION

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

The manufacture of parenteral solutions also continued in full swing.

In addition to the above, the department provided general facilities for sterilization, cleaning of glasswares etc. The ampoule section supplied cut and sterilized ampoules to Department of Bacteriology, Immunology and Antirabic Department. The production figures of the different items are presented below :—

Production of Culture Media

Product			Quantity manufactured.	
			Litres	
	Plague	..	695·00	
1. Casein Hydrolysate	Cholera	..	19897·5	
2. Meat Digest Agar (Typhoid)		..	510·00	
3. Agar Medium for Cholera Research		..	114·8	
4. Sterility Medium	Anaerobic	..	102697)	Bottles of 80 ml. each.
	Aerobic	..	101832)	

Production of Parenteral Solutions

				Litres
1. Glucose Saline	..	..	..	49,371·00
2. 5 per cent. Glucose Solution	..	..	..	48,834·5
3. Normal Saline	..	..	..	21,550·00
4. 25 per cent. Glucose Solution	..	..	..	1,635·5
5. A. C. D. Solution	..	..	..	2,406·5
6. Hypertonic Saline	..	..	..	1,133·5
7. Distilled Water	..	..	..	8,213·5
8. Sodium Lactate	..	..	..	2,105·00

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

Media and Stains and Solutions

Product			Amount manufactured in litres	
1. Peptone Water	..	..	..	207·1
2. Standard Broth	..	..	..	310·9
3. Sterility Media (Acid Broth)	..	..	..	13·43

Product	Amount manufactured in litres		
4. Cooked meat media (Anaerobic Broth).	..	442.92	
5. Standard Agar	..	642.5	
6. Acid Agar	..	1110.00	
7. Hartley's Broth	..	3.5	
8. Mac Conkey's Medium ..	..	14.7	
9. Mac Conkey's Agar ..	..	45.6	
10. Löffler's Medium ..	..	26.0	
11. Lowenstein and Jenson's Medium	..	6.00	
12. Sabouraud's Medium	..	46.5	
13. Sabouraud's Agar	..	33.00	
14. Typhoid Agar	..	484.00	
15. Cholera Agar	..	114.8	
16. Glucose Broth	..	41.5	
17. Sugars for Biochemical Tests :—			
(a) Glucose	..	28.35	
(b) Lactose	..	35.65	
(c) Sucrose	..	25.35	
(d) Mannite	..	26.85	
(e) Maltose	..	0.95	
(f) Arabinose	..	1.15	
(g) Xylose	..	1.15	
(h) Dulcitol	..	24.25	
18. B. S. A. Medium	..	Nil	
19. Taurocholate Medium ..	..	29.40	
20. Citrate Saline	..	5.00	
21. Dubo's Medium	..	Nil	
22. Lab-lemco Broth	..	26.5	
23. Lab-lemco N. Broth ..	..	9.1	
24. Potato Agar	..	Nil	
25. D. E. C. Medium	..	11.9	

Products				Amount manufactured in litres
26.	Glucose Phosphate	..	..	7.00
27.	Normal Saline	..	..	19881.00
28.	Selenite F. Medium	..	..	16.00
29.	17 per cent. Sodium Citrate	..	..	Nil
30.	Plague Agar	..	..	11.00
31.	Koser Citrate	..	..	2.25
32.	Thioglycholate Medium	..	..	70.9

STAINS AND SOLUTIONS

1.	Benedict's Solution (Qualitative)	..	686.00
2.	Albert's Stain No. 1	..	15.00
3.	Albert's Stain No. 2	..	12.00
4.	J. S. B. Stain No. 1	..	37.00
5.	J. S. B. Stain No. 2	..	43.00
6.	Löffler's Methylene Blue	..	15.6
7.	Indole Reagent No. 1	..	00.00
8.	Indole Reagent No. 2.	..	00.00
9.	Carbol Fuchsin Ziehl Neelsen's Stain.	..	108.00
10.	Carbol Gention Violet	..	9.00
11.	Gram's Stain No. I	..	14.00
12.	Gram's Stain No. II	..	27.00
13.	Hayem's Fluid for R.B.C.	..	24.00
14.	Methylene Blue (Aqueous)	..	83.00
15.	Oxalic Acid Solution for W.B.C.	..	27.00
16.	Saffranine 0.5 per cent. (Aqueous).	..	7.00
17.	Basic fuchsin	..	14.00
18.	Carbolic Acid 5 per cent.	..	00.00

BLOOD BANK DEPARTMENT

The year under report has been one of intense activity.

A. National Emergency.—During the National Emergency, there was sudden upsurge in all the activities of the whole department. A large number of persons responded to the call for blood donation for this cause and number of voluntary donors suddenly shot up. Facilities had to be created to receive these donors both at Institute and to arrange Mobile Blood Donation Camps at various parts of the City and the State. All the blood collected by all the other Institutions in the city was also sent to this Institute for processing into dry plasma, as that was the only way of preserving such large quantities of blood. The army authorities placed an order for a large quantity of lyophilised blood plasma. The blood collected from various distant places like Ahmedabad and Aurangabad also started coming in large quantity. As the capacity of the drying plants and cold storage were limited, proper planning and phasing out of collection programme had to be arranged. All these activities placed a heavy strain of work on the department. Obviously, it was beyond the capacity of the limited staff of the department. Fortunately, the staff (of all cadres) from other departments of the Institute responded to this emergency load of work and with esprit-de-corps put the shoulder to the wheel as one man. They shouldered the responsibility equally as the members of the staff of this Department. The staff deserves congratulations for the spirit of team-work shown :—

(1) *Blood Collection Work.*—At the Institute, extra facilities had to be created to collect blood donation from large number of donors, calling at the Institute. The working hours were extended from 8-00 A.M. to 7-00 P.M. A total of 8 bleeding cots were arranged. The main entrance verandah was utilised as reception and recuperation room for want of space. Three mobile bleeding units were organised and they visited various centres in the city and the State and collected blood and transported it back to the Institute for processing. In all, 74 Mobile Sessions were held.

(2) *Training of medical and paramedical personnel.*—Many State Governments and Institutes deputed their medical men and paramedical personnel for training in blood bank methodology. They were given training facilities.

(3) *Blood Group Reagent.*—There was unprecedented demand for blood grouping sera from all over the country and almost all the

demands were met. These sera could be obtained by iso-immunising a large number of donors and by utilising some of the voluntary donor sources.

B. *Staff*.—Dr. K. V. Kulkarni was deputed to U.S.S.R. under Indo-Soviet Cultural Exchange Programme to study the Blood Transfusion Service in that country. He was away from 21st December 1965 to 6th January 1966. Dr. S. S. Sabnis, Senior Scientific Officer, Immunology Department, was in charge of the department during this period.

C. *Production Activities*.—The following statement shows the details of the work carried out:—

I. PLASMA PRODUCTION

1. Number of Blood Donors—

1. Voluntary	..	..	10,557
2. From Prisons	..	..	3,924
3. On payment	..	..	1,877
4. From hospitals	..	..	15,688

Total	..		32,046
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2. Number of places visited by the Institute Mobile Blood Collecting Unit for Voluntary Blood Collection. 74

3. Plasma prepared—

Haffkine Institute	..	..	433·750 litres.
Defence	..	..	1590·750 litres.

Total	..		2024·500 litres.
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4. Plasma supplied—

(i) Defence supply	..	..	1354·750 litres.
(ii) Other parties	..	..	570·000 litres.

Total	..		1924·750 litres.
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5. Ampoules of antivenin dried .. 28,822 ampoules.

II. BLOOD GROUP REAGENT PRODUCTION

6. *Blood Grouping Sera prepared :*

Anti-A	..	..	24546 ml.
Anti-B	..	..	25761 ml.
Anti-A plus B	..	..	612 ml.
Coomb's Reagent	..	..	160 ml.
Bovine albumin	..	..	511 ml.
Total			51590 ml.

7. Rh tests done (All the voluntary blood donors were tested for Rh. and the results communicated to them ..

$$10,557 + 371 = 10,928$$

8. *Plasma Fractionation Unit :*

*No. of batches of plasma taken for fractionation	..	..	17
*Quantity of plasma processed	..		1364 litres.
Ampoules of gamma globulin produced	..	..	1100 2 ml.
Bottles of Pasteurised Plasma	..		1000 1 ml.
Protein Solution produced	..		169 × 250 ml.
Bottles of Albumin produced	..		35 × 100 ml.

*Some of the batches of plasma are partially processed so far. They will be processed further later.

DEPARTMENT OF CHEMOTHERAPY

GENERAL

As in the previous years, all the activities of the department showed good progress during the year. The research problems taken in hand in the field of antimicrobial agents, synthetic diuretic, psychotropic substances, cytotoxic agents etc., were pursued. It was possible to extend the activities of the Drug Screening Units to the screening of antiviral substances by the appointment of Scholar. There was slight fall in the routine production due to emergency but some staff was switched over during this period to the organisation of facilities for blood collection and processing of plasma by collaboration with the Blood bank of the Institute and the facilities of the department were also fully utilised for this purpose.

During the period under review, three trainees received advanced training in the department and eleven postgraduate students including scholars worked on various research problems. Three theses were submitted and accepted for the postgraduate degrees of M.Sc. from the department. Seven research papers were sent for publication. Two C. S. I. R. units were sanctioned in the department, one for synthesis of diuretics and another for the studies of antimicrobial substances.

ROUTINE AND PRODUCTION

Micro-analytical Section.—Micro-analytical section carried out micro analysis of 218 compounds for carbon and hydrogen and of 823 compounds for nitrogen from among the substances synthesised in the department during the year.

*Statement showing the products manufactured during
the period April 1965 to March 1966*

Serial No.	Name of the product.	No. of tablets manufactured.
1.	Vitamin AD tablets (pediatric).	1,23,79,200
2.	Vitamin BC tablets ..	3,08,55,600
3.	Vitamin B1 tablets ..	27,63,240
4.	Vitamin B2 tablets ..	4,16,708

Serial No.	Name of the product.	No. of tablets manufactured.
5.	Vitamin C tablets	65,85,300
6.	Vitamin K tablets	2,56,100
7.	Acid Nicotinic tablets	10,00,298
8.	Vitamin BC No. 3 tablets	8,34,415
9.	Multivitamin Tablets.	2,62,21,562
10.	Vitamin A forte tablets	1,51,000
11.	Vitamin AD tablets (Coated).	16,84,314
12.	Magnesium Trisilicate Tablets	8,71,600
13.	Nicotinamide Tablets	24,000
14.	Calcium with vitamin D tablets	17,46,980
15.	Tolbutamide tablets	8,89,180
16.	Sulphathiazole tablets	55,66,500
17.	Sulphadiazine tablets	1,23,73,500
18.	Sulphaguanidine tablets	82,36,000
19.	Quinine Sulphate Tablets	2,96,300
Total ..		11,31,51,797
20.	Pemon	26,879 lit.
21.	Solapsone Strong, B.P.C. Solution	378 × 50 cc.
22.	Sulpha Cream	168 pounds.

Analytical Section.—The Analytical Section of the Department analysed 20 samples of raw materials and finished products during the period.

DEPARTMENT OF CLINICAL PATHOLOGY

GENERAL

Short-term training courses were continued during the period. Twelve students were admitted for D. M. L. T. Course.

ROUTINE

The department continued to render diagnostic facilities to hospitals and the public as before. Following tests were performed :—

Tests performed				Total
Routine Examination :—				
1. Smear for T. B.	..	..	..	87
2. Culture for T. B.	..	..	..	146
3. G. Pig inoculation for T. B.	..	..	..	53
4. Differential W. B. C.	..	..	..	480
5. Total B. C.	..	..	..	470
6. Haemoglobin	..	..	..	356
7. Total R. B. C.	..	..	..	354
8. Blood Culture for Typhoid	..	..	..	1,133
9. Urine Culture	..	..	..	233
10. Blood Culture for others	..	..	..	82
11. Stool Culture	..	..	..	44
12. C. S. F. Culture	..	..	..	3
13. Miscellaneous Culture	..	..	..	152
14. Autovaccine	..	..	..	64
15. Diphtheriae	..	..	..	299
16. Wassermann Test	..	..	..	166
17. Kahn Test	..	..	..	603
18. V. D. R. L. Test	..	..	..	427
19. Stool for Cholera	..	..	..	30
20. Malarial Parasites	..	..	..	14
21. Prothrombin time	..	..	..	14
22. Throat Swab for Culture	..	..	..	40
23. Filaria	..	..	..	7

Tests performed			Total
24. Antibiotic Sensitivity	..	..	237
25. C. S. F. Cell Count	..	..	39
26. Ascitic and other body fluids	..	..	51
27. Cold Agglutination Test	..	..	Nil
28. Widal and Cultural Tests for Typhoid	..	..	1,216
29. Stool for Routine	..	..	252
30. Urine for Routine	..	..	358
31. Smears for G. C.	..	..	3
32. Semen Examination	..	..	13
33. Pregnancy Tests	..	..	2,848
34. Rideal Walker Co-efficient Test	..	..	145

<i>Tests performed.</i>	<i>Total No. of cases.</i>
Histopathological Tests ..	4,023

Some of the interesting specimens received during the year were as follows :—

1. Epidermoid carcinoma of cervix.
2. Fibrosarcoma of axillary gland.
3. Papillary adenocarcinoma of the ovary.
4. Adenocarcinoma of breast.
5. Vesicular mole.
6. Lymphosarcoma of intestine.
7. Undeveloped testes.
8. Elephantiasis of vulva.

Production of Diagnostic Reagents		Quantity
Killed suspensions for Widal Tests	..	3,30,245 ml.
High-titre sera and amboceptor	..	2,914 ml.
Kahn Antigen.	..	15,960 ml.
Wassermann Antigen	..	2,810 ml.
Russell's Viper and C. V. Solution	..	1,102 ml.
Casoni's Antigen	..	478 ml.
Biological Stains.	..	11,52,600 ml.
Cobra and Viper Venom Solutions.	..	5,672 ml.

DEPARTMENT OF ENTOMOLOGY

The Assistant Director attended the quarterly meetings for the co-ordination of plague work in three States of Andhra Pradesh, Mysor and Madras. He also attended I.S.I. meeting in connection with the transport of live animals. He toured the district of Chanda for work on snakes in connection with District Gazetteer and plague studies in Maharashtra State. The department, with the help of staff from the Director, Public Health, Government of Maharashtra, carried out studies on plague in the Vidarbha region of the Maharashtra State.

Shri G. C. Chaturvedi was promoted as Junior Scientific Officer; a new post sanctioned by the Government. Dr. (Smt.) Rajadhyaksha, C.S.I.R. Pool Officer and Kum. M. R. Sathc, I.C.M.R. Fellow, left the department in September and October 1965 respectively.

Academic Activities.—Four students continued their work for a Ph.D. degree while one more student was registered for M.Sc. degree in the department. Kun. R. R. Hingorani was trained in the Department for advanced training in entomology, as a short-term trainee. Eight other students of Technicians' Training Course were trained in the department. Kum. K. M. Karnikar continued as C.S.I.R. Scholar and Shri R. S. Prasad continued as Government of India Scholar.

Routine.—Approximately 105 inquiries were attended during the course of the year in connection with the identification of rats, insects and snakes, and also for their control. Three samples received from the Milk Colony were also examined. Specimens of insects of medical importance and slides were supplied to three institutions for educational purposes. Besides this, colonies of fleas, flies and cockroaches were maintained in the laboratory. One bait box was supplied as a sample to Director of Public Health, Madras State.

Production and Supply.—The total quantity of snake venom extracted and supplied during the year was 309.6 gms. and 189.85 gms. respectively. Seven thousand eight (7,008) snakes of different varieties were purchased for the purpose, while 12 snakes were received as gratis. Two thousand three hundred twenty-seven (2,327) scorpions were also purchased during the year. The quantity of scorpion venom extracted during the year was 3.06 gms., while 0.10 gm. of it was supplied. Besides this, 1,185 frogs were purchased for feeding the snakes.

DEPARTMENT OF IMMUNOLOGY

GENERAL

All the sera manufactured by the department were processed by the enzyme refining method from August 1965. The enzyme refined sera as a great improvement over the previous method as the rate of serum reaction diminishes and the unitage and quality of sera is improved hereby. The production of sera was adversely affected during August and September 1965 because the horses had to be given rest during preventive vaccination against South African Horse sickness disease. Since July 1965, the horses from one Bombay Stable were shifted to impri. There was progressive utilization of $2\frac{1}{2}$ stables built last year at Pimpri, though purchase of horses had to be delayed during emergency restrictions.

No new construction could be taken up during the year due to emergency. For the next year also, little has been sanctioned and thus the urgent requirements for expansion of serum production, such as the construction of quarantine stables, ordinary stables, main laboratory, housing for workers etc. has been held up. Increase in production or completion or progressive shifting of the serum production activity to impri will not be possible unless the construction of these is taken up. There is increased turnover and shortage of trained manpower, owing to opportunities elsewhere.

The work carried out in the department on the role of phage and oxinogenic factor in converting non-toxinogenic strains into toxin producing strains has been published and has received recognition as work of fundamental interest.

A new strain of diphtheria organism has been isolated which can produce diphtheria toxin on sucrose instead of on imported Maltose. The yield of toxin has to improve still further before it can be used for routine production.

A new method of isolating anaerobic organisms was developed. Using this method, a strain of nontoxic clostridia was isolated which was producing a bacteriocine type of antibiotic against *Clostridium tetani*. This interesting finding is being further investigated for its implications.

Production

				LITRES
1.	Tetanus Toxin—			
	(a)	Peptic digest medium		540
	(b)	Protein-free medium		680
2.	Tetanus Toxoid—			
	(a)	Peptic digest medium		170
	(b)	Protein-free medium		580
3.	Diphtheria Toxin—			
	(a)	Papain digest medium		196
	(b)	Protein-free medium		90
4.	Diphtheria Toxoid—			
	(a)	Papain digest medium		214
	(b)	Protein-free medium		96
5.	Gas Gangrene (Welchii) toxin			142
6.	Pertussis Vaccine			30
7.	Triple antigen			22
8.	Tetanus antisera			422
9.	Enzyme digested Tetanus Antisera			343·7
10.	Diphtheria antisera Enzyme digested			63·3
11.	(a)	Gas Gangrene Antisera		72·5
	(b)	Enzyme digested Gas Gangrene Antisera		78·6
12.	(a)	Antivenin		112·5
	(b)	Enzyme digested Antivenin		219·8

DEPARTMENT OF NUTRITION AND BIOCHEMISTRY

A. NUTRITION

Dr. V. N. Jai, Senior Scientific Officer (I) in the department has been deputed for 13 months' Diploma Course in Nutrition in London and Ibadan under an International Fellowship. Dr. S. D. Ambegaonkar has been promoted temporarily as Senior Scientific Officer (I). Dr. S. D. Ambegaonkar attended a course in Nuclear and Radiation Chemistry for 6 weeks organised by the Atomic Energy Establishment. Kum. M. S. Kotnis, Senior Lady Nutritionist, was appointed as Factory Canteen Adviser (M.M.S. II) in the Department. Dr. (Kum.) S. Arora, Senior Lady Nutritionist of the Department, was deputed to serve with the F.A.O. of the United Nations, Rome, as a Nutrition Consultant, in food consumption and planning branch, Nutrition Division, for four months.

During the year, one of the Senior Lady Nutritionists of the Department, resigned and two Senior Lady Nutritionists—one for the Regional Nutrition Unit, Poona, and one Junior Lady Nutritionist were appointed.

The training of the various functionaries under the Applied Nutrition Programme of the Maharashtra State was started during the year at the Public Health Institute, Nagpur.

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.

Routine.—193 tests were carried out on 48 samples which included 27 assays for vitamins.

Field Studies—Diet-cum-nutrition surveys.—The diet and nutrition survey carried out in the rural areas under the Applied Nutrition Programme was completed. The data was analysed and the detailed report has been submitted to Government.

Hospital Dietetics—(a) Hospital visits.—Routine visits to Government Hospitals for surprise-checks were continued.

(b) *Therapeutic Diets.*—A standard diet scale for peptic ulcer patients was formulated and experimented in one of the local hospitals earlier proved effective and was recommended for implementation in Government Hospitals in the State. Similarly, a diet scale for acute nephritis was worked out.

Social Dietetics.—Study of dietary conditions in residential institutions, industrial and Square Meal Canteens, Secretariat, was continued.

Nutrition Education.—Nutrition education programme, such as lectures, display of posters and charts, cooking demonstrations, screening of films etc. was continued and were further developed as a major activity of the Department.

Human Feeding Trial with unfamiliar foods.—This department has also been interested in carrying out human feeding trials with unfamiliar foods. During the year, many recipes from banana root (banana rhizome flour) and leaf proteins were tried and acceptability trials were carried out.

Formulation of recipes from subsidiary foods.—In view of the acute shortage of staple cereals, wheat and rice, the department undertook work on formulating new recipes from non-cereal subsidiary foods. The recipes from subsidiary foods were demonstrated to various organisations with a view to educate the public, particularly house-wives, in the use of these foods for their daily menu with the object of supplementing the rationed cereals.

Applied Nutrition Programme—Training of the functionaries.—The training course for the different categories of functionaries — instructors in gramsevak and non-gramsevak training centres, Development Officers for horticulture, fisheries, principals of training institutions and Regional Lady Project Officers, etc. was commenced at the Public Health Institute, Nagpur.

In addition to the tutorials on the subject of nutrition, practical cooking demonstrations and screening of films on nutrition were also arranged for all the batches.

The training of functionaries at gram sevak training centres was also carried out. They include gramsevaks, gramsevikas and representatives of local mahila mandals.

Consultative Service.—The department continued to render help in an advisory capacity to Y.W.C.A., S.N.D.T. University, K.E.M. Hospital and the like. Technical advice continued to be given (by correspondence) to institutions and individuals regarding their queries on nutritional merits of foods, problems in practical dietetics, etc.

B. BIOCHEMISTRY

General.—The laboratories continued to give effective services to the hospitals and attended to some special work whenever necessary. More or less only special tests are referred to the department.

Shri A. R. Advaney was allowed to proceed on study leave for one year to U.S.A. to finish a course in Medical Technology. Kum. Shantha Nair was selected to undergo one year's advanced training at the Institute of Microbiology, Prague, Czechoslovakia. Dr. (Kum.) D. H. Shah joined the department as a Pool Officer from September 1965 — January 1966. She was for some time spared to do collaborative work at the Tata Institute of Fundamental Research.

Kum. S. Nair, Shri M. J. Modak and Kum. M. Advaney have obtained their M.Sc. degree of the Bombay University in Biochemistry.

The department took the additional work of training 5 students for the D.M.L.T. Course of the Bombay University.

Routine.—The total number of tests on clinical materials carried out during the period under review was 3,238 as shown below :—

Blood	..	..	..	..	..	2,300
Urine and stool	..	..	..	..	..	190
C.S.F. and body fluids	..	..	..	..	..	103
Special Tests	..	..	..	..	..	645

DEPARTMENT OF PHARMACOLOGY

GENERAL

The Department's activities, during the past years' have centered round its efforts towards fundamental and applied researches on drugs, testing of drugs with the view to eliminate substandard or spurious drugs from the market, training of a band of scientific workers in research and technological jobs and providing facilities for post-graduate studies. Similar efforts were continued during this year.

Dr. N. K. Dutta, Head of the Department, was selected for the G. J. Watumall award for 1965, by the Watumall Foundation, Honolulu, U.S.A., for his distinguished contributions in the field of medicine. Shri K. G. Anantanarayanan, M.Sc., rejoined the Department. Shri K. M. Mehta, M.Sc., joined as Junior Scientific Officer and Assistant Chemical Examiner. The Research Section was strengthened by the appointments of Shri M. Y. Mhasalkar, M.Sc., Kumari P. H. Marker, M.Sc., Shri A. N. Dohadwala, M.Sc., Kumari Dr. Amita Mukerjee, M.B.B.S., Kumari R. P. Motwani, M.Sc., Smt. Usha Kashyap, M.Sc. Several experienced workers left the department. They were : Shri K. V. Bhide, M.Sc., Smt. M. M. Lotlikar, M.Sc. (Tech.), Shri V. P. Kulkarni, B.Sc., Dr. R. B. M. Colah, M.Sc., Ph.D., and Shri A. D. Seth, M.Sc.

Besides the eight graduates who attended the Technician Training Course, Shri C. S. Joshi, B.Sc., had spent several months in the Department as a trainee. Shri V. J. Thomas, B.Sc., B. Pharm., Bacteriologist, Drugs Control Administration, Kerala State, joined as a trainee.

ANALYTICAL SERVICES

Routine activities.—This Department has specialised in the fields of analyses of biological and chemical products. The samples were received from various sources, namely Drugs Control Administration, Government of Maharashtra, Department of Prohibition and Excise and Police, Government Hospitals of Maharashtra State, Pharmaceutical Firms, etc. A variety of samples that were received belonged to pharmacopoeia and non-pharmacopoeial drugs, which required pharmacological chemical, biochemical, pharmacognostic, microbiological and serologica testing depending upon the nature of samples. The testing of sample from Drugs Control Administration, Government of Gujarat, was discontinued from 1st April, 1965. During the time of hostilities, priori was given to testing of those drugs meant for our Armed Personnel. During the year, 3,817 samples were tested, out of which 14.2 pe

cent were found to be of non-standard quality. The drugs analysed under the Drugs and Cosmetics Act were 1942. About 12 per cent of these were found not to conform to the required specifications. The samples that were examined for Police and Prohibition and Excise Departments amounted to 780. These were mainly spirituous preparations and some of them were claimed to be *Allopathic* preparations. There was a notable increase in the testing of samples from commercial pharmaceutical firms, the total number being 539, as against 381 during the year 1964-65.

GENERAL COMMENTS

Biological Section.—Owing to the improvement in the supply of laboratory animals, 279 drugs could be tested for toxicity and 29 samples of insulin. All the insulin samples were found to be of good quality. The presence of depressor substances in the liver extracts were carried out in 92 preparations, out of which, one sample did not comply with the test. One hundred and seventy-nine samples were tested for pyrogens but 16 of them did not satisfy the test.

Chemical Section.—This section dealt with a large number of samples of varied nature. Those were fine chemicals, preparations containing fixed oils, surgical dressings, insecticides, disinfectants, unlabelled compounds etc. Several interesting observations were made. Some ointments claimed to be *Ayurvedic* preparations were found to contain drugs like boric acid, zinc oxide, sulphur or sulphathiazole. A few *Ayurvedic* preparations claimed to alleviate cough and cold, contained eucalyptus oil. There was an example of a wrong dispensing. Calcium sulphate was dispensed instead of calcium hypophosphate. Whitening agents are not permitted to be used in surgical dressings, but 50 per cent of the samples gave fluorescence test. Besides these, several samples did not comply with the test for absorbancy. It was observed that some manufacturers of surgical dressings marketed gauze made of bandage cloth according to their own specifications for warp and weft instead of keeping the material according to the official standards. Official standards are more rigid.

The disinfectants that were examined were generally labelled as "Phenyl", "Sanitary Fluid", "Cleaners", "Essence of Phenyle". Out of 36 samples of disinfectants, in 35 samples the phenol coefficient was less than 5.

Biochemical Section.—Four hundred and fifteen antibiotic preparations were examined microbiologically. Besides injectable preparations there

were ointments, ear drops, lozenges, oral suspensions and capsules. Only 2 per cent of these samples were of substandard quality. Among the new antibiotics that were tested, were Hamycin, Nystatin and Erythromycin. The problem of standardisation of oral multivitamin preparations, containing vitamin A, ascorbic acid, thiamine salt, and folic acid remained as critical as before. Generally speaking, a good number of these oral preparations were found not to conform to the claim made. The stability of some of the preparations containing enzymes needs careful investigation by manufacturers. For example, 23 per cent of pepsin preparations failed to pass the requisite standard. A few samples were examined for diastatic activity but none of them was found to sustain the claim. Twenty-three samples of vaccines and 49 samples of antitoxin and sera were analysed. All the antitoxin samples conformed to the claim made. The methods of assay of calciferol (Vitamin D₂) and vitamin E₁ were standardised. A collaborative study for the assay of chlortetracyclin (to be established as a national reference standard) was carried out.

DEPARTMENT OF VIROLOGY

GENERAL

Construction work on the new polio building is in progress. Phase I consisting of testing laboratories and epidemiology unit is likely to be ready for occupation by July 1966.

Dr. Nanavati, Assistant Director, proceeded to U.S.A. on a study leave for one year from April 1965. Dr. K. H. Dave held the charge.

Work of sheep inoculation, housing of inoculated sheep and slaughters has been shifted to the newly constructed sheep pen.

The K.F.D. Inquiry was closed down on completion of work. The K.F.D. Block was given to I.C.M.R. Polio Research Unit for starting vaccinia viral control laboratories as desired by Central Government.

Government of Maharashtra have sanctioned some staff for starting separate Polio Department, but owing to staff, equipment and building position, the polio department could not get separate existence as a unit during the year.

ROUTINE

Poliomyelitis in Ceylon.—During the period under report, four batches of stool samples from clinical cases of polio-myelitis in Ceylon were referred to this Institute for virological studies.

One hundred and fifty-eight fecal samples were received and 140 have been tested in monkey tissue cultures. Of these 25 were identified as type—I Polio virus and 8 as type—III polio virus. Three were found to be non-polio viruses. Results of typing of 5 strains are awaited. Eighteen samples are awaiting inoculation.

Influenza.—Twenty-five throat gargles and swabs were collected from suspected influenza cases which, after processing, were inoculated and passaged into eggs. No virus agent was isolated.

PRODUCTION

1. *Processing of Oral Polio Vaccine.*—During the course of the year under report, 3,71,700 doses of oral polio vaccine were processed from the imported concentrated vaccine at the Haffkine Institute.

Out of this, 3,60,460 doses of the vaccine were distributed during this year; 97,060 doses of the vaccine were distributed at the polio vaccine

centre at the Haffkine Institute ; 82,600 doses were supplied to the Municipality and 1,80,800 doses to other agencies.

2. *Production of Rabies Vaccine—*

Antirabic Vaccine.		Production.
1. Semple's Vaccine	..	40,15,162 ml.
2. Canine Vaccine	..	37,170 ml. (6,1
3. Number of sheep used	..	3,312 sheep.
4. Number of centres	..	351 (4 new

The supplies to Antirabic Treatment Centres in G continued during the year. The Antirabic Treatment Centres have been requested to keep a minimum balance of 2 months' stock of indenting fresh vaccine.

ANIMAL FARM

Following last year's Salmonella infection in one colony of mice, this section had a heavy task of raising the disease-free stock of mice and reduce the suspected diseased colony without affecting the day-to-day supply of mice to the departments.

This year, main supply of mice was from Swiss Strain (Kasauli). The strain was bred with concentrated efforts. The improved method of mice breeding over the past method used in this animal house proved to be economic from the cost point of view as well as for maintenance of requisite supply of mice to the using departments according to their needs. The strength of breeding colony was maintained at about 20,000 against 36,000 in past while the issue to the departments has increased by about 20 per cent over that of previous year.

Inbred strain of mice from NIH strain susceptible to cholera and plague is being bred. The inbreeding will continue in future. Experiments in caesarian delivery technique are initiated.

Guineapig colony has increased twofolds and all the demands even in large numbers were met without purchasing Guineapigs from other breeding centres.

Training.—The Technician Trainees and D.M.L.T. Course students were trained in the maintenance and handling of Laboratory Animals. Following persons were trained in all the aspects of the Animal House work during this year :—

1. Shri T. K. Senguptha .. Indian Institute of Biochemistry
and Experimental Medicine,
Calcutta.
2. Shri V. M. George .. S.P. Medical College, Bikaner.
3. Shri Ram Prakash Singh .. Patiala Medical College.
4. Shri Ranjit Singh .. Medical College, Rohtak, Punjab.

Total number of laboratory animals bred during the year 1965-66 in comparison with that of the year 1964-65 is given in the following table :—

Species.	No. of Animals produced during 1965-66	No. of Animals produced during 1964-65
White Mice	.. 1,62,840	1,36,909
White Rats	.. 11,390	9,372
Rabbits	.. 3,544	3,023
Guineapigs	.. 4,391	3,458
Hamsters	.. 1,189	1,043
Cotton Rats	.. 71	90

Besides these animals, following species of animals were maintained in this Animal House and issued to departments :—

Sheep	..	.. 3,261
Fowls	..	.. 26 average per month.
Monkeys	..	.. 27 average per month.
Cats	..	.. 10 average per month.
Horses	..	.. 100 average per month (besides Pimpri Stables).

MAINTENANCE ENGINEERING SECTION

This year, both the Desivac and A.E.C. Plant were constantly used. This Section is also maintaining cold rooms, centrifuge machine, deep-freeze, ovens, vacuum pumps, air compressors, air conditioners, refrigerators, etc.

The carpenter shop continued to prepare the varieties of crates and small requirements for all the departments. Plants and machinery which have been imported and purchased locally were tested, installed and put to commission.

Some new arrangements like Annual Contract for rewinding work, airconditioning work, have been started. Preventive maintenance work is initiated by staggering the weekly holidays for the staff in place of Sundays.

LIBRARY SECTION

The Library of this Institute caters to the needs of our research and research workers of the other scientific and technical institutions. It also participated in the Inter-Library Loan System, which is in vogue in other neighbouring libraries. It participates in exchange programme under American Medical Library Association, British Medical Library Association and UNESCO.

The following statistics speak of the work attended to by the library during the year under report :—

A. *Readers—*

- | | |
|---|-------|
| (1) Number of visitors in the Library for reference only .. | 2,360 |
|---|-------|

B. *Loan of Books and Periodicals—*

- | | |
|--|-----|
| (1) Number of books, and periodicals issued to our staff. | 768 |
| (2) Number of books and periodicals issued under Inter-library Loan to other libraries | 500 |
| (3) Number of books and periodicals obtained for our staff from other libraries | 80 |

C. *Additions of library material during the year—*

- | | |
|--|-----|
| (1) Total number of books (Accn. Nos. 10584 to Accn. No. 10808) | 290 |
| (2) Total number of bound volumes of periodicals (Accn. Nos. 8615 to 9409) | 789 |
| (3) Total number of current periodicals subscribed .. | 109 |
| (4) Total number of current periodicals in exchange .. | 200 |
| (5) Total number of current periodicals as gratis .. | 107 |
| (6) Total number of periodicals in the current list .. | 438 |
| (7) Total number of reports of scientific institutions received in the library | 90 |
| (8) Total number of papers published | 38 |
| (9) Total number of papers under publication .. | 20 |

LIST OF RESEARCH PAPERS PUBLISHED FROM HAFFKINE INSTITUTE DURING THE YEAR 1965-66

ANALYTICAL AND QUALITY CONTROL

Habbu, M. K. and Crampitz, H. E. :

Die Pestschutzimpfung, Handbuch der Schutzimpfungen Herausgegeben von.

A. Herrlich, P. 657, 1965.

BIOCHEMISTRY AND NUTRITION

Guttikar, M. N., Shah, D. H., Adhikari, H. R., Ambegaonkar, S. D. and Radhakrishna Rao, M. V. :

Studies in Nutritive Value of Indian Foodstuffs – III :

Magnesium and copper content of some foods. Journal of Nutrition and Dietetics, Jan. 1966.

Venkataraman, A., and Nair, Shantakumari :

A Critical Study of the conditions for colorimetric estimation of Serum Transminases : Part I :

Estimation of Small amounts of Pyruvic Acid in the presence of Ketoglutarate using dinitrophenylhydrazine. Indian Journal of Biochemistry, Vol. 2, No. 3, pp. 174-178, 1965.

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

Estimation of Phenol in Vaccines. A Note in the Indian Journal of Pharmacy, Vol. 27, No. 7, pp. 200-201, 1965.

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

Colorimetric Estimation of Phenol in Rabies Vaccine. Indian Journal of Pharmacy, Vol. 127, No. 12, pp. 336-337, 1965.

Jai, V. N. :

Dietary Prevention of diabetes mellitus and Integral aspect of educating prospective diabetic. "Madhumeha," Vol. 6, No. 2, pp. 210-212, January 1966.

Arora, Shanti :

Diet and Diabetes.

"Madhumeha", Vol. 6, No. 2, pp. 141-147, Oct. 1965.

Kotnis, M. S. :

Educational Approach in Industrial Nutrition.

Indian Journal of Occupational Health, Vol. VIII, No. 4, pp. 88-91,
April 1965.

Deshmukh, Kalindi and Ganapathi, K. :

The Biochemistry of *Vibrio Cholerae* : Part IV — Acid Soluble
Nucleotides Constituents.

Indian Journal of Biochemistry, Vol. 2, No. 4, Dec. 1965,
pp. 234-236.

CHEMOTHERAPY

Kum. Bundeally, A. E., Kum. Kanga, Dolly, H., Bellare, R. A. and
Deliwala, C. V. :

Comparative Bactericidal Activities of four different preparations
on a wide range of common pathogenic micro-organisms.

Indian Journal of Medical Sciences, Vol. 19, No. 6, June 1965.

Kum. Bundeally, A. E., Kulkarni, B. S. and Bellare, R. A. :

In-vitro Antitubercular Activity of P-(4-Thiazolyl)-P-alkoxy
Thiocarbanilides.

Indian Journal of Experimental Biology, Vol. 3, No. 4, pp. 247-
248, 1965.

CLINICAL PATHOLOGY

Modi, B. G. and Sant, M. V. :

Pregnancy Diagnosis Hemagglutination Inhibition Method Com-
pared with Rat Test.

Indian Journal of Medical Sciences, Vol. 19, No. 10, October 1965,
pp. 745-749.

Kum. Iyer, L. S., Modi, B. G. and Sant, M. V. :

Studies on Bacteriology of Ice.

Journal of the Indian Medical Profession, Vol. 12, No. 8, 5513-
5515, Nov. 1965.

Kum. Gokhale, Vidya K. and Sant, M. V. :

Studies on Immunofluorescence Test for Syphilis.

Journal of Post-Graduate Medicine, Vol. 12, No. 1, Jan. 1966.

ENTOMOLOGY

Deoras, P. J. :

A Note of the Trial of Two Modern Ratticides.
Current Science, Vol. 34, No. 11, June 1965.

Deoras, P. J. :

Studies on Bombay Rats : A Modified Technique for Flea Indices.
Current Science, Vol. 34, No. 18, 20th Sept. 1965.

Deoras, P. J. :

Snakes of India, 1965.

IMMUNOLOGY

Bundeally, Amina, E. and Rao, S. S. :

Stimulation of Growth of *Bordetella pertussis* by Acetate.
Indian Journal of Experimental Biology, Vol. 4, No. 1, January 1966,
pp. 23-24.

Patel, A. A. and Rao, S. Srinivasa :

Action of Tetanus Toxin on Brain and Liver Mitochondria of
Resistant and Susceptible Animals.
Indian Journal of Biochemistry, Vol. 2, No. 2, July 1965, pp. 135.

Patel, A. A. and Rao, S. Srinivasa :

Action of Adrenaline on Tetanus Toxin.
Indian Journal of Medical Sciences, Vol. 19, No. 11, November 1965,
pp. 818-820.

Rao, S. Srinivasa, Mehta, P. R. and Cooper, S. N. :

Conversion of x-Crystallin of Bovine Lens into Insoluble Protein
in vitro.
Experimental Eye Research, 1965, vol. 4, pp. 140-147.

Sardesai, Sulabha, K. and Rao, S. Srinivasa :

Induction of Toxin and other Antigens in *Corynebacterium*
Diphtheriae by Phage.
Indian Journal of Experimental Biology, Vol. 3, No. 3, July 1965,
pp. 205.

Rajadhyaksha, A. B. and Rao, S. Srinivasa :

Induction of Toxin in *Corynebacterium Diphtheriae*.
Indian Journal of Experimental Biology, Vol. 3, No. 3, July 1965,
pp. 205.

Rajadhyaksha, A. B. and Rao, S. Srinivasa :

The Role of Phage in the Transduction of the Toxinogenic Factor in *Corynebacterium Diphtheriae*.

Journal of General Microbiology, vol. 40, 421-429, 1965.

PHARMACOLOGY

Panse, M. V. and Dohadwalla, A. N. :

Serum Protein Changes by Paper Electrophoresis in Rabbit and Human Cholera.

Indian Journal of Pathology and Bacteriology, Vol. 8, No. 2, April 1965, pp. 138-144.

Dutta, N. K. and Oza, N. B. :

A New Approach to the Treatment of Cholera based on Experimental Evidence.

British Journal of Experimental Pathology, Vol. 46, No. 4, pp. 418, August 1965.

Norris, R. T. Finkelstein, R. A., Dutta, N. K. and Sprinz, H. :

Intestinal Manifestations of Cholera in Infant Rabbits. Morphological Study.

Laboratory Investigation, Vol. 14, No. 7, 1965, pp. 1428-1436.

Madiwale, M. S., Rao, S. S. and Dutta, N. K. :

Stability of Folic Acid in Pharmaceutical Preparations.

Indian Journal of Pharmacy, Vol. 27, pp. 114, 1965.

Oza, N. B. and Dutta, N. K. :

Choleraenic Activity in Infant Rabbits of El Tor Vibrios from south-east Asia.

Proc. Cholera Res. Symposium, pp. 271, 1965.

VIROLOGY

Lopes, John, Dave, K. H. and Gharpure, P. V. :

Serological Evidence of Coxsackie and Polio Virus Infection.

Indian Journal of Medical Sciences, Vol. 119, No. 7, July 1965.

Mansharamani, H. J., Dandawate, C. N., Nanavati, A.N.D. and Jhala, H. I. :

Experimental Vaccine against Kyasanur Forest Disease (KFD)
Virus from Mouse Brain Source.

Indian Journal of Pathology and Bacteriology, Vol. 8, No. 3,
July 1965, pp. 159-177.

Dandawate, C. N., Mansharamani, H. J., and Jhala, H. I. :

Experimental Vaccine against Kyasanur Forest Disease (KFD)
Virus from Embryonated Eggs : I. Adaptation of Virus to Developing Chick Embryo and Preparation of Formolised Vaccines.

Indian Journal of Pathology and Bacteriology, Vol. 8, No. 4,
October 1965, pp. 241-260.

Dandawate, C. N., Mansharamani, H. J. and Jhala, H. I. :

Experimental Vaccine against Kyasanur Forest Disease (KFD).
Comparative Immunogenicity of Vaccines with Formalin and Beta-propio-lactone (BPL).

Indian Journal of Pathology and Bacteriology, Vol. 8, No. 4,
October 1965, pp. 261-270.

Ver, Binie A :

Adaptation of Rabies Street Virus Strains in Tissue Culture.

Indian Journal of Pathology and Bacteriology, Vol. 8, No. 4,
October 1965, pp. 271-279.

Desai, Ramesh, N., Jhala, H. I., Dave, K. H. and Gharpure, P. V. :
Identification of Non-polio Human Enteroviruses Isolated from
Healthy and Sick Children in Bombay.

Indian Journal of Pathology and Bacteriology, Vol. 8, No. 4,
October 1965, pp. 297-303.

MISCELLANEOUS

Jhala, H. I. :

Review of a book entitled "The Brilliant and Tragic Life of
M.W.M. Haffkine."

Nature, July 1965.

Jhala, H. I. :

Medical Marvels.

Journal of the Gujrat Res. Society, Vol. XXVII, No. 3/107, July 1965.

Jhala, H. I. :

Story of Small-pox Vaccine.

Bombay Hospital Journal, Vol. 7, No. 4, October 1965, pp. 24/
175-25/177.

Jhala, H. I. :

Shall we live with or outlive Poliomyelitis?

Calcutta Medical Journal, Vol. 63, No. 1, January 1966, pp. 1-12
(Sir Nilratan Sircar Memorial Oration, 1965).

PART IV
MISCELLANEOUS

CRITICS CLUB

During the year under review (1st April 1965 to 31st March 1966), 35 meetings were held under the auspices of the Critics Club, out of which 2 were addressed by Scientists from outside. At these meetings, members of the staff, scholars and research fellows of the Institute either presented original research work or reviewed topics of scientific interest. The meetings were followed by critical discussions on the subject.

The following is the complete list of the speakers and the subjects :—

1. Shri J. K. Kamath .. Microbiological evaluation of protein quality.
2. Kum. L. S. Iyer .. Observations on serological phage types and sensitivity patterns of the pathogenic staphylococci.
3. Kum. M. Sathe .. Fungii collected from *Musca domestica* nebulosa Fabr. and their possible role in biological control.
4. Shri H. K. Sevani .. Genetic markers of poliovirus strains.
5. Shri A. K. Bhat .. Antifungal drugs.
6. Shri A. B. Rajadhyaksha .. Role of phage in induction of toxin in *C. diphtheriae*.
7. Shri K. M. Thomas .. Flask combustion technique in the analysis of drugs.
8. Shri M. J. Modak .. Biochemical studies on *Salmonella* organisms.
9. Shri C. J. Karkhanis .. Fractions of Human Plasma for clinical purposes.
10. Dr. S. V. Huprikar .. Plant Hemagglutinins.
11. Shri K. N. Joshi .. Review on anonymous Mycobacteria and their present status in chemical field.

12. Shri S. M. Desai .. Immunisation against Small-pox, ancient and modern.
13. Shri S. S. Sabnis .. Synthesis of compounds for possible anti-tumour activity (preliminary screening data).
14. Shri A. A. Patel .. Mechanism of action of Tetanus Toxin.
15. A. D. Nadkarni .. Accelerated stability testing of pharmaceuticals.
16. Dr. (Kum.) S. Arora .. Effect of nutritionally balanced mid-day meal on pre-school going children.
17. Shri G. C. Chaturvedi .. Pulicidal action of certain common insecticides on Bombay fleas.
18. Kum. N. K. Ghag .. Fluorescent antibody studies in *C. diphtheriae*.
19. Dr. H. J. Mansharamani .. Vaccine against K.F.D. Virus from tissue culture sources.
20. Shrimati M. R. Patel .. Antitubercular studies on activity of *cæalpinia digyna* (Vakeri root).
21. Shri A. B. Rajadhyaksha .. Role of iron in the control of toxin production by *C. diphtheriae*.
22. Shri J. A. Fernandez .. The antisterility vitamins.
23. Dr. P. J. Deoras .. Impressions of the symposium on the husbandry of laboratory animals at Dun-Logbaine.
24. Shri M. J. Modak .. Metabolism of Glucose and Xylose by *Salmonella* organisms.
25. Kum. J. R. Joshi .. Attenuation of cultures and vaccine production.
26. Dr. K. V. Kulkarni .. Trends in blood transfusion.

- | | |
|--|--|
| 27. Shri K. K. Das | .. Serum agglutinins following a T.A.B. Inoculation. |
| 28. Shri Q. R. Miranda | .. Extraneous agents of monkey kidney tissue cultures (Rhesus). |
| 29. Shri Umarji | .. Study of manganese content in hair. |
| 30. Kum. A. E. Bundeally | .. Nutritional requirements of <i>B. pertussis</i> . |
| 31. Shri A. N. Dohadwalla | .. The antigenic interrelationship of live cholera vaccines and method of Bio-assay. |
| 32. Prof. H. R. Cama (Institute of Science, Bangalore). | Recent work on Vitamin A Metabolism. |
| 33. Dr. D. S. Murray (American Fellow, N.H.S., England). | Organisation of laboratory services. |
| 34. Kum. M. S. Kotnis | .. Applied Nutrition Programme in Maharashtra State. |
| 35. Dr. P. J. Deoras | .. Trial of certain ratticides. |

DISTINGUISHED VISITORS

1. Dr. P. V. Cherian, Governor of Maharashtra State.
2. Smt. Tara Cherian.
3. Prof. R. H. S. Thompson, Guy's Hospital Medical School, London, England.
4. Dr. A. R. Hakimi, M.D., Director-General of Public Health Services in Afghanistan, Kabul.
5. Dr. Hashmulullah, Assistant Director of Health Services in Afghanistan.
6. Dr. Abdul Satar Seraj, Director of General Medical Legislation and Director of Foreign Relation in the Health Ministry, Afghanistan.
7. Dr. S. Murtaza Said, Director, General Communicable Diseases Control, Afghanistan.
8. Dr. A. Samad, Chief Pharmacist.
9. Dr. Chom Debyasuarn, Chief, The Communicable Disease Control Division, Health Department, Bangkok, Thailand.
10. Mr. Randall S. Thompson, National Institute of Health, American Embassy, New Delhi.
11. Dr. Rintaro Okamoto, M.D., P.D., Acting Director, International Quarantine, Ministry of Health and Welfare, Japan.
12. Shri Shantilal H. Shah, Minister for Public Health, Maharashtra.
13. Dr. B. B. Yodh, Bombay.
14. Smt. Leela Moolgaonkar, Hon. Organiser, Blood Bank Scheme.
15. Shri Ganesh Ram Anant, Minister for Public Health, Madhya Pradesh, Bhopal.
16. Dr. Dioryz Blaskoria, Director, Institute of Virology, Czechoslovakia Academy of Sciences, Bratislava.
17. Mr. N. Sakrica, Institute of Immunology, Zagrab, Head, Department of Antigens.
18. Dr. H. Quastal, F.R.S., McGill University, Montreal.
19. Mr. Ucalb K. G. Vilebaskobe L.S., Doorayas Aohhayada, Cobla.
20. Mr. D. Stark, Murray, District Pathology Consultant, N.H.S., U.K. American Bevan Fellow (Indian Government).

21. Dr. (Mrs.) S. Rangelove, Director of Institute of Epidemiology and Microbiology, Sofia, Bulgaria.
22. Mr. Angel Panayofov., Sofia (Bulgaria), Chief of Export Department, Sera and Vaccines of the Stat. Comm. Enterprise, Chim im port, Sofia.
23. Dr. V. Demin (W.H.O., Geneva, Switzerland).
24. Mr. Theadore Oleson, Consultant W.H.O. Professor of Public Health Biology, University of Mennesota, U.S.A.
25. Mr. Director Georgi — Institute of Chemical Technology, Sofia, Bulgaria.
26. Friederids Yung, Institute of Pharmacology, University of Berlin, Berlin EAST.

ACADEMIC COURSES AND TRAINING OF HEALTH PERSONNEL

The Institute is affiliated to the University of Bombay for post-graduate training courses for the following degrees :—

M.Sc. and Ph.D. in Microbiology, Bacteriology, Biochemistry (including Nutrition), Zoology, Organic Chemistry and Applied Biology.

M.Sc. (Med.) and Ph.D. in Pathology, Bacteriology, Parasitology and Pharmacology.

The following are post-graduate teachers recognised by the University of Bombay :—

1. Dr. H. I. Jhala.
2. Dr. N. K. Dutta.
3. Dr. P. J. Deoras.
4. Dr. M. V. Sant.
5. Dr. C. V. Deliwala.
6. Dr. A. N. D. Nanavati.
7. Dr. (Smt.) A. Venkataraman.
8. Shri K. G. Anantanarayanan.
9. Dr. S. S. Rao.
10. Dr. S. D. Ambegaokar.
11. Dr. R. B. M. Colah.
12. Dr. K. H. Dave.
13. Dr. B. G. Modi.
14. Shri R. A. Bellare.

The Institute is also recognised by the College of Physicians and Surgeons of Bombay for training candidates for (a) F.C.P.S. Examination in Pathology and Pharmacology and (b) D.P.B. Examination of the College. The following officers of the Institute are recognised as teachers of the College :—

1. Dr. H. I. Jhala.
2. Dr. N. K. Dutta.
3. Dr. N. V. Sant.

During the period under review, 16 more Science Graduates, who registered at this Institute, were accorded facilities for prosecuting their post-graduate studies. Their departmentwise analysis is given below :—

Department/Section.	1965-66 Newly admitted.		Total No. of students working during the year.	
	M.Sc.	Ph.D.	M.Sc.	Ph.D.
1. Chemotherapy	..	..	10	6
2. Clinical Pathology	..	3	7	..
3. Entomology	..	1	1	4
4. Immunology	..	1	8	7
5. Nutrition and Biochemistry	..	..	6	2
6. Pharmacology	..	1	2	1
7. Analytical and Control Department	..	1	1	..
8. Virology	..	4	5	3
9. Bacteriology	..	..	1	..
10. Blood Bank	..	1	2	1
11. Animal House	..	2	2	..
	14	2	45	24

The following post-graduate students got their M.Sc. and Ph.D. Degrees during the year :—

Post-graduate Student.	Subject.	Degrec.
1. Kum. S. B. Nayen ..	Microbiology.	M.Sc.
2. G. M. Govekar ..	Microbiology.	M.Sc.
3. Kum. N. K. Ghag ..	Microbiology.	M.Sc.
4. Kum. S. K. Nair ..	Biochemistry.	M.Sc.
5. Shri M. J. Modak ..	Biochemistry.	M.Sc.
6. Shri U. S. V. Acharya ..	Biochemistry.	M.Sc.
7. Shri M. S. Sant ..	Microbiology.	M.Sc.
8. Shri G. M. Umarji ..	Chemistry.	M.Sc.
9. Kum. M. H. Advani ..	Biochemistry.	M.Sc.
10. Kum. V. P. Kalokhe ..	Biochemistry.	M.Sc.

Post-graduate Student.	Subject.	Degree.
1. Shri G. P. Kamat	.. Biochemistry.	Ph.D.
2. Shri P. D. Mehta	.. Biochemistry.	Ph.D.
3. Shri A. B. Rajadhyaksha	.. Microbiology.	Ph.D.
4. Kum. B. A. Ver	.. Microbiology.	Ph.D.
5. Shri M. D. Gadgil	.. Microbiology.	Ph.D.

SHORT-TERM TRAINING COURSE

Following are the personnel who received training in various Departments during the period under review—

- | | |
|--------------------------|---|
| 1. Pharmacology | <p>.. 1. Shri C. S. Joshi, Bai Gopibai Damani Blood Bank, Sholapur.</p> <p>2. Shri C. R. Rajwade, R. A. Podar Medical College, Bombay.</p> <p>3. Shri M. N. Mahambrey, Bassein Drugs and Pharmaceuticals Bassein.</p> <p>4. Shri V. J. Thomas, Drugs Inspector, Kerala.</p> |
| 2. Clinical Pathology | <p>.. 1. Kum. D. J. Lathi.</p> <p>2. Shri Ramswarup Ramdin, Laboratory Assistant, Jabalpur Hospital, Western Railway.</p> <p>3. Dr. S. A. Jamdar.</p> |
| 3. Chemotherapy | <p>.. 1. Dr. Estevan Afouse, Director, Vaccine Institute, Goa.</p> |
| 4. Immunology Department | <p>.. 1. Dr. D. S. N. Raju, Quality Control Chemist, Biological Evans Pvt. Ltd.</p> <p>2. Dr. R. S. Raju, Over all Production Manager, Biological Evans Pvt. Ltd.</p> <p>3. Dr. P. Y. Dighe, Chougule & Co.</p> <p>4. Shri R. S. Choksi, Lyka Labs., Bombay.</p> |

5. Entomology .. 1. Kum. Rajakumari Hingorani.
6. Bacteriology .. 1. Dr. Chandrashekhar, Alembi Chemicals, Baroda.
2. Shri F. M. Poonawala, Alembi Chemicals, Baroda.
7. Animal House .. 1. Shri D. S. Ganguli.
2. Shri T. K. Sengupta, Indian Institute of Biochemistry and Experimental Medicine.
3. Shri V. M. George, Animal House Keeper, S. P. Medical College, Bikaner (Raj.).
4. Dr. Shivaramakrishnan, Veterinary Officer, Biological Evans Pvt. Ltd.
8. Blood Bank .. 1. Shri M. L. Bhavsar, L. G. General Hospital.
2. Shri P. R. Patel.
3. Dr. K. Chaturbhuj, Laboratories Grimautt, Bombay-18.
4. Dr. T. D. Seetharam, Assistant Surgeon, Grade II, S.D.S. Sanatorium, Bangalore.
5. Shri E. J. Joseph, Technician, S.D.S. Sanatorium, Bangalore.
6. Dr. M. K. Suryanarayana Rao, Lady Bowering and Curzon Hospital, Bangalore.
7. Shri H. V. Padmanabha Rao, Technician, Lady Bowering and Curzon Hospital.

The following Pool Officers were working at this Institute during the period under review :—

No.	Name of Officer	Department	Period
1	Kum. D. H. Shah	Nutrition & Biochemistry.	2nd July 1965 to 31st January 1966.
2	Dr. (Smt.) N. S. Rajadhyaksha	Entomology	5th August 1964 to 8th September 1965.

MEDICAL LABORATORY TECHNICIANS' TRAINING COURSE

During the period under review, sixteen Science Graduates were admitted in the seventh batch of this training course and the following 13 candidates, who completed the course satisfactorily, were awarded the certificates :—

1. Shri M. K. Nair.
2. Kum. V. G. Mehta.
3. Kum. P. V. Chitalia.
4. Kum. M. N. Lala.
5. Kum. S. B. Rajadhyaksha.
6. Kum. A. S. Redkar.
7. Kum. S. S. Kulkarni.
8. Kum. K. S. Bandodkar.
9. Kum. U. P. Naik.
10. Kum. N. V. Karnik.
11. Kum. A. I. Bhatt.
12. Kum. P. M. Gupte.
13. Shri V. A. Aroskar.

The facilities were extended to the following foreign trainees during the year :—

Trainees

Sr. No.	Name of Trainee	Subject in which the training was imparted	Period
1	Mr. Georgi Dimitroff Assistant, Institute of Chemical Technology, Sophia, Bulgaria.	Specialization in the methods of Fractionation of Snake Venom's and investigation on Snake Venom's Enzyme.	For one year from 22nd March 1966.

DIPLOMA IN MEDICAL LABORATORY TECHNOLOGY

During the year, this Institute has been recognized for conducting course for Diploma in Medical Laboratory Technology by the University of Bombay.

The following members of staff of this Institute have been recognised as Teachers by the University of Bombay for Diploma in Medical Laboratory Technology :—

1. Dr. H. I. Jhala.
2. Dr. M. V. Sant.
3. Dr. A. N. D. Nanavati.
4. Dr. S. S. Rao.
5. Dr. (Smt.) A. Venkataraman.
6. Dr. K. V. Kulkarni.
7. Shri Y. S. Nimbkar.
8. Dr. K. H. Dave.
9. Dr. B. G. Modi.

During the year, twelve candidates were admitted in the first batch of Diploma in Medical Laboratory Technology Course. Their names are as follows :—

1. Kum. N. G. Annachat্রে.
2. Kum. S. K. Desai.
3. Kum. S. M. Desai.
4. Kum. C. K. Kulkarni.
5. Kum. U. R. Mahadkar.
6. Kum. M. B. Makani.
7. Shri B. G. Patel.
8. Kum. M. V. Phadnis.
9. Kum. L. Pinto.
10. Kum. G. S. Sabnis.
11. Kum. P. R. Sarangdhar.
12. Kum. S. P. Tillu.

GRANTS

Following Research Fellowships and Scholarships were attached to the Institute during the period under review :—

Serial No.	Sponsors	Subject of Fellowship and Scholarship	Name of the Scholar/Fellow	Period	Amount per mensem
					Rs.
1	Council of Scientific and Industrial Research.	Biochemical and Immunological Investigations of the toxins of clostridia.	Shri A. A. Patel, M.Sc.	From 1st August 1962 to 28th February 1966.	250
2	Do.	.. Study of flies with special reference to Musca Neculo Endocrine Glands.	Kum. K. M. Karnikar, M.Sc.	From 27th August 1963 up-to-date.	250
3	Government of India Research Training Scholarship.	For carrying out Research in Entomology.	Shri R. S. Prasad, M.Sc.	From 16th August 1962 up-to-date.	250
4	Do.	.. For carrying out Research in 'Organic Chemistry.'	Shri R. B. Petigara, M.Sc.	From 1st February 1964 up-to-date.	250
5	Do.	.. For carrying out Research in 'Organic Chemistry'.	Shri M. G. Dhaplapur, M.Sc.	From 24th June 1964 up-to-date.	250
6	Government of Maharashtra Research Scholarship.	For carrying out Research in Biochemistry.	Shri M. J. Modak, M.Sc.	From 22nd February 1964 to 31st March 1966.	150
7	Government of Maharashtra Junior Research Training Scholarship.	For carrying out Research in Clinical Pathology.	Shri K. N. Joishy, B.Sc.	From 1st March 1965 up-to-date.	150

GRANTS—contd.

Serial No.	Sponsors	Subject of Fellowship and Scholarship	Name of the Scholar/Fellow	Period	Amount per mensem
					Rs.
8	Government of Maharashtra Junior Research Training Scholarship.	For carrying out Research in Biochemistry.	Shri P. Divakaran, B.Sc.	From 4th December 1963 up-to-date.	150
9	Do.	.. For carrying out Research in Nutrition and Biochemistry.	Vacant ..		
10	Unichem Research Fellowship	To carry out work on Indigenous Drugs.	Kum. R.P. Motwani M.Sc.	From 1st September 1964 to 10th May 1965.	350
11	Do.	.. Do. do. ..	Shri A. N. Dohadwala, M.Sc.	From 10th September 1965 onwards.	350
12	Do.	.. To carry out Research on Cholera	Smt. Usha Kashyap, M.Sc.,	From 1st March 1965 up-to-date.	350
13	Blood Group Reference Centre	Preparation of Blood Grouping Reagents	Shri V. D. Kulkarni	From 6th June 1963 up-to-date.	250
14	Sir Dorabji Tata Trust	.. Research on Cataract ..	Shri P. D. Mehta, M. Sc.	From 1st January 1963 to 8th September 1965.	300
15	Indian Council of Agricultural Research.	Immuno Studies in Animal Viruses with special reference to Pox Group of Viruses and mucosal disease complex viruses.	Kum. Thankam Bastion, M.V.Sc.	From 15th December 1963 up-to-date.	250
15A	Sir Dorabji Tata Trust	.. Research on Cataract ..	Kum. N. G. Gogwekar, M.Sc. December 1965.	From 1st January 1965 to 31st December 1965.	300

16	Haffkine Institute Diamond Jubilee Commemoration (Research Unit) Senior Research Fellowships.	Biochemistry of <i>Vibrio cholerae</i>	Dr. (Smt.) K. Deshmukh.	From 1st August 1963 up-to-date.	560
17	Do.	.. Studies on Lysis of <i>Vibrio cholerae</i> .	Dr. S. V. Huprikar	From 7th December 1964 up-to-date.	530
18	Do.	.. Studies on Toxin-producing Strains of <i>C. diphtheriae</i> .	Dr. A. B. Rajadhya-ksha.	From 19th October 1965 up-to-date.	500
19	Do.	.. Continuation of Studies on Rabies Virus.	Dr. (Kum.) B.A. Ver.	From 26th November 1965 up-to-date.	500
20	Do.	.. Nutritional requirement of <i>P. Pestis</i> and their relation to vaccine production.	Dr. M. D. Gadgil ..	From 5th March 1966 up-to-date.	500
21	Haffkine Institute Diamond Jubilee Commemoration (Research Unit), Junior Research Fellowships.	Studies on toxin-producing strains of <i>C. diphtheriae</i> .	Shri A. B. Rajadhya-ksha.	From 20th February 1961 to 18th October 1965.	400
22	Do.	.. Nutritional Requirement of <i>P. pestis</i> and their relation to vaccine production.	Shri M.D. Gadgil ..	From 1st March 1961 to 4th March 1966.	400
23	Do.	.. Purification and other Studies on Rabies Virus in Tissue Culture.	Kum. B. A. Ver ..	From 1st March 1961 to 25th November 1965.	400
23A	Do.	.. Biochemical Composition of <i>P. pestis</i> , in relation to virulence and immunogenicity.	Smt. S. M Gadgil	From 6th July 1963 up-to-date.	350
24	Haffkine Institute Diamond Jubilee Commemoration (Research Unit) Junior Research Fellowships.	Studies on <i>Bordetella Pertussis</i>	Kum. A. E. Bunde-ally.	From 1st July 1963 up-to-date.	350

Serial No.	Sponsors	Subject of Fellowship and Scholarship	Name of the Scholar/Fellow	Period	Amount per mensem
					Rs.
25	Haffkine Institute Diamond Jubilee Commemoration (Research Unit) Junior Research Fellowships.	Studies on Hypersensitivity to Horse Serum.	Kum. S. K. Sarde- desai.	From 1st December 1964 up-to-date	325
26	H. I. D. J. (Commemcration Scholarship Unit) Unichem Scholar.	Studies in Chemotherapy of Viral Infection.	Kum. N.A. Varaiya	From 4th January 1965.	250
27	H. I. D. J. (Commemcration) Pftizer Scholarship	Studies on Folic Acid	.. Shri A.D. Seth ..	From 1st February 1965 to 31st Aug- ust 1965.	250
28	H. I. D. J. (Commemoration) Unichem Scholarship.	Studies on the Chemotherapeutic Drugs.	Shri J.D. Modi ..	From 19th Septem- ber 1965 up-to- date.	250
29	Council of Scientific and Industrial Research.	Synthetic Diuretics	.. Shri H. V. Patel ..	From 3rd January 1966.	492
30	Indian Council of Medical Research.	Post-graduate Studies in Micro- biology.	Shri R. K. Bhatnagar	From 7th August 1965.	150
31	Do.	.. Do.	.. Shri Stanley Jessu- dass.	From 14th June 1965.	150

NAMES OF INDIAN COUNCIL OF MEDICAL RESEARCH AND OTHER ENQUIRIES

<i>I.C.M.R. Enquiries—</i>					Grants Rs.
1.	Pharmacological Research Unit under Dr. N. K. Dutta	..	..	..	29,720
2.	Preparation of Type Specific Antisera under Dr. A. N. D. Nanavati	..	..	..	20,500
3.	Isolation of Cholera Toxin under Director, Haff- kine Institute	..	..	..	5,760
4.	Hyperimmune Rabies Serum under Dr. A. N. D. Nanavati	..	..	..	3,750
5.	Polio Research Unit under Dr. P. V. Gharpure	..			1,32,670
6.	Blood Group Reference Centre under Director, Haffkine Institute	..	..	..	50,000
<i>Other Research Schemes—</i>					
1.	Research Grant for work on Cholera from World Health Organisation	..	..	..	18,000

BUSINESS SIDE

This was an extremely trying period for the Institute, beset with all kinds of unusual difficulties. Due to the emergency, there was a sudden scarcity of funds. The import position was extremely difficult and so also the local market position, the prices having gone up several-fold. Due to the economy drive, it seems our sales were also affected. Coupled with all the above factors, the Institute's long un-resolved difficulty connected with the shortage of Senior Officers on the Business Side and absence of adequate Cost Accounting Section, continued.

The sales during the year under report amounted to Rs. 80,81,467. As compared to the last year, there is a fall of about Rs. 10,67,400. This is due to the fact that this year, the cholera vaccine sales were less by about Rs. 5 lakhs. Also, scarcity of raw materials on the one hand and economy drive of the customers on the other, affected the sales. The budget expenditure during the year amounted to Rs. 83,61,576. This, for the first time, includes a provision for Depreciation Fund to the extent of Rs. 2 lakhs.

Sufficient stocks of raw material stores and finished products could not be maintained due to the inadequate storage space. The Cost Accounts Officer is not available to the Institute since July 1964.

Below are given productwise supply figures for the year 1965-66 followed by the financial picture of the Institute for the year 1965-66.

Statement of supplies made by the Institute for the year 1965-66.

Serial No.	Name of the Product	Quantity supplied
1	Cholera Vaccine	1,32,56,776 ml.
2	T.A.B. Vaccine	3,07,984 ml.
3	Plague Vaccine	1,05,000 ml.
4	Antirabic Vaccine	38,66,604 ml.
5	Tetanus Antitoxin	14,01,487 ml.
6	Tetanus Toxoid	2,44,706 ml.
7	Diphtheria Antitoxin	1,07,925 ml.
8	Diphtheria Toxoid (P.T.A.P.)	4,082 ml.
9	Gas Gangrene Antitoxin	1,64,030 ml.

Serial No.	Name of the Product	Quantity supplied
10	Anti-snake Venom Serum ..	2,47,800 ml.
11	Normal Horse Serum ..	19,420 ml.
12	Triple Antigen ..	23,387 ml.
13	Intravenous Solutions ..	2,50,256 bottles.
14	Vitamin Tablets ..	8,57,51,090 tabs.
15	Sulpha Tablets ..	2,55,59,600 tabs.
16	Pemon ..	20,920 lit.
17	Cobra Venom ..	31·9456 gms.
18	Russell's Viper Venom ..	13·2839 gms.
19	Krait Venom ..	5·0100 gms.
20	Echis Venom ..	5·0043 gms.
21	Dead Emulsions ..	3,51,375 ml.
22	High-titre Sera ..	3,413 ml.
23	Kahn Antigen ..	18,690 ml.
24	W.R. Antigen ..	1,960 ml.
25	Casoni's Antigen ..	354 ml.
26	Cobra Venom Solution ..	5,651 ml.
27	Russell's Viper Venom Solution ..	782 ml.
28	Biological Stains ..	10,69,000 ml.
29	Blood Plasma ..	1,924·750 litres.
30	Blood Grouping Sera :	
	Anti-A ..	24,184 ml.
	Anti-B ..	24,303 ml.
	Anti-A plus B ..	584 ml.
	Coomb's Reagent ..	131 ml.
	Bovine Albumin ..	212 ml.
31	Human Gamma Globulin, 1 ml.	150
	amps. ..	
	Human Gamma Globulin, 2 ml.	149
	amps. ..	
32	Normal Human Serum Albumin ..	5 bottles.
33	Pasteurised Plasma Solution ..	16 bottles.

WELFARE ACTIVITIES

GENERAL

The welfare activities in the year under report were quite energetic. Several indoor and outdoor games and tournaments were played both with outside teams as well as on interdepartmental basis under aegis of Sports Club.

The distribution of skim milk to the children and expectant and nursing mothers under the UNICEF Skim Milk Distribution Scheme in the workers' residential quarters of this Institute is being carried out and, on an average, 75 beneficiaries take advantage of this scheme every day.

Showing documentary films is a regular feature and the employees and their families residing in the Institute Compound derive benefit therefrom.

With the kind co-operation of the Indian Cancer Research Centre, a five-day Family Planning Orientation Training Course was organised at this Institute. Thirty-two members of the staff of this Institute attended the course.

Three members of the staff of this Institute were admitted to the Workers' Education Scheme, conducted by the Bombay Labour Institute (Government of Maharashtra), Parel, Bombay-12.

Lectures on useful topics were arranged. With the kind co-operation of the I.C.R.C., Film Strips on 'Human Reproduction' and 'Methods of Family Planning', were shown and explained to the members of the staff.

Collection for the following funds was arranged :—

1. Jawaharlal Nehru Memorial Fund.
2. Flag Day on 7th December 1965 for benefit of the Ex-Servicemen and for providing amenities to service people of our country.
3. Flag Day for the Blind on 8th February 1966.

With the co-operation of the officers of the Small Savings Organisation, the Small Savings Scheme was explained to the members of the staff and about four-hundred employees have taken advantage of this Scheme. Response from the staff has been encouraging.

Some of the important days of national festivity were celebrated in the residential campus of this Institute. Full-length films were shown

d entertainment programmes like Mimiory, Light, Devotional and Film music by famous artists was arranged for the benefit of the employees and their families.

HAFKINE INSTITUTE SPORTS CLUB

The Sports Club of the Haffkine Institute was constituted in the year 1959 in order to bring recreational atmosphere among the employees and social relationship with each other. The Sports Club has various activities, viz. cricket, football, table tennis, volley ball, tennis, badmington etc., besides indoor and Indian Games. For those who cannot take part in physical sports activities, other recreational activities such as : cinema shows, library etc., have been instituted.

The activities of the club are increasing day by day and more and more members are coming forward in enhancing the Sports Club Activities.

Inadequate space for more recreational activities is a handicap for further improvement of the Club. However, this matter is under active consideration of the Sports Club Managing Committee and all efforts are being made for progress in this direction.

Last year, the annual prize distribution was done by Shrimati Tara Cherian and H.E. The Governor of Maharashtra presided.

The Haffkine Institute Sports Club is further happy to report that it has started Drama Activity and the artists taking part in the drama are employee members of the Institute.

During the year under review, a drama "PREMA TUZA RANGA KASA" by Shri Vasant Kanetkar was staged at Damodar Hall, Parel.

DISPENSARY

The Institute Dispensary continued to serve the needs of the staff of the Institute and their dependents as usual. A full-time Pharmacist is now in charge of dispensing. This has facilitated the work of the dispensing greatly and the services of Blood Bank Staff Nurse are utilised in attending to female patients and to help in giving injections.

A new more spacious room has been allotted for the Clinic. The department of Blood Bank and Clinical Pathology co-operated in running this service. Cases requiring expert guidance were referred to the teaching hospitals.

Total of 19,140 patients were attended to.

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