



सत्यमेव जयते

GOVERNMENT OF MAHARASHTRA

ANNUAL REPORT
OF THE
HAFFKINE INSTITUTE
FOR THE YEAR 1974-75



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PART I

GENERAL REVIEW

Haffkine Institute completed its Platinum Jubilee Celebrations. In all, six scientific seminars were held during the entire Platinum Jubilee Year. These seminars have now been compiled for publication through the pages of "Progress in Drug Research" edited by Dr. E. Jucker and published by Sandoz, Basel, Switzerland. These books should provide guidelines for further research in tropical diseases.

The Institute continued its expansion, both in the fields of production and research. The Government of Maharashtra have taken a decision to organise the Institute into two autonomous bodies, viz. Haffkine Biopharmaceutical Corporation Limited to be registered as a Public Sector Undertaking, taking over the activities of production of vaccines, sera, blood products, non-biologicals etc. ; the research, training and testing activities will be carried out by Haffkine Institute for Training, Research and Testing, registered as a Society under the Societies Registration Act, 1860 and the Bombay Public Trusts Act, 1950. Thus, these two autonomous bodies will start functioning with effect from 1st April 1975, each taking over the production activities or research, training and testing activities of Haffkine Institute respectively. It is hoped that this step will serve to streamline production and organise it on sound commercial principles. Research will also get impetus. The Government of Maharashtra will contribute to the tune of 1 crore as equity capital for the company.

The Research Society will be given a grant of Rs. 25 lakhs which will be tapered off to nil in the next five years, by which time it is expected that the profits of the Corporation will be able to sustain the research activities of the Institute.

The production turnover showed a rise of 64 per cent over that of the previous year. However, due to unprecedented rise in the prices of raw materials and high overheads, the production of drugs and medicines became grossly uneconomical, resulting in heavy losses. The annual turnover of the drugs rose to Rs. 164 lakhs, i.e. a rise of about 50 lakhs over that of the previous year. The retail prices of various products which were plugged in 1964, could not be revised. Efforts were being made to obtain revision of prices in view of the high cost of production.

The Institute continued its training programme in various disciplines of biomedical sciences. This year, 11 students obtained their Master's degree from University of Bombay and 10 were awarded Ph.D. degree in Organic Chemistry, Microbiology, Biochemistry and Applied Biology. D.M.L.T. and M.L.T. courses were reorganised and put under the charge of a Senior Scientific Officer Cl. I. Eighty-two students were trained under short-term training

programme scheme. Institute offers four courses for short-term training : Clinical Pathology, Quality Control. Bacteriology, Media, Blood E k, Biochemistry, Maintenance and care of laboratory animals, Entomc gy and Parasitology.

Cholera carrier studies were undertaken in non-endemic areas near V ur, since this area had a high incidence of diarrhoea and dysentery, particu ly during monsoon, i.e. from the month of July to September. Although clal V. cholerae was isolated from only one patient, NAG vibrios were iso ed from a number of healthy persons. These NAG were found pathogen to infant rabbit. This is an extremely interesting finding and gives a lea or further studies in the epidemiology of the disease.

Studies were continued for improvement in the methods of productio of cholera vaccine. Attempts are being made to prepare cholera toxoid by Ga ra irradiation using 60Co as the source.

Epidemiological studies in Filariasis were continued. FST 3-1 an supplied by Prof. Sawada of Japan was used for screening test antige in endemic and non-endemic areas. Results showed a high degree of speci ty for the antigen. Only 4.31 per cent of population gave positive skin te in non-endemic area.

Sixty-five compounds belonging to R-series were screened for effectiv ss in rats infected with *L. carini*. Two compounds were found active in in ro studies but were ineffective *in vivo*. Further work is in progress.

Plague surveillance is one of the most important epidemiological program es of the Institute since the last several years. Such a programme is necessa in view of the presence foci of plague in India and the neighbouring coun s. *X. cheopis* was found to be the prevalent flea on all species of rodents exam ed both in "D" and "E" wards of Bombay city. Fraction I antigen sup ed by the W.H.O. was used to find out evidence of in-apparent plague infe on in rats. None of the rat sera tested was positive. Susceptibility of ra to *P. pestis* infection was tested. It was interesting that amongst the rats coll ed from Satara district, 53 to 56 per cent of the rodents were resistant to plagi

Ricketts is still prevalent in the infants and children in our country. Fifty ve blood samples were examined for biochemical parameters for eviden of ricketts. The data was correlated with radiological and clinical findings.

Study Group on snake venoms carried out further investigations on the chemistry of Elapid snake venom. Three neurotoxins were isolated om venom of *Bungarus caeruleus*. Most interesting finding is a non-toxic p s- pholipase-A of high purity. Neurotoxins and cardiotoxins were isolated m the venom of *Naja naja*. Studies on blood coagulation factors revealed at *Naja naja* venom contains a fraction which exhibits a strong anticoag nt activity. It acts in the extrinsic system of blood clotting mechanism and s ci- fically inhibits the activity of Factor VIII, antihæmophilic globulin. Fun r, the venom also possesses fibrinolytic activity.

Research in the Department of Bacteriology consisted mainly of improvements in the techniques of vaccine production. Important achievements during the year were : (a) standardisation of fermentation technology and (b) development of submerged continuous culture methods. Standardisation of the technique will help adoption of the submerged culture technology in the production of vaccines.

Use of radiation in the preparation of vaccine was initiated last year. It was established that killed cholera vaccine is as potent as formaline-killed vaccine. Further, the rate and dose of radiation within the prescribed limits did not affect the potency of vaccine.

Production technology of diphtheria vaccine was improved to make the vaccine production economical. Imported Casamino acids were substituted by locally made casein hydrolysates. Further work on improving the medium for growth of organisms will be taken up vigorously.

Pertussis vaccine production, which was taken up on a trial basis, has now been routinely manufactured in the production line. The department is now in a position to produce 2 million doses of vaccine.

Use of radio-labelled compounds in microbiological research was initiated. A significant advance has been made in this field. Thus, in collaboration with the Radiation Medicine Centre of B.A.R.C., a method for utilising ^{14}C labelled glucose, to detect bacterial and fungal contamination has been devised. B.A.R.C. is also working on a suitable monitoring system. The method enables detection of bacterial contamination within 15 hours. The method will contribute significantly in early diagnosis, detection of bacterial sensitivity to antibiotics and also early detection of contamination in the manufacture of vaccines in the production line.

Department of Biochemistry continued to function with depleted staff. The posts of Assistant Director and Senior Scientific Officer remained vacant. During the year, two new programmes were initiated :—

- (a) use of waste material for preparation of media
- (b) isolation of insulin and other enzymes from glands and organs collected from slaughter house

Chemotherapy Department synthesized about 250 compounds for screening against tuberculosis and parasitic diseases. Compounds were tested *in vitro* against *Mycobacterium tuberculosis*. Similarly, new compounds were tested against *A. lumbricoides*, *N. dubius* and *H. nana*. The new drugs comprised of methyl imidazole substituted imidazothiadiazoles, halogenated phenols, certain isothiocyanate group, mercaptooxadiazoles, etc. Further, the department undertook two new projects for standardizing technology for synthesis, first on laboratory scale and then scaling up on the pilot plant of metronidazole and tetramizole. A pilot plant committee constituted under the Chairmanship of Director with Commissioner, Food and Drug Administration as its member, has been monitoring the programme of the project, providing valuable guidelines for further work.

Clinical Pathology Department continued its laboratory service activities viz. examining clinical material including histopathology work, referred to it by various hospitals. The department continued its research activities such as studies on Typhoid Carrier, Rheumatoid Arthritis, bacterial resistance etc. Two new projects : (1) Epidemiology of anaerobic infections and (2) Studies of Gonococcal infection, which were initiated last year, were continued during the year.

Immunohaematology research is mainly in the field of blood enzymes, abnormal haemoglobin, Australia antigen studies and preparation of specific immunoglobulins. A new radioimmunoassay technique for detection of Australia antigen has been standardised. This provides a sensitive method for screening all blood donors and blood products for the presence of Australia antigen.

The original Department of Immunology was reorganised and converted into two wings, viz. the Department of Immunology and the Department of Antitoxins and Sera, the first one purporting to research activities and the second one purporting to production of antitoxins and sera. Several immunological projects were initiated under the direct supervision of the Director.

For the first time, an antitoxin was prepared from varieties of poisonous snakes, i.e. Cobra, Krait, Echis and Russell's Viper. ^{60}Co was used as a source of radiation. It was established that the venom toxicity was marked by attenuation but antigenicity was retained. Preparation of a toxoid will be useful for active immunization of the rural population exposed to a high risk of snake bites.

Scorpion venom was subjected to biochemical and pharmacological studies. For the first time, it was established that black scorpion venom contains large quantities of histamine. Attempts are being made to prepare a specific antivenin.

Leprosy has become a grave national problem. Haffkine Institute is closely associated with the Acworth Leprosy Hospital. A new project on the study of immunology of leprosy has been initiated.

Pharmacology Department screened over two hundred synthetic compound and a few plant extracts for their pharmacological activities against helminth infections and amoebiasis. The department has standardised the Sheper mouse foot pad technique. An extensive programme has been undertaken to find out the extent of DDS resistance in Leprosy. A new project to study toxicity of sea snakes has been initiated during the year under review.

The Pharmacology Department has specialised in the study of drugs as anti-allergic agents. Several techniques for the study of such drugs have been standardized.

Radiobiology Unit, which was organised last year, was further strengthened during the year. Three projects were initiated : Radioimmunoassay method for detection of Australia antigen, estimation of plasma corticoids using C^{14} labelled corticosterone and effect of radiation on biological and microbiological material. Whole blood irradiation to study the effect on preservation and stability of RBC has been initiated. The unit has also been engaged

studies on a virus isolated from the infant rabbit liver inoculated with human serum obtained from a patient of jaundice.

Department of Virology, under the leadership of Dr. Shirodkar who joined last year, has made significant contribution to the researches on hepatitis B type. A strain of hepatitis B type virus, isolated from a patient of jaundice admitted to Kasturba Hospital, could be serially passaged six times in the newborn (40-50 g) rabbit *Oryctolagus cuniculus* strain Haffkine. The virus has been named "HIBOMA-I". The route of choice for injection appears to be intrahepatic. The acute syndrome closely mimicks human acute hepatic necrosis. This is the first time that human hepatitis B virus has been grown in a common laboratory animal. Further intensive work is in progress.

Host-virus interaction studies have been undertaken. It was shown previously that Rabies virus (RB) produced a blockade of Rous sarcome (RSV), the Bryan Master Standard Strain. It was further shown that rabies virus produces a serum factor in chickens which blocks the RSV. It has now been established that there is a plasma factor which shows this type of specific blockade. The findings are extremely interesting, as they indicate the presence of a new antihuman factor.

Host-virus interaction studies on Rubella were continued. The new Antirabic Vaccine trials in volunteers were completed. Post-exposure trials have been planned.

Poliomyelitis surveillance programme in the population of Greater Bombay revealed that a total of 811 cases of paralytic poliomyelitis were reported—an increase of 84 cases over those of the previous year. Ninety-three per cent of the cases were in the age group of 3 months to 3 years. About 60 per cent of the cases were reported during the months of June to October, with a peak incidence in August. It is disturbing to note that 131 out of 811 (16.1 per cent) paralytic cases had a history of receiving oral poliovaccine. Virological studies were done on 123 out of these 131 cases, 80 per cent showed virus excretion and out of these polio virus constituted 57 per cent of the isolated strain. Further investigation on the failure of protection in the vaccinated cases is in progress.

The Viral Vaccines Department has standardised the preparation of powdered media. Use of such media has several advantages like storage, transportation and uniformity of preparation. These media will be useful for tissue culture and virological work in different centres in the country.

A project for preparation of a freeze-dried antirabic vaccine has been initiated. Ten batches of vaccine have been prepared. If the technique is successful, shelf life of the vaccine will be longer.

Studies on the epidemiology of Guinea worm disease were continued. Effect of three anthelmintic drugs in the treatment of guinea worm was assessed. Mebendazole and Bitoscanate were shown to be the more effective drugs in the treatment of this condition.

It was possible to distinguish between toxicity-producing fractions and those showing maximum protective activity as tested by the I.P. method. Intensive investigations are in progress towards obtaining a chemical vaccine.

The work on (Lf₁) plant extract was continued. Alcoholic fraction of this plant was shown to produce antifertility effect in rats, mice and rabbits. The activity was dose related: 100 per cent effect can be induced with large doses which were non-toxic. The drug is effective during the period of zygote formation as only three doses are enough to induce sterility. Further, the drug has no abortifacient or teratogenic activity.

V. parahaemolyticus.—may be a causative agent of mild to moderate diarrhoea seen in individuals particularly after eating shell fish. Strain of *V. parahaemolyticus* was isolated from variety of fish collected from Bombay coast. Further epidemiological work is in progress.

The Library organised a new section on the biography of Dr. W.M. Haffkine. A large number of manuscripts and papers written by Dr. Waldemar Morde Haffkine as well as articles written by different persons on his life and contributions to science were collected from different parts of the world. For a long time, the library had no facilities for obtaining photostat copies of various articles published in the journals. This facility has now been created. Copies of articles are made available at a nominal price not only to scientists of this Institute but also to outsiders.

Labour Welfare activities were further spurred up during the year. Labour Education Scheme was started. The book bank section has been strengthened further. Two hundred and seventy-seven college books were purchased.

With the co-operation of the Central Board of Workers' Education, the first Unit Level Class was conducted at this Institute. It is most gratifying that the class was conducted by one from amongst the workers, Shri P.G. Pillai, who deserves all praise for this active interest.

All scientists, technicians and workers jointly contributed to the achievement of the Institute, such as production, research and training. Without their co-operation, enthusiasm, abiding faith in the greatness of the Institute and to shoulder joint responsibility, the Institute would not have been able to render substantial contributions to science. The Director takes this opportunity to thank all the fellow scientists, administrators and workers for their unstinting support and the loyalty and the sense of involvement which alone are responsible for the efficient discharge of the onerous duties, both administrative and scientific.

PART II

A. RESEARCH PROJECTS CHOLERA PROJECT

Cholera Carrier Studies

Cholera carrier studies were undertaken in a non-endemic area near Virar in Thana District. The study area consisted of Bolinj, Agashi, Navapur-Vatar and Nandakhal villages near Virar, Vasai Taluka, Thana District. These villages are attached to the Primary Health Centre (PHC), Virar, with sub-centres at the villages. The data collected during the last five years show a high incidence of diarrhoea and dysentery cases as reported by the Primary Health Centre, Virar. Incidence of cholera cases has been reported to occur during the monsoon season, especially from July to September. No cholera cases are generally reported during the inter-epidemic period.

Stool samples were collected from (i) healthy population in the study area and (ii) at the out-patients of Primary Health Centre (P.H.C.), Virar and (iii) Mission Hospital, Agashi. Also, samples from well water in the study area were examined.

One thousand six-hundred and ninety-seven human stool samples were processed. The following pathogens were isolated :

V. cholerae Ogawa (one), *Non-agglutinable vibrios* (thirteen), *Salmonella typhi* (three) and *Shigella* sp. (two). Other organisms isolated were *E. coli*, *Proteus* sp., *A. aerogenes*, *Alk. faecalis*, *Pseudomonas*, *Staphylococci*, and *Streptococci*.

V. cholerae isolated from a patient and few of the NAG vibrios from healthy persons were found pathogenic to infant rabbits.

These investigations are being continued in the different villages under study.

Use of peanut protein as a substitute for casein in the production of cholera vaccine

About 600 litres of peanut protein hydrolysate, containing 200 mg of nitrogen per 100 ml were prepared by the improved procedure of digestion of protein with hydrochloric acid alone. The medium was tested for the cultivation of two strains of classical vibrios (Inaba, 569-B and Ogawa S/121/58) and two *El tor cholerae* strains (Inaba and Ogawa, Phil.) The yield of cholera vaccine containing 12,000 million organisms/ml in the peanut protein hydrolysate agar medium was the same as that in the casein hydrolysate medium.

Utilisation of horse red blood cells

Preliminary work of isolation of proteins from the horse red blood cells was carried out. Isolated protein was then used for the preparation of hydrolysate medium. This liquid medium (135 mg N/100 ml) was used to study the growth of classical *Vibrio cholerae*. The results showed that the growth response of *V. cholerae* in this medium is about 70 to 80 per cent of that obtained in the casein hydrolysate medium. Further work is in progress.

Preparation of toxoid by irradiation of toxin

Crude cholera toxin was irradiated with gamma rays in order to detoxify it for the preparation of toxoid. The dose of irradiation required to detoxify exceeded 3 megarads. Crude toxin was freed from media constituents and concentrated. This toxin could be detoxified by doses of 0.4 to 0.6 megarads.

Study of immunity induced in experimental animals by vaccine intraintestinally administered

Conventional cholera vaccine prepared by formalin treatment was used to immunise mice by intraintestinal administration after abdominal operation. Immunised mice were subjected to challenge after a period of one month.

Intra-peritoneal challenge.—Groups of immunised and control mice were challenged intra-peritoneally with mucinized living vibrios with both Ogawa and Inaba strains. All control animals succumbed to challenge, while the immunized animals were fully protected against Ogawa as well as Inaba challenge.

Intra-intestinal challenge.—Ligated loops were prepared in the small bowel of both the immunised and control animals. Live vibrio strains (Inaba and Ogawa) were introduced into the loops. Fluid accumulation in treated and normal animals was compared after sacrifice at 18 hours. Immunised animals were protected against both Inaba and Ogawa challenge.

Formalin-treated vaccine was found unsuitable for intraintestinal immunisation of adult rabbits as, in the quantities administered, it caused the formation of adhesions and yellowing of the intestinal viscera.

Therefore, a vaccine was prepared by sterilisation of cholera vibrios by irradiation.

Rabbits were immunised intra-intestinally with this irradiated cholera vaccine. Vibriocidal titres were obtained by this route of immunisation. Further, when challenged with living organisms by the ligated loop technique, immunity to both Inaba and Ogawa strains was noted, though the results were not as consistent as in mice. Loops in which toxin was instilled, instead of living organisms, gave full-blown response indicating that immunisation with a vaccine of irradiated organisms did not give protection against toxin challenge.

FILARIASIS PROJECT

1. Epidemiology

Epidemiological survey of filariasis was carried out in Mankhurd. Twenty-nine microfilaria carriers were detected amongst six-hundred and eighty-six people staying in the campus of Mankhurd Children's Home, Bombay.

2. Immunology

FST 3-1 antigen of Prof. Sawada was used to screen the population of Mankhurd Children's Home in Bombay by intradermal test. Out of a total of 441 cases tested, 39.97 per cent were found positive.

The same antigen was used in 301 persons of Karegaon village near Sirur in Poona District. Only 4.31 per cent of the population gave positive results by skin test. Tests carried out on blood samples collected at night did not reveal the presence of any microfilaria carriers.

Studies on drugs against filariasis

In vitro screening:

(a) against microfilariae of *Litomosoides carinii*:

Sixty-five compounds received from Chemotherapy Department were screened. Five out of these viz. R-998, R-2768, R-2916, R-3504 and R-3536 were found active at 15 ug/ml concentration.

(b) against microfilariae of *W. bancrofti*:

The compounds which showed activity against microfilariae of *L. carinii* were also tested against microfilariae of *W. bancrofti*. All the compounds so tested were found active at 31 ug/ml concentration.

In vivo drug screening:

White rats infected with *L. carinii* were used for screening.

(a) Hetrazan at 6 mg/kg for six days brought the microfilarial count to zero, but after stopping the treatment, the microfilariae reappeared. L-tetramisole at a dose of 8 mg/kg for six days reduced the microfilarial count to zero but reappeared slowly after stopping the treatment, while at higher dose level of 32 mg/kg for six days, the microfilariae did not reappear at a level of more than 10 per cent of the normal value. DL-tetramisole was effective at 8 mg/kg and 32 mg/kg for six days. Mebendazole at 8 mg/kg for 6 days reduced the microfilarial count to 25 per cent of the normal, whereas at 32 mg/kg for 6 days it reduced to 3 per cent and remained constant at this level.

(b) Compounds R-3504 and R-3536, which were found active in *in vitro* studies, were tested in rats at a dose of 60 mg/kg given orally for six days. However, no effect on microfilariae and adult worms was observed. Further work to elucidate the action of these drugs in rats is in progress.

PLAGUE PROJECT

Surveillance against plague is necessary in view of presence of plague foci in India and neighbouring countries. Plague project studies, launched as a part of plague surveillance programme in 1965, have two objects in view: (i) to study the distribution of various species of rodents and ectoparasites and (ii) to detect reservoirs of infection by bacteriological and serological methods.

The study of distribution of various species of rodents in 'D' and 'E' wards was continued. Prevalence of *Rattus rattus*, *Rattus norvegicus* and *B. bengalensis* in 'D' and 'E' ward areas of Greater Bombay was continued. *Mus musculus* and *B. gigantea* were also collected in good numbers. Seasonal variation in prevalence of various types of rodents and their significance from the point of view of plague epidemiology are being studied. In all, 718 rats from 'D' ward area and 566 from 'E' ward area were examined. *X. cheopis* flea was found to be prevalent on all the species of rodents examined in both 'D' and 'E'. Amongst the fleas collected in 'D' and 'E' wards *X. cheopis* constituted 64 per cent and 73 per cent respectively.

In view of the reports of rat nuisance, rodents from Sholapur area were examined for evidence of inapparent plague infection by using Fraction antigen supplied by W.H.O. Thirty serum pools from Madha, Niphad, Miraj and Dindhori were examined. None of these pools was found to be serologically positive.

Rat fleas from various localities reporting heavy rat nuisance were collected for flea pool studies. No evidence of plague infection was observed by flea pool animal inoculation tests.

Laboratory trials with RH 787

Laboratory trials with RH 787, for assessing its efficacy as a rodenticide, were continued. The compound was evaluated against four species of rats, viz. *Rattus rattus*, *Rattus norvegicus*, *B. bengalensis* and *M. musculus* (albino). Four bait formulations were employed for evaluating comparative acceptability and efficacy. These bait formulations consisted primarily of wheat flour, rice flour, corn and ground-nut cake.

Susceptibility of rats to anti-coagulant rodenticides

Assessment of susceptibility status of various species of rodents to warfarin and other anti-coagulant rodenticides is in progress.

Earlier studies carried out in 'D' and 'E' ward areas had revealed that *B. bengalensis* is tolerant to warfarin. Studies on the assessment of susceptibility to warfarin in different areas of Greater Bombay were continued during the year. However, during the year, no new areas showing resistance to warfarin were detected.

Susceptibility of rodents to experimental plague infection

During the year under review, studies on the susceptibility status of rats (*R. rattus*) collected from Satara district were carried out. The rats collected from Dahifal, Madha and Rindhori villages were challenged subcutaneously with a usual fixed dose of about 5000 organisms of *Y. pestis* (195/P strain) suspended in 0.5 ml acid broth and their susceptibility status was determined. Results presented in Table 1 show that the resistance level varied from 53 to 56 per cent.

Flourescent antibody staining technique for *Y. pestis*

For rapid detection of plague infection, both direct and indirect methods using fluorescent antibody staining technique were reported last year. During the year 1974-75, more strains were employed to confirm the reliability of these methods in the early diagnosis of plague. Different strains employed in both the methods were Ronur A and Ronur B received from Mysore and J-2, J-2a, J-4, J-5a and J-6 from Indonesia. Both the methods showed specific staining for all the *Y. pestis* strains, whereas the negative controls viz. *Y. pseudotuberculosis* (PR-I & PR-II strains) did not exhibit any fluorescence.

Results obtained in the indirect method were more specific than in the direct method.

TABLE
Susceptibility of rats (*Rattus rattus*) from Satara district to *Yersinia pestis*

Place of collection	No. of rats inoculated			No. of rats found dead within fifteen days			No. of rats found positive to plague			Percentage Susceptibility	Percentage Resistance
	Male	Female	Total	Male	Female	Total	Male	Female	Total		
Dahifal	16	18	34	8	7	15	8	7	15	44	56
Madha	16	18	34	6	9	15	6	9	15	44	56
Rindhori ..	16	18	34	7	9	16	7	9	16	47	53

PROJECT STUDY ON RICKETS

A joint project with J. J. Group of Hospital, Bombay, includes the following points :—

- (a) to determine the incidence of rickets in children ;
- (b) to establish reliable criteria for early diagnosis ;
- (c) to study the metabolism of Vitamin D in children with or without rickets ;
- (d) to evaluate the treatment schedule of Vitamin D in the prevention and treatment of rickets.

Last year, about fifty-five samples of blood were tested for biochemical parameters pertaining to rickets. The data collected was correlated with radiological and clinical findings. Biochemical investigations have indicated that out of 55 cases, 23 cases had low serum calcium and high serum alkaline phosphatase level, which is an indication of rachitic condition. However, the age group could not be discriminated, as these cases were found in all the age group (0-3 months, 4 cases ; 4-6 months 5 cases ; 7-9 months 3 cases ; 10-12 months 7 cases ; 13-18 months 5 cases and 19-24 months nil). At the same time, only three cases had shown radiological and clinical symptoms of rickets. Hence, a definite conclusion could not be arrived at.

The gas liquid chromatographic (G.L.C.) method was used for the estimation of Vitamin D in liver samples, because the liver is primary site of Vitamin D metabolism. Eighteen liver samples were analysed. The mean value of Vitamin D in the liver was found to be 71.4 micrograms per 100 gms. of wet liver weight with a range of 46.2 to 116.3 micrograms per 100 gms. However, it was difficult to obtain liver samples after administration of massive dose of Vitamin D. Further work is in progress.

VENOM PROJECT

The method for holding and milking of snakes was standardized. The condition in cages was much improved by providing separate trays for each of the cages. Ten Kraits, ten Cobras and ten Russell's vipers were kept separately to study the monthly changes in the output and biochemical properties of the snake venoms. These snakes were milked every month and their output was recorded. Venom collected every time was pooled separately and lyophilised. The toxicity of the monthly pools was determined in terms of MLD by animal test.

Further biochemical studies were carried out on the venoms of elapid snakes. Enzymatic and toxicity studies were carried out on the venoms of *Bungarus caeruleus*, *Bungarus fasciatus*, *Naja naja* and *Ophiophagus hannah*. Three neurotoxins were isolated from the venom of *Bungarus caeruleus*. All these toxins were found to be homogenous. Two of these were free from enzymic activities and the third contained phospholipase-A activity. A non-toxic phospholipase-A of high purity was isolated and characterised from the venom of *Bungarus caeruleus*. A neurotoxin was also isolated from the venom of *Naja naja*. A simple method was devised to isolate cardiotoxin from *Naja naja* venom. All the purified fractions were of low molecular weight and have been biochemically characterised.

In vivo and *in vitro* studies of anti-coagulant and coagulant principles were carried out on *Naja naja* venom. *In vitro* studies showed that *Naja naja* venom contains a powerful anti-coagulant activity and that it acts at the intrinsic system of blood clotting mechanisms and specifically inhibits the activity of factor VIII (Antihaemophilic globulin). *In vivo* studies carried out in rabbits by injecting sub-lethal doses of venom by subcutaneous route indicated that the sub-lethal dose of the venom (0.2 mg to 0.8 mg/kg) produces prolongation of whole blood clotting time and recalcification time at the end of 6 to 8 hours. Levels of factor VIII go down with the increasing Cobra venom dose. Fibrinolytic activity increases with the increase in Cobra venom dose.

B. DEPARTMENTAL RESEARCH

DEPARTMENT OF BACTERIOLOGY

During the year under review, studies on the "Development of sub-merged and continuous culture methods for the growth of microorganisms" were continued. The department also continued the studies on "Susceptibility of rodent population in Maharashtra State to *Y. pestis*", "Radiation-killed Cholera Vaccine", "Use of labelled glucose for sterility testing of biologicals", and "Antibody response to cholera vaccines in volunteers". Experimental work was undertaken to seek a substitute for the imported and costly casamino acids for growing *C. diphtheriae* for the production of diphtheria toxin. Various modifications of the existing media and methodology used in the production of tetanus toxin were also tried.

Development of sub-merged and continuous culture method for the growth of micro-organisms.

Typhoid vaccines manufactured at this Institute are being assayed by the method of Habbu (1958) which consists of using two immunising doses subcutaneously. Recently, one dose immunising method has been recommended (Public Health Service Regulation, 1971, U.S.A.) in order to avoid the booster effect of the second dose in case where "two dose" technique is used. It was thought worthwhile to test few vaccines repeatedly by using one and two dose techniques and to find out if there was any discrepancy between these two methods. With this end in view, tests were carried out in two sets, one for the "one dose" and the other for "two dose" technique. The animals in both these sets were divided into three groups and were immunized subcutaneously. In one dose set, the first group of animals received heat-killed and phenol-preserved vaccine, the second received alcoholised vaccine and the third received standard acetone-killed and dried (A.K.D.) vaccine.

In the other set, animals in each group were immunized with the same vaccines as mentioned in one dose technique but with two doses of the vaccines at an interval of 7 days. Total volume of the vaccine injected in each mouse was the same in both the techniques.

Animals were challenged 15 days after immunisation in "one dose" and 7 days after the second dose in "two dose" techniques with Tyz (5/P) strain of *S. typhi* suspended in mucin. Deaths and survivals were recorded for three consecutive days after the challenge and ED₅₀ was calculated accordingly for each vaccine. In all, six tests were carried out to get a clear idea of the discrepancy between the two methods.

It will be seen from the results presented in Table 1 that the mean ED 50 value in respect of any of the vaccines is more or less the same in both the techniques,

though there is some difference in one or two tests in regard to heat-killed and A.K.D. vaccines. The results indicate that the ED_{50} of the vaccine remains almost the same, irrespective of whether one dose or two dose immunizing technique was employed for ascertaining its efficacy in mice.

As mentioned in the last year's report LD_{50} of *S. paratyphi-B*-a challenge strain received from Central Reserach Institute, Kasauli, for the standardization fermentor para typhoid B vaccines was determined in white NIH mice weighing 18-20 gms. The challenge strain was injected in numbers varying from 2·0 to 44050 organisms into the group of animals. To get proper values of LD_{50} three tests were carried out. The mean LD_{50} for mouse was found to be between 8 and 9 organisms.

In addition to the work mentioned above, tests on typhoid vaccines were repeated for their efficacy in mice. These included seven fermentor vaccines and three prepared from the growth of *S. typhi* on solid fortified medium. Results obtained were in accordance with the previous tests performed.

Radiation-killed cholera vaccine

It has been reported previously that the radiation-killed cholera vaccine was found to be as potent as the formaline-killed cholera vaccine, when tested by three different methods *viz.* the agglutinin response in rabbits, vibriocidal activity of immune sera and the ileal loop challenge method.

With a view to see if the rate and dose of radiation had any effect on the potency of the vaccine, suspension containing 60×10^9 organisms/ml of *Vibrio cholerae* Inaba strain 569-B and Ogawa strain S/12/1/58 were exposed to two different Cobalt-60 sources of 0.1 M.rad/hour and 0.5 M. rad/hour respectively. Four different doses ranging from 0.1 M. rad to 0.5 M. rad were used. The irradiated suspensions were then diluted to contain 60×10^9 organisms/ml and tested for their potency by active mouse protection test, along with a standard cholera vaccine. The results are given in Table 2, which indicate that within the ranges used, the rate and dose of radiation had no influence over the potency of the vaccines.

Quantitative toxicity tests were carried out on formalin-killed and phenol-killed types of cholera vaccine by injecting groups of mice with three different doses, *viz.* 30×10^9 , 6×10^9 and 1.2×10^9 organisms per mouse. Both subcutaneous and intraperitoneal routes were tried. The mice were observed and weighed every day for 8 days.

The toxicity of the different types of vaccines appeared to be almost the same. The LD_{50} when injected intraperitoneally, varied between 12×10^9 and 18×10^9 organisms. The LD_{50} by the subcutaneous route was more than 30×10^9 organisms (highest dose used) in all the cases.

Antibody response to two cholera vaccines in volunteers

Using two-dose schedule, antibody response of two cholera vaccines A and B, prepared from the same strains of organisms and having about the same mouse protective ability, but differing in the method of preparation, was discussed last year. It was also mentioned that for immunization of the volunteers, a two-dose schedule was used.

The work was extended further to study the antibody response of the same vaccines using one dose schedule and collecting blood samples at different periods after immunisation.

Volunteers

The volunteers included were the persons residing at Bassein and the surrounding vicinity. The volunteers for each vaccine group were allotted by random reference to table.

Dosage schedule

A one-dose schedule was used. A dose of 1.0 ml of the vaccine was administered subcutaneously and before injection, a sample of blood was collected. Samples were again collected 15 days, 1½ months and 3 months after immunization and analysed for their antibody response.

Agglutinin response

The agglutinin response was determined by tube agglutination test using heated suspensions of the vaccine strains. Titres, therefore, represent the 'O' antibody titres. Tables 3 and 4 show the agglutinin response pattern of the two vaccines against Inaba and Ogawa serotypes.

Out of 58 volunteers inoculated with vaccine A, 41 had pre-immunization titre of less than 1/10, whereas 17 had a titre varying between 1 : 10 and 1 : 40 for Inaba. In case of vaccine B, out of 61 volunteers, 15 had pre-immunization titre ranging from 1/10 to 1/80 for Inaba.

Fifteen days after immunization with vaccine A, 28 out of 40 volunteers showed a response varying from 1 : 10 to 1 : 160, whereas in 12 volunteers, the titre was less than 1 : 10 for Inaba. Out of 41 volunteers immunized with vaccine B, 28 showed a titre ranging from 1 : 10 to 1 : 160.

As far as absence of response to Inaba antigen is concerned, both the vaccines failed to produce response in one volunteer.

With Ogawa antigen, similar pattern of agglutinin response was observed. After injection of vaccine A, the titres varied between 1 : 10 and 1 : 640 except in one case where there was no response even up to a period of three months after immunization. The vaccine B showed almost similar response, except that the titres varied between 1 : 10 and 1 : 160.

The results showed that the difference in response to both the antigens in relation to the production of agglutinins, with the use of A or B vaccine, is insignificant.

Vibriocidal antibody response

After determining the agglutinin titre, all sera were subjected to vibriocidal antibody titration. Both A and B vaccines induced vibriocidal antibody titre by 1 to 4 logs equally in almost all sera tested. Highest number of volunteers showed a 2 log increase in titre with both Inaba and Ogawa antigens and with both A and B vaccines. Few volunteers failed to show response, but they had already a titre of 10⁻³ to 10⁻⁴ against both antigens. The results indicate that there is no difference in response to both A and B vaccines, showing similar vibriocidal response with both the vaccines.

Diphtheria Toxin production in Casein Hydrolysate medium

The medium at present used for growing the diphtheria organisms for the production of Diphtheria Toxin consists mainly of imported casamino acids. Difficulties are encountered in importing the casamino acids. In order to overcome these difficulties, attempts were made to investigate the possibility of substituting the casamino acids by casein-hydrolysate which is being regularly used at this Institute in the preparation of cholera vaccine.

There was a variation in the yield of the toxin almost from lot to lot. Moreover, the titres of the toxoids came down more rapidly on keeping. It is therefore, proposed to carry out more experiments to study the different factors affecting the yield and the stability of the toxoids. Attempts will also be made to investigate the possibility of using another substitute like peanut-llysate. Studies to develop a medium and to find out the factor responsible for the production of diphtheria toxin by submerged culture method in a fermentor will also be undertaken.

Studies in Pertussis vaccine

Various lots of pertussis vaccines were prepared and tested in order to compare the different media and methods of preparation used in the standard laboratories. Modifications of the media were also tried and the vaccines were tested for sterility, potency and toxicity. The two different media used were Verwey's medium and Cohen and Wheeler's medium. Both these media gave potent pertussis vaccine as tested by the active mouse protection test. Cohen and Wheeler's medium yielded a slightly higher potency of the vaccine than the Verwey's medium, but the difference is not significant.

Casein hydrolysate (acid-digest of casein) was also tried as a substitute for Casamino acids (Difco) in both the abovementioned media preparations and was found to give good results.

Pertussis vaccines were prepared by two different methods using two different killing agents, viz. (1) heating at 56°C for 20 minutes and (2) addition of 0.2 per cent formalin. Both the preparations gave good vaccines and it was difficult to establish the superiority of one type of vaccine over the other.

Both these vaccines passed the toxicity test as judged by the weight gain in mice.

Tetanus toxin production by different methods

Experiments were carried out to compare the toxin production in Massachusetts's Medium (routinely used for the production of tetanus toxin at this institute) and a slightly modified medium. The modified formula differed from Massachusetts's formula in its contents of tryptone (25 gms. instead of 30 gm/L), glucose (10 gms instead of 8.0 gms/L), and beef infusion (10 ml/litre) was also incorporated in the modified medium. Experiments were carried out in 250.0 ml capacity beaker, taking 125 ml of the medium. Using this new formula, an average of 85 Lf/ml with a maximum of 110 Lf/ml of toxin was obtained as compared to the average of 65 Lf/ml obtained in Massachusetts's medium.

Further attempts to obtain high titres of toxin using new formula in routinely used production beakers of 5.0 litre capacity and taking 3.5 litres of medium were completely unsuccessful. However, when experiments were carried out using the same ratio of surface area to volume for 5.0 litre beaker, it was observed that only 900 ml. of the medium could be taken and this gave a toxin titre of 100 Lf/ml. Thus, the results obtained with these experiments indicate that the old convention of producing tetanus toxin in tall and narrow containers filled two-third with the medium, no longer holds good to obtain optimum yield of the toxin. There should be wider containers if larger quantities of the medium are to be employed. It could be inferred from above results that the trials in fermentor are likely to give better yields of tetanus toxin.

Fluorescent antibody staining technique for *Y. pestis*

In an attempt to develop a rapid method for the detection of plague infection, both the direct and indirect methods were tried with more number of plague strains. Details are furnished under plague project included in this report.

TABLE 1

ED 50 values of different vaccines tested by different dose techniques

Test No.	VACCINES					
	Heat-killed		Alcoholised		A.K.D.	
	One dose	Two dose	One dose	Two dose	One dose	Two dose
1	.. 0.001468	0.002470	0.002710	0.002827	0.004949	0.004949
2	.. 0.002293	0.001650	0.002222	0.002222	0.007934	0.007062
3	.. 0.001250	0.001984	0.001766	0.002207	0.006305	0.01081
4	.. 0.001060	0.008450	0.002832	0.001428	0.007634	0.003473
5	.. 0.002182	0.001576	0.003271	0.003152	0.005891	0.003339
6	.. 0.001486	0.001486	0.001577	0.001971	0.01009	0.004277
Mean	.. 0.001623	0.001668	0.002396	0.002301	0.007184	0.006768

TABLE 2

Results of active mouse protection tests of cholera vaccines irradiated at two different rates to different doses Vaccine irradiated

No.	Strain of the vaccine	at the rate of 0.1 M rad/ hour to a total dose of				at the rate of 0.5 M rad/ hour to a total dose of				Standard vaccine killed and mercuric (P.M.N.)	cholera [Formalin- phenenyl nitrate] preserved
		0.1 M rad		0.2 M rad		0.17 M rad		0.5 M rad			
		ED ₅₀	*Relative	ED ₅₀	*Relative	ED ₅₀	*Relative	ED ₅₀	*Relative potency		
1	569 B Inaba	0.0000314	2.2			0.0000393	1.76			0.0800691	1
2	"			0.0000357	2.46			0.0000368	2.42	0.000089	1
3	"			0.000023	2.17	0.0000284	1.76			0.00005	1
	Mean	0.0000314	2.2	0.00002935	2.315	0.00003385	1.76	0.0000368	2.42	0.0000694	1
4	S/121/58 Ogawa	0.0000597	1.68			0.0000525	1.9			0.0001	1
5	"			0.000089	1.36	0.000063	1.9	0.0000724	1.67	0.00012	1
6	"	0.0000559	2.67	0.00005	2.98	0.0000668	2.23	0.0000691	2.16	0.0000149	1
*Relative potency		ED ₅₀ of the standard vaccine									
		ED ₅₀ of the vaccine under test									

TABLE 3

Agglutinin response of volunteers to two vaccines using one dose schedule

Vaccine	INABA			TITRES							
	Number of volunteers	Number of non-responders	Time of collection of sample	10	10	20	40	80	160	320	640
				Number of volunteers							
A	58	1	Pre-immunization ..	.. 41	6	5	5	..	..	..	..
	40		15 days after immunization ..	.. 12	7	8	8	3	2	..	..
	21		1½ months after immunization ..	.. 11	4	2	2	1	1	..	..
	23		3 months after immunization ..	.. 21	1	1	..	..	..	..	..
B	61		Pre-immunization ..	.. 46	6	7	1	1	..	..	..
	41	1	15 days after immunization ..	.. 13	6	8	9	4	1	..	..
	18		1½ months after immunization ..	.. 10	1	6	..	..	..	1	..
	25		3 months after immunization ..	.. 20	2	2	1	..	..	..	..

TABLE 4

Agglutinin response of volunteers to two vaccines using one dose schedule

Vaccine	OGAWA			TITRES							
	Number of volunteers	Number of non- responders	Time of collection of sample	10	10	20	40	80	160	320	640
				Number of volunteers							
A	58		Pre-immunization ..	.. 51	5	2	..	..	..	..	..
	40	1	15 days after immunization ..	.. 11	5	7	9	4	3	..	1
	21		1½ months after immunization ..	.. 7	4	2	6	2	..	..	..
	23		3 months after immunization ..	.. 21	1	1	..	..	..	..	..
B	61		Pre-immunization ..	.. 48	8	5	..	..	..	..	..
	41		15 days after immunization ..	.. 11	7	8	6	6	3	..	..
	18		1½ months after immunization ..	.. 6	4	5	2	..	1	..	..
	25		3 months after immunization ..	.. 21	2	1	..	1	..	..	..

COLLABORATIVE RESEARCH

Sterility testing by use of labelled glucose

Sterility testing of biologicals

Work on the sterility testing of biologicals using labelled glucose was continued. During the year under review, 8 samples of cholera vaccine, 4 of rat vaccine, 2 of A.T.S. and 2 of normal saline were tested. As usual, 10 μ of C labelled glucose was incorporated in 10.0 ml of nutrient broth in flask and 1 ml of the test sample was then added to it and the flasks incubated at 37°C. The counts were taken 24 hours after incubation.

Out of 8 cholera vaccine samples, one gave count, indicating thereby presence of contamination in the sample. Of the four rabies vaccine samples one gave the count, whereas the remaining samples of A.T.S. and normal saline were found to be sterile. All the results of sterility tests, using labelled glucose, were in conformity with the results obtained by I.P. test carried out simultaneously.

Antibiotic sensitivity testing using labelled glucose

The method of incorporating labelled glucose for ascertaining the sterility of the biologicals was also extended to the antibiotic and drug sensitivity tests for micro-organisms. The test organism employed was *Staphylococcus aureus* resistant to penicillin. Various antibiotics and drugs used were : kanamycin, streptomycin, sulphadimidine, erythromycin, ampicillin, gentamycin and tetracycline. The test was carried out by incorporating antibiotic or a drug into the medium containing labelled glucose and a culture of the test organism. Counts were taken 6 hours after incubation at 37°C. There was a considerable reduction in counts in the flasks containing antibiotic or of drug as compared to culture control without antibiotic or a drug. The antibiotics found to be active by this method are listed below in order of their activity :

(1) Erythromycin (2) Gentamycin (3) Tetracycline and Kanamycin (4) Sulphadimidine, Ampicillin and (5) Streptomycin.

DEPARTMENT OF BIOCHEMISTRY

Utilisation of horse blood cells after removal of serum or plasma from blood

Thousands of litres of horse blood cells remain unutilized after the removal of serum or plasma from the blood. It was thought worthwhile utilizing this waste material, which is abundantly available at this Institute, for the maintenance and growth of pathogenic organisms used in the production of vaccines and antitoxins.

Preliminary work was carried out to isolate proteins from the blood cells. Proteins were isolated from five different batches of horse blood cells and tested for their purity by determining total nitrogen. About 165 gms. of proteins (90 to 94 per cent) isolated from 1,000 ml. of blood cells were obtained. These proteins were hydrolysed by digestion only with hydrochloric acid. The residue (12 to 15 per cent) left out after digestion may be due to the hemin portion of the hemoglobin, still adhering to the protein molecules. The hydrolysates prepared were analysed for alpha amino acid nitrogen. The degree of hydrolysis was found to be the same as that of casein hydrolysate prepared under identical conditions. It appears from the results of alpha amino acid nitrogen (67 to 78 mg per 100 mg of total nitrogen) that the isolated proteins contain the minimum amount of hemin.

The liquid medium of blood cell protein hydrolysate containing 135 mg N per 100 ml was tested for the growth of *vibrio cholerae* and was compared with casein hydrolysate medium. It appears from the results that the growth response of *V. cholerae* in this medium is about 70 to 80 per cent as compared to that of the casein hydrolysate medium. The 30 to 20 per cent reduced growth may be due either to the excessive amount of iron still remaining in the proteins used for the preparation of medium or the deficiency of essential nutrients required for optimum growth. The estimation of the mineral elements and amino acids of the isolated proteins is being pursued further.

Isolation of insulin and other glandular products from glands and organs collected from slaughter house

The entire requirements of glandular products like insulin, corticosteroids, enzymes like trypsin, pepsin etc. are at present being met only by imports, although the raw materials for their production are available in the country. So far, the major difficulty in obtaining the raw materials, collected properly and at proper time, was that the slaughter houses of the country were not set up on modern lines for this purpose and the entire raw materials, which could save very valuable foreign exchange, were being wasted. However, with the increasing awareness of the utilization of these products, an up to date slaughter house working on modern lines has been established at Deonar in Bombay. Availing of this facility, the work of isolation and purification of insulin was taken up on a small scale to start with.

Initially, the work of isolation and purification of insulin was undertaken using sheep pancreas. As the yield was poor, it was thought worthwhile using bovine pancreas available at Deonar slaughter house. So, far five different

batches have been used for the isolation of crude insulin by the improved method of Pollock *et al.* (1968). For further purification of crude insulin, attempts are being made to standardize a suitable method.

Mode of action of drugs on the enzyme pattern of the Nematode (*Nematospiroides dubius*)

Infection due to *Ancylostoma* species is very common in India but therapeutic treatment is not well advanced, since the knowledge about the biochemistry of this nematode is meagre. In view of this, work was undertaken to standardize methods for determining the activities of tricarboxylic acid (T.C.A.) cycle enzymes of the parasite. Once the methods of estimation of enzymes are standardized, certain drugs found to be anthelmintic, will be screened to see their effect on these enzymes. It is also proposed to study the changes in serum protein pattern in animals infected with *Nematospiroides dubius*.

Studies on rickets with special reference to vitamin D and Calcium metabolism

It is well documented in the literature that Vitamin D is necessary for activation of intestinal calcium transport. The active transport is achieved by Vitamin D₃ through the synthesis of calcium binding protein. The protein synthesis is generally enhanced by cyclic adenosine monophosphate (cAMP). Initially, a method for the estimation of cAMP has been standardized using a radioassay technique.

Studies on the regulatory mechanism of calcium absorption from the lumen of the intestine of albino rats fed on low, high and normal calcium diet, will be carried out.

DEPARTMENT OF CHEMOTHERAPY

In the field of synthesis of organic compounds for possible therapeutic activity, the work reported previously was continued and some new problems were also taken in hand. More attention was paid to synthesis of compounds for antiparasitic and antitubercular activity as these are the major health problems faced by the country.

Synthesis of antiparasitic compounds

A large number of compounds were synthesised and studied for their anthelmintic activity *in vitro* against *A. lumbricoides* and *in vivo* against *N. dubius* and *H. nana*. Some of them are enumerated below :—

5 (or 6) Substituted-2-(N⁴-substituted—N¹-piperazinyl)methyl-benzimidazole derivatives.

As benzimidazole derivatives are known to exhibit good anthelmintic activity, a series of 5 (or 6)-substituted-2-(N⁴-substituted—N¹-piperazinyl) methyl-benzimidazole derivatives were synthesised and submitted for their anthelmintic activity. The compounds containing-NO₂ group on benzimidazole with benzyl or o-tolyl-piperazine have shown the mortality rate of the adult worm up to 95 per cent when tested against *H. nana* in mice.

2,6-Disubstituted imidazo (2,1-b) thiadiazole

Tetramisole, an imidazo-thiazole derivative, is a broad spectrum anthelmintic compound. Further, since some of the imidazo-thiazoles synthesised in this laboratory have shown good anthelmintic activity *in vitro*, a new series of, imidazo (2,1-b) thiadiazoles having various substitutions on 6-phenyl ring were synthesized. These compounds are being tested for their *in vitro* and *in vivo* activity.

2-Substituted-thiadiazole (3,2-a)-s-triazine-5,7-diones derivatives

Some tricyclic triazino derivatives, e.g. pyrido-thiazolo-s-triazine, had earlier shown good anthelmintic activity. However, the majority of compounds of this series were very sparingly soluble and a true activity pattern could not be judged during *in vitro* testing. A new similar bicyclic series of 2-substituted thiadiazolo-(3, 2-a)-s-triazine-5, 7-diones was then undertaken and a representative compound, when tested *in vitro*, showed significant anthelmintic activity. The series is now being exploited further.

Halogenated phenols containing isothiocyanato group

On the basis of structure of Bitoscanate, Bithinol and Dichlorophene, which are wormicidal drugs, synthesis of new series of compounds containing halogenated phenolic nucleus and isothiocyanato group has been started. These compounds will be screened for anthelmintic activity.

Anti-amoebic compounds :—5-Nitro-2-formylimidazoles

In search for potential anti-amoebic compounds, 1-substituted-5-nitroimidazole-2-carboxaldehyde and related azomethines and acid hydrazones were synthesised. Among these, a few compounds have shown anti-amoebic activity up to 5 µg/ml. The series is being further expanded for full evaluation.

Anti-tubercular compounds :— 2-Mercapto-1,3,4-oxadiazoles

As the 2-mercapto-1,3,4-oxadiazoles synthesised in this laboratory have shown promising activity, the structure was further exploited to get two series of compounds, viz.-[5-(2-substituted phenyl)-1,3, 4-oxadiazole-2-yl-thio] acetohydrazides and-[5-(2-substituted phenyl)-1,3,4-oxadiazole-2-yl-thio] acetamides.

All these compounds were tested *in vitro* for anti-tubercular activity and among them 2,4-dichlorophenyl derivative has shown activity up to 2 µg/ml.

5-Nitro-2-thienyl-N⁴-substituted piperazinyl sulphones

In our previous study, N¹-(5-nitro-2-furoyl)-N⁴-pyrimidyl piperazine has exhibited very good antitubercular activity. Similar derivatives, namely, thienyl-N⁴-substituted piperazinyl sulphones have now been prepared. The results of preliminary screening have shown 2-thienyl-N⁴-p-tolylpiperazin sulphone to possess anti-tubercular activity at 2 µg/ml concentration.

Anti-bacterial compounds

2-Substituted-4-(4'-methoxy-3'-aminophenyl) thiazoles and their Schiff-bases

Thiazoles are known for their anti-bacterial activity. Some new thiazoles viz. 2-substituted 4-(4'-methoxy-3'-amino-phenyl) thiazoles, were synthesised and submitted for anti-bacterial activity. The results are awaited.

Keeping in mind the anti-bacterial property of 5-halo-or 5-nitro-salicylaldehyde, Schiff bases of the above aminophenyl thiazoles using these aldehydes were prepared. All the compounds were submitted for preliminary screening and the results are awaited.

Anti-fungal compounds N¹-(5-substituted salicylideneaminoethyl)-N⁴-substituted piperazines

5-Halo and 5-nitro-salicylaldehyde are known for their anti-bacterial and anti-fungal activity. The Schiff bases were prepared using these aldehydes with N¹-substituted-N⁴-(β-aminoethyl) piperazines. Among these, some have shown anti-fungal activity against *Candida albicans* at a concentration 40 µg/ml while some others were active at 10 µg/ml against *T.rubrum* and *menta grophytes*.

Study of compounds for anti-tumor activity

Additional screening reports for the anti-tumor activity of the compounds in the series of bimethanesulphonates, benzaldehyde nitrogen mustards and pyrimidine derivatives, which were previously sent to National Cancer Research Institute (NCI), Bethesda, U.S.A. are still being received. About 40 reports were received. Two bimethanesulphonate compounds showed confirmed activity when tested in different laboratories against L1210 lymphoid leukemia. None of the compounds in benzaldehyde nitrogen mustard series showed appreciable anti-tumor activity.

DEPARTMENT OF CLINICAL PATHOLOGY

Typhoid Scheme

Under the Typhoid Scheme Unit, 681 calls were attended to Blood samples were collected for blood culture and Widal tests. An analysis of the data showed the following results :

Total No. of Samples			Widal +ve Culture —ve	Widal—ve Culture +ve	Widal +ve Culture +ve	<i>S.</i> <i>typhi</i> isolated	<i>S.</i> Para A isolated	Stool Culture +ve
Blood	Urine	Stool						
681	344	209	27	231	5	54	24	8

Fifty-four strains of *S. typhi* were sent to the Salmonella Phage Typing Centre at Lady Hardinge Medical college, New Delhi. The following phage types were reported :

							Pages each	Nos.
A4		A6,	J1	D2	V1	K1	Vi-ve	1
C5	..							7
A	..							12
D1	..							3
O	..							3
E1	..							12
46	..							3
VII+IV	..							3
Deg. Vi	..							4

Typhoid carrier study

Blood and stool samples from 86 typhoid patients were collected on the day of discharge from the Kasturba Infectious Diseases Hospital, Bombay. During the peak months of July, August and September, *S. typhi* was isolated from the blood of two patients at the time of discharge and *S. para A* was isolated from the stool of one patient. Vi haemagglutination test was negative in these three cases. A month later, the stool and clot cultures as well as Vi haemagglutination test of the three patients were found to be negative.

Two-hundred and ten strains of *N. gonorrhoea* were tested against routine antibiotics, viz. sulphonamides, penicillin, streptomycin, tetracyclin, chloromycetin and erythromycin and sixty strains were tested against the combination of sulphamethoxazole and trimethoprim.

Haemagglutination test using alkaline extract of *N. gonorrhoea* and fluorescent antibody test have been standardized for the serological diagnosis of gonorrhoea. Serology has a definite role in the diagnosis of gonorrhoea, particularly in female cases, wherein the isolation rate is not satisfactory in spite of using the best possible media. So far, 225 sra have been collected and further work in progress.

Study of *Candida* infection in female patients attending the V.D. Clinic has been recently undertaken. Final identification of *Candida* species and preparation of suitable antigens for serological diagnosis are in progress.

Anaerobic culture

For this work, clinical materials from nearby hospitals were collected. The materials originated mostly from infected female genital tract and septic wounds. This year, 170 specimens were collected, out of which 28 per cent were positive for anaerobic bacteria. The anaerobic bacteria isolated belonged mainly to *Streptococcal* 56 per cent and *Clostridial* 34 per cent species.

Rheumatoid arthritis

The work on the detection of R.A. factor in the sera of rheumatoid arthritis patients by using locally prepared reagents was continued and sera from various Government and non-government hospitals were tested.

During the last year, endotoxin of *S. typhi* was extracted and labelled with ^{51}Cr by the method of Felsenfeld (I.A.E.A. report, 1973). The labelled antigen and 17 sera samples of suspected cases of typhoid fever were radio-immuno-assayed on filter papers. Widal test and clot cultures of these cases were also done.

Out of 17 cases, 13 were found to be positive for Widal test and in 2 cases *S. typhi* was isolated in clot culture. In the radio-immunoassay, 12 sera samples gave a positive reaction but the radio-activity of the samples did not vary according to the agglutination titre.

This year, polysaccharide of *S. typhi* was labelled and the labelled antigen gave a better result as compared to the previous one. Thus, 10 sera samples positive for widal test for *S. typhi* also gave a positive result in the radio-immuno-assay and in one case where *S. para A* was isolated, failed to show any positive radio-activity for the labelled antigen.

A standard *S. typhi* antiserum (titre 1600) was diluted and radio-activity and agglutination titre of the diluted samples were determined. The radio-activity corresponded to the agglutination titre of the samples.

Venereal diseases

Gonorrhoea

Clinical material is collected regularly from the V.D. Clinic, Belasis Road, Bombay. During this year, 978 cervical specimens were collected and *N. gonorrhoea* was isolated from 170 male and 291 female Patients respectively.

DEPARTMENT OF IMMUNO-HAEMATOLOGY

Studies on Australia antigen

The screening of the sera of patients suffering from various clinical conditions for Australia antigen (HB_{Ag}) and antibody (HB_{Ab}) was continued by counter immuno-electrophoresis technique (CIEP). Results are given in Table 1.

Screening for Australia antigen by Auscell test

The department obtained, through the courtesy of Messers. Abbott Laboratories, U.S.A., kits for carrying out the screening for HB_{Ag} by the newly developed technique of "Auscell" reversed passive haemagglutination technique and the sera of 247 persons were screened simultaneously by this technique as well as by other techniques, viz. A.G.D. and C.E.P. The samples of sera found positive by RIA technique were also included in this series to compare the sensitivity of both the methods. The method has the advantage of better sensitivity than A.G.D. and C.E.P. techniques, viz. simplicity and avoidance of false positives as in the RIA method. The results are given in Table 1.

Relationship of variant lactate dehydrogenase (LDH) isoenzyme in HB_{Ag} positive individuals

This fascinating work is the result of a coincident finding in the cases of hepatoma, which showed the presence of an anomalous LDH band occurring between LDH₄ and LDH₅. All these cases having anomalous LDH band were also positive for Au antigen and this led to further evaluation of 20 more non-hepatitis cases having Au antigen. All the 20 cases showed the presence of anomalous LDH band. This double-blind study was further supported by the removal of HB_{Ag} and anomalous LDH band by absorption with the corresponding antibody *in vitro*. These observations certainly demonstrate the association of LDH isoenzyme with HB_{Ag}. Further studies are in progress to evaluate the significance of this unique finding.

Red cell enzymes in haematological disorders

In the last year's report, a summary of the data was presented. The detailed analysis of these large data has now been completed and the conclusions drawn are given below.

A project on red cell enzyme profile in haematological disorders was undertaken (i) to detect the abnormality of key enzymes, if any, and emergence of any specific pattern, typical of a particular disorder; (ii) to unearth specific enzyme defects, hereditary or acquired, in connection with the haematological disorder studies.

The following conclusions have emerged from these investigations: (i) increased activity of the enzyme G-6-PD was observed in all the categories of haematological disorders studied. The high mean values were found in iron deficiency anaemia, dimorphic anaemia, megaloblastic anaemia, haemolytic anaemia, leukemia and in thalassemia, whereas normal G-6-PD activity was observed in aplastic anaemia and the absence of the enzyme activity in G-6-PD deficiency anaemia; (ii) high PK activity was observed in all the haematological

disorders except in aplastic anaemia. The increase in the activity of these enzymes could be due to the reticulocytosis, (iii) high levels of GSH were found in iron deficiency anaemia and dimorphic anaemia and also in leukemia and thalassemia. Normal or lower levels were found in G-6-PD deficiency anaemia, (iv) GSH stability test of Beutler has proved to be an important marker in the study of various haematological disorders. GSH was found to be more unstable in cases of iron deficiency anaemia, dimorphic anaemia and G-6-PD deficiency. On the other hand, GSH was stable in cases of haemolytic anaemia, leukemia and thalassemia, (v) these variations in the red cell enzyme profile help in understanding the metabolic changes in red cells in different haematological disorders on the basis of biochemical parameters.

Enzymes in haemolytic anaemia

In continuation of the previous studies, an attempt was made to determine the role, if any, played by important intermediates, namely, ATP and 2, 3-DPG. The methods for these enzyme assays have been standardized as also levels in normal males, females and new born infants established. Results are given in Tables 2 and 3.

Results in the normals indicate a slightly lower mean ATP level in females as compared to males, though these differences are not statistically significant. ATP levels in new-born infants appear to be within the normal range of adults; however, 2, 3-DPG levels in newborns were significantly lower than in the adults. Further work on ATP and 2, 3-DPG levels in haematological disorders has just been taken up.

Comparison of two screening techniques for the G-6-PD deficiency

There have often been reports in Indian literature that the incidence of G-6-PD deficiency is somewhat higher in different parts of India as indicated by the methemoglobin reduction test. Although this test has also been reported in W.H.O. Technical Bulletin, as one of the standard techniques, a question has often been raised as to its comparative value using BCB dye decolorization test of Motulsky. It was, therefore, thought necessary to undertake the evaluation of the two techniques, so that the drawbacks of each test could be brought out in comparison with the quantitative assay method, which is the ultimate answer to the levels of G-6-PD. Table 4 gives the comparative findings of the two methods. It would be evident that 5 of the 65 normals identified as deficient by MR test, were normal by BCB dye test and enzyme assay. Similarly, though 4 of the 15 new born jaundiced infants showed deficiency by MR test, they were normal by the other two techniques. All the eleven G-6-PD cases identified by BCB dye test were also deficient by MR test and enzyme assay technique. Further work on this problem is being continued with a larger series in order to evaluate the possible causes of the discrepancy by MR test.

Red cell preservation

This project was initiated last year. Anticoagulants like CPD and ACD were used. These studies were continued using biochemical parameters like glucose

and haemoglobin levels and osmotic fragility in ACD, CPD and ACD (Russian formula) as given below :—

Russian Formula ACD

Citric acid	..	..	..	..	0.75 gm.
Dextrose	..	..	..	..	3.00 gm.
Na ₃ PO ₄	..	..	..	..	0.75 gm.
Lavomycetin	..	..	..	..	0.015 gm.
Distilled water	..	..	..	..	100 ml.

Preservation by ACD and CPD appeared to be superior over the Russian formula. Preliminary studies have also been carried out on intermediates like ATP and 2, 3-DPG in the preservatives and also the efficacy of adding purine derivatives like adenosine and inosine. Some beginning has also been made to study the effect of radiation sterility using 0.08 M/rads of gamma radiation (from radio cobalt source). The sterility was maintained for 3 weeks, other biochemical changes are being studied.

Specific immunoglobulins produced after tetanus toxoid immunizations

A fresh batch of 105 prison donors was taken up for hyper-immunization against tetanus. Out of these prisoners, only 35 completed the course of immunization. Even after 4 doses of the toxoid (5 Lf), titre of the anti-tetanus antibody was 4 to 5 units. This is not considered sufficient for plasma pool for fractionation. The blood of these donors was collected and plasma separated and preserved. There is a recent reference in W.H.O. publication wherein Lf of the tetanus toxoid used for this purpose is 25 instead of 5. Hence a trial will be given by using this type of toxoid. Scientists from Pasteur Institute, Paris, also stated that special type of toxoid is used for this purpose. Attempts in this direction will be made.

Anti-D : A batch of anti-D prepared sometime back by the modified method of Kabi was stored for 1 year to study its stability. It was tested by B. G. R. C. and found to be quite stable. A bigger batch is now under process.

Biochemical and immunological studies of human fibrinogen and cryoprecipitate

Fibrinogen and anti-haemophilic globulin were prepared by different methods. Anti-sera against both these products were prepared by purification.

Estimation of fibrinogen was carried out by Biuret's method, thrombin clotting time method (Farrel and Wolf 1973) and by radial immuno-diffusion technique. A quantity of 0.8 ml of anti fibrinogen was used for 10 ml. agarose. One-hundred samples were tested by this technique.

Fibrinogen was also prepared from cryoprecipitate supernatant plasma by adding 10 per cent alcohol. Two bottles of such fibrinogen were pooled together to give one bottle of fibrinogen. This method is helpful in preparing both cryoprecipitate and the fibrinogen.

Cryoprecipitate was taken for purification by Hoyer's method. Thrice re-precipitation was done and fibrinogen was almost removed and tested immunologically against anti-fibrinogen. Radial immuno-diffusion technique

of AHG was used was with 0.8 ml. of anti AHF for 10 ml. agarose. Standard graph was plotted-AHG level of 100 samples was estimated together with AHF activity. This technique was developed in this laboratory.

Immuno-biochemical aspects of rheumatoid syndrome

Rheumatoid disorders are collagen disorders supposed to be autoimmune in origin, leading to various clinical features like polyarthritis fibrositis, the exact nature of which is not definitely known, but is presumed to be immunoglobulin M with sedimentation of 19-22 S. Many diagnostic tests have been evolved to diagnose this condition. Latex fixation test is one of the sensitive and dependable tests. The latex particles coated with active gamma globulin molecules produced by this department have been found to be suitable for this test.

One-hundred and seventy two samples were tested last year for R. A. factor test, haptoglobins and immunoglobulins. The investigation was continued and two-hundred and forty-five samples were tested. Results are given in Table 5.

Anti-coagulant activity of cobra venom

Naja Naja venom possesses a powerful anti coagulant activity. *In vitro* studies of the *Naja Naja* venom showed that the venom acts in the intrinsic system of blood clotting mechanisms, particularly on Factor VIII (anti-haemophilic globulin.) *In vivo* studies were carried out on rabbits by injecting sub-lethal doses of venom (starting dose 0.2 mg. kg.) subcutaneously. Samples of blood were collected after a period of 30 minutes, 1 hour, 4 hours and 8 hours. The dose of venom was increased to 7 mg/kg. of the rabbit weight. This venom dose was found sufficient to kill the animal within 1 hour.

The results indicate that the action of Cobra Venom with a sub-lethal dose (0.2 mg. to 0.8 mg/kg.) produces prolongation of whole blood clotting time and recalcification time at the end of 6 to 8 hours. Levels of factor VIII go down with the increasing dose of Cobra Venom. Fibrinogenolytic activity was seen when 0.2 mg. to 0.8 mg/kg. of the Cobras Venom was injected.

TABLE 1
Incidence of HBAG in different categories of patients

Clinical condition	No.	HBAG	Percentage
(1) Multitransfused Patients	60	5	8.0
(2) Jaundice	130	8	6.0
(3) Renal Patients on dialysis	42	1	2.4
(4) Post-transfusion hepatitis	15	1	6.5

TABLE 2
G-6 PD deficiency among 123 cases

Clinical state	No. of cases	Number showing G-6-PD Deficiency
Haemolytic anaemia	171	12
Neonatal Jaundice	60	6
Total	231	18

TABLE 3

Levels of ATP and 2, 3-DPG in normal adults and new-borns

	No. tested	ATP moles/gm. Hb		2,3-DPG moles gm.Hb	
		Range	Mean	Range	Mean
Males ..	20	1.615 — 4.7	3.008	5.64 — 14.96	8.91
Females ..	20	1.03 — 4.35	2.636	5.8 — 15.7	8.719
New-born males ..	10	1.194 — 4.52	2.698	1.005 — 9.457	3.359
New-born females ..	10	1.378 — 4.19	2.665	1.008 — 8.321	3.82

TABLE 4

Comparison of two screening methods : BCB dye test and micro methemoglobin reduction for the detection of G-6-PD

	No. tested	Methods			
		BCB DYE Test		MR MICRO Method	
		Normal No.	Deficient No.	Normal No.	Deficient No.
Normal controls ..	65	65	Nil	60	5*
G-6-PD deficient ..	11	Nil	11	Nil	11*
Hyper bilirubinemia cases ..	15	15	Nil	11	4*

* These samples showed the presence of enzyme by quantitative assay.

** Samples showed zero level of G-6-PD enzyme by quantitative assay.

TABLE 5

Immunological findings in suspected rheumatoid cases

	Percentage distribution of haptoglobin				Immunoglobulins						
	2-2	2-1	1-1	0-0	IGN	G	IG	1gG	1gG	1gN	1gN
In RA positive..	78	17	0	5	60	28	12	4	11	8	1
In RA negative	81	15	1	2	50	40	10	8	8	8	1

DEPARTMENT OF IMMUNOLOGY

Studies of snake venoms

In view of study of biochemical aspects of components of elapid venoms, this work was taken up under Diamond Jubilee Fellowship.

Cobra and Krait venoms were studied. In case of *Bungarus caeruleus*, mild physico-chemical methods like electrophoresis and chromatography, using dextran gels and ion-exchange cellulose were tried. Three different components of biochemical character were obtained, two of which were free of any tested enzyme and the third had phospholipase-A activity. A nontoxic highly purified phospholipase-A was also purified from *Bungarus caeruleus* venom. The preparation was 40 times the original venom activity.

A toxic component from *Naja naja* venom was purified to a high degree.

A simple method was devised for purification of cardio-toxin from venom of *Naja naja*.

Toxoidation of snake venoms

Experiments were carried out to obtain a toxoid of venoms of four Indian snakes, viz. Cobra, Krait, Echis and Russell's viper, which could be used for active immunization of horses for the production of antivenin and if so required, for active immunization of human beings.

Cobalt 60 was used as a source of irradiation for toxoidation. Preliminary study was carried out to immunize guinea-pigs. Two horses also were used for immunization.

Studies on scorpion venoms

Symptoms of envenomation were studied in mice, guinea pigs and cats as well as on isolated auricle and ileum. Possibilities of preparing antivenin against the black scorpion venoms were explored. Precipitating type of antibodies were detected, but no protection was observed even to 1 MLD in mice.

Biochemical aspects of *Echis carinatus* venom

Attempt was made to study crude *Echis carinatus* venom and fractions in order to study biochemically the characteristic components. The venom was found to contain the following activities :—

- (1) Proteolytic (2) Phospholipase-A (3) Phosphodiesterase (4) ATPase
- (5) L-amino acid oxidase (6) DNase (7) RNase (8) Coagulant Principle
- (7) Haemorrhagic principle.

Intravenous MLD was approximately 35 Mcg. The disk electrophoresis showed 13 bands. Zone electrophoresis on pevicon indicated anodic mobility for toxic component. By gel filtration, toxic fraction indicated high molecular weight. Ion-exchange chromatography on DEAE cellulose gave 5 times the purification of toxic component.

Immunological studies on leprosy

In view of the grave leprosy problems faced by the world, immunological studies were taken up on this problem.

Standardization of lymphocyte transformation test with non-specific activator phytohaemagglutinin, was carried out using normal human peripheral blood lymphocytes. It is proposed to evaluate the immunological response of leprosy patients, their contacts and non-contacts by *in vitro* method of lymphocyte transformation to PHA and *M. leprae*.

It is also proposed to purify and characterize antigen specific to *M. leprae* which could be used in place of the crude bacillary lepromin to test the hypersensitivity of *M. leprae*.

DEPARTMENT OF PHARMACOLOGY

The Department has been involved in the screening of synthetic compounds and plant materials for their potential medicinal properties. The compounds were received from the Chemotherapy Department. Indigenous plant materials were received from Dr. M. V. Subbarao of Osmania University, Hyderabad, tested under a project supported by the Central Council for Research in Medicine and Homeopathy. A few compounds were received from Central Drug Research Institute, Lucknow. A small section in the Department is also responsible for both synthesis of new compounds and extraction and purification of plant material.

The screening tests used at present are as follows :—

- (1) Antibacterial effects, (2) Anti-Leprosy, (3) Anti-filarial, (4) Anthelmintic (Hook-worm, Tape-worm), (5) Anti-amoebic, (6) Anti-inflammatory.

The department actively participates in the following research projects —

- (i) Venom Group,
- (ii) Soil-transmitted nematodes,
- (iii) Filariasis.

The outstanding feature of this year's work was that a very promising anti-asthmatic activity was observed in one of the synthetic compounds received from Dr. Subbarao. Further work is in progress.

Study of new anthelmintics

Over two-hundred synthetic compounds and few plant extracts were screened in mice against *Hymenoleps nana* and *Nematospiroides dubuis* infection. The results are shown in the accompanying tables. None of the compounds showed a promising activity.

The effects of Bephenium naphthoate in *N. dubuis* infection in mice were studied by performing an autopsy four-hourly and looking for the presence of worms in the intestine and for eggs, larvae or worms in faeces.

Anti-amoebic screening

The screening of organic compounds received for testing from Chemotherapy Department for hepatic infection in hamsters and intestinal infection in rats was continued.

Compound number 2768 showed promising activity in hepatic amoebiasis and compound number 3097 showed promising activity in caecal amoebiasis.

Study of anti-leprosy drugs

(i) *Screening of new compounds.*—Shepard's method of cultivation and harvesting of Leprosy bacilli was standardised and the following four

compounds were studied. All of them showed some activity. Further work is in progress.—

- (a) 4-4' Di (chloroacetylamine) sulphone,
- (b) 4-4' Di (glycolamide) diphenyl sulphone,
- (c) Schiff base of indole-3-aldehyde,
- (d) Schiff base of substituted aldehyde.

(ii) *Clinical trial on Rifampicin*.—A clinical trial was undertaken with the help of Dr. R. Ganapathy of Acworth Leprosy Hospital. The patients were given Rifampicin for a short period (7 days) followed by DDS. It was found that the patients did not become non-infective (as tested by mouse foot-pad technique) within 7 days.

Drug resistance in leprosy

A few cases of leprosy, which were not responding to DDS, are now found to be cases of drug resistance to DDS as proved by the use of Shepard's foot-pad technique. There is more likelihood of emergence of drug resistance if the treatment is not adequate and regular. It was, therefore, decided to find out the extent of the problem of resistance to DDS and treat the resistant cases by alternative method of treatment. This work has been undertaken under project supported by the Indian Council of Medical Research.

Anti-inflammatory drugs

Nearly one-hundred compounds received from Chemotherapy Department were tested by the method of carrageenin induced edema in rat hind paw. Three compounds in a dose of 100 mg/kg showed an effect equal to that of phenylbutazone used in a dose of 100 mg/kg.

Venoms from sea-snakes

Marine toxicology is not given due attention in our country. With a view to initiate the work, a small group was first formed to study venoms from sea-snakes. A rapport was established to obtain different species of sea-snakes in required quantities.

The unit has been involved in testing indigenous plant extracts and synthetic compounds for their anti-asthmatic activity. A battery of tests has been employed for the work, which includes *in vivo* as well as in *in vitro* techniques. The following methods are used for the screening :—

- (i) Passive cutaneous anaphylaxis (Ovary 1952),
- (ii) Passive peritoneal anaphylaxis (Kaliner *et al* 1972),
- (iii) Guinea-pig respiratory resistance (Konzett and Rossler 1940),
- (iv) Guinea-pig tracheal chain (Castillo and de Beer, 1947),
- (v) Chopped lung anaphylaxis (Vane, 1969),

In addition to these techniques, the drugs were also subjected to various studies in order to find out their pharmacological properties, such as anti-histaminic and anti-cholinergic effects.

The unit tested the following drugs using the abovementioned technique –

Indigenous

(i) Sub-fractions of the chloroform extract of *Tylophora indica*

TA/PL-1

TA/PL-2

TA/PL-3

TA/PL-4.

(ii) Fractions of *Mesua ferrea* seeds and stems

C2/MF/S

C3/MF/S

C4/MF/S.

(iii) A new indigenous plant extract :

KR/NVS/H

Synthetic : KR/NVS/E

KR/NVS/F

KR/NVS/G

KR/NVS/I

KR/NVS/J

KR/NVS/K

KR/NVS/L.

Promising anti-asthmatic activity was exhibited by the fractions of *Tylophora indica*, KR/NVS/H and two synthetic compounds KR/NVS/E and KR/NVS/L. Synthetic drugs 'E' and 'L' demonstrated potent anti-anaphylactic activity and potentiated the bronchodilator effects of Isoprenaline, but were devoid of *per se* effect. Synthetic drug 'E' was equiactive to Disodium chromoglycate in inhibiting histamine release during antibody antigen interaction. It was less potent (about 60 per cent as potent as DSCG). *Tylophora* extracts were found to possess *per se* bronchodilator activity, potentiated isoprenaline, and inhibited histamine release. Further work on these fractions is in progress.

DEPARTMENT OF QUALITY CONTROL

Study on *Vibrio parahaemolyticus* in Bombay

Work on this problem was continued. Strains of *V. parahaemolyticus* could be isolated from a variety of fish like shell fish, oysters, shrimps and crabs from the coast of Bombay. Investigation on faecal samples of animals had culminated in isolation of *V. parahaemolyticus* from dogs. But *V. parahaemolyticus* could not be detected in stool samples collected from 1,005 cases of gastroenteritis. The reason for this non-isolation could be that the fish is properly cooked before consumption and not raw as in other countries or because the stool samples are collected after administration of antibiotics and other drugs to the patients.

Further work on other aspects of the problem will be undertaken.

Studies of immunoglobulins with special reference to antibody patterns in pooled and purified mono-specific immunoglobulins

These studies have been taken up from the public health point of view in order to investigate the feasibility of mono-specific immunoglobulins as a protective measure against bacterial and viral diseases.

The immunoglobulins IgG, IgA and IgM were separated from human γ -globulin injection manufactured by the Haffkine Institute, by employing column chromatography. One-hundred and five samples of Plasma, γ -globulin and immunoglobulins were tested for antibody titres of tetanus, small-pox and typhoid.

Tetanus antibody titres have been determined "*in vivo*" by neutralisation technique in animals. The titres were found to be high in IgG varying from 0.1 to 0.5 I.U./ml. The titre of other fractions is negligible.

Small-pox titres were done by haemagglutination inhibition technique on Perspex plates. The IgG type immunoglobulins exhibited high titre (varying from 64 to 256).

The antityphoid levels have been determined by the Widal Test. The IgM fraction showed high up to 1:80.

Antibody studies in filarial cases

This work was undertaken to investigate into the immunological aspect of filariasis which is a project of the Institute.

The antibody levels were estimated on the tripartigen plates (against IgG, IgA and IgM) and mono-specific antisera (against IgG, IgA and IgM) supplied by Behringwerke, Germany. Ninety-three samples of sera of MF-positive, MF-negative and disease positive cases of filariasis collected from Murdhe, Rai and other villages in Maharashtra were used for this investigation.

The samples were put up for estimations on the tripartigen plates and antisera slides which were standardised for immuno-diffusion.

After 48 hours, the results were read.

The IgG and IgM levels were found to be high in all the filarial stages, ranging above 1591 mg per cent and 235 mg per cent respectively, while the IgA levels were almost normal.

Dynamics of disinfection

The study covers the behaviour of two disinfectants, viz. formalin and phenyl mercuric nitrate, at different pH levels (5.5, 7.5 and 9.0) and temperature levels at 4°C, 27°C and 37°C on *Staphylococcus aureus*, *Shigella dysenteriae* Flexner and *V. cholerae* Inaba and Ogawa.

Experiments were conducted to find out optimum quantity of the sodium metabisulphite required to neutralise the formalin, so as to get the maximum revival of organisms.

The following parameters were selected for the study :—

(1) Mean death time 't'—mean of the time interval at which the sub-culture showed growth and the next time interval at which the sub-culture failed to show any growth. A study of this factor, using two different media, viz. the routine sterility testing medium for aerobic organisms and the ordinary nutrient broth, reveals that this 't' varies with each medium.

(2) The death rate 'K'. $K = \frac{1}{t} \log \frac{A}{B}$: where A and B are the number of survivors at the beginning and at the end of the time interval 't'.

The remaining parameters, viz. concentration exponent 'n' and the temperature coefficient are to be studied.

Estimation of the amount of formalin absorbed by the organisms has been made using the method given in N.I.H. Manual.

Similar work is in progress in respect of the disinfectant Phenyl Mercuric Nitrate with the antagonist Sodium thioglycollate. But the work has been hampered due to non-availability of good quality of sodium thioglycollate. An alternative method, i.e. by diluting the reaction mixture, is being studied.

Biological standardisation of antivenin

About 7 individually pooled venom samples of Cobra, Russell's Viper, Krait and Echis were collected. The LD₅₀ dose of each of the venoms, viz. Cobra, Krait, Russell's viper and Echis was determined by using 5 mice for each dilution. Four dilutions of each venom were used and the LD₅₀ was calculated by the method of Reed and Muench.

The neutralisation capacity of polyvalent antivenin against each of the abovementioned venoms is estimated separately using 5LD₅₀ of the venom as the test dose. Appropriate amount of the dilution of the antivenin was mixed with the venom, so that 0.5 ml. of the mixture contained one test dose of the venom.

The mixtures were allowed to stand at 37°C. for 30 minutes and then mice were injected intravenously in 0.5 ml. amounts. Calculations were made to determine the LD₅₀ of the venom neutralised by 1 ml. of reconstituted polyvalent antivenin.

Seven polyvalent antivenin batches, out of the 61 batches collected, were tested as above ; 5 more batches were tested with respect to only Cobra and Russells Viper.

The study revealed that the batches tested so far neutralise the same amount, of Krait venom. They vary in their neutralisation capacity in comparison with other three venoms.

Study of contaminants in biologicals and sterile area

An investigation was initiated to isolate and identify the micro-organisms that are encountered during the sterility testing of bacterial and viral vaccines antitoxins, antivenins, toxoids, transfusion fluids, etc. Aerobic, anaerobic organisms and fungi will be isolated and identified ; so also, the organisms that lurk in the sterile area. So far, fungi belonging to *Penicillium* (*Asymetrica* *Velutina*, *Asym. divertica*, *Biverticilate symmetrica*, *digitatum*) *Aspergillus* sp. and *Curvularia lunata* were isolated. The study is in progress and will also be extended to other aspects of the problems.

A comparative biochemical and immuno-chemical study of anti-bodies against tetanus and diphtheria

During the period under review, rabbits and guinea-pigs were immunized with diphtheria and tetanus toxoids. By using Ref's method, IgG was isolated from the anti-tetanus and anti-diphtheria sera of rabbits and guinea-pigs.

The anti-diphtheria rabbit serum, differs from the anti-diphtheria guinea-pig serum in respect of protein concentration, antibody titre and carbohydrate content of IgG.

The concentration of fractions (IgG) isolated from antisera of rabbits and guinea-pigs does not differ much.

The rabbit anti-diphtheria serum has protein concentration of 8.0 mg/ml, antibody titre 10.4 I.U./ml., IgG content 0.6 mg./ml. and carbohydrate content of IgG 61.0 r/ml., whereas the anti-diphtheria serum has protein concentration of 6.7 mg./ml., antibody titre 7.8 I.U./ml., IgG content 0.6 mg./ml. and carbohydrate content of IgG 47.3 r/mg.

Using the methods of immuno-diffusion and immuno-electrophoresis, samples of sera as well as their fractions (IgG) were identified.

RADIATION BIOLOGY UNIT

radioimmunoassay for hepatitis associated antigen

The unit undertakes this test for detection of hepatitis-B antigen in respect of commercial samples. Presently, it is being done with materials available from Messrs. Abbot Laboratories, U.K. However, after availability of antisera and purified antigen, the unit intends to manufacture the kit for testing this antigen. The kit will also be made available to other Institutions on request. Preliminary immunisation of rabbits is in progress. The purification of virus by ultracentrifugation is also being done.

irradiation of hepatitis-B virus

The unit contributed significantly to the break-through in virus research, namely lapinization of strain of hepatitis associated antigen. The major part of inactivation and identification of virus has been carried out by the unit. The virus is inactivated for the control experiments with 60 Cobalt gamma irradiation at a dose of 0.25 Mrads to more than 2.5 Mrads. It was shown that this process destroys the infectivity of the virus and retains most of its antigenic property. The successful serial passage in rabbits was identified by radioimmunoassay procedures with suspensions of the selected rabbit tissues. An "*in vivo*" administration of labelled antibody has also shown multiplication of virus in infant rabbits. The studies are still in progress.

Whole blood irradiation

Aliquots of whole blood collected in different anticoagulant solutions were given graded doses of 60 Cobalt gamma irradiation to see the effect on preservation and stability of red blood corpuscles. The doses administered ranged from 0.05 to 0.25 Mrads. Periodic sterility test, measurement of enzyme levels and red blood cell survival were carried out with periodic samples. It was shown that low dosage brings about more survival of red blood cells even after 3 weeks. However, the high dosage may haemolyse the red corpuscles with hydrolysis and thus cannot be considered suitable for such a process. The study is still in progress.

Immune response in malnutrition

A group of selected rural population in Thana District exhibiting classic signs of undernutrition was given tetanus toxoid on an almost standard clinical trial basis. The individuals were subsequently bled and bacterial, immunological and haematological parameters were estimated. The cellular immunity was tested by the method of tuberculin testing and by tritiated thymidine uptake by the lymphocytes. A preliminary paper on this project was presented in the Fourth International Conference on Tetanus held at Dakar, Senegal.

Adrenocortical function in protein malnutrition

The study on the effect of protein deprivation stress on the adrenocortical function has been continued. The investigations mentioned earlier, such as estimation of 24-hour urinary 17-keto-steroid, urinary creatinine, plasma corticosterone, serum proteins and albumin etc., along with histo-pathology of adrenal and liver tissues, were carried out at 1, 2, 3 and 4 week intervals.

The urinary creatinine shows significant rise in the protein-deficient group at the end of first week and thereafter, the level was found to have been lowered and comparable with that in normal group. Urinary excretion of 17-ketosteroid did not show any correlation with the period of treatment in either of the groups and the results were not consistent in all the studies. The levels of plasma corticosterone were higher at all the intervals in the protein deficient group as compared with those in the normal group, though they were within the normal range. The plasma proteins and albumin indicated gradual fall in level with the advancement in the period of treatment in the protein deficient group. A new parameter, namely, estimation of adrenal ascorbic acid, has been standardised. At present, studies are being carried out in order to see the effect of other types of stress phenomena (induced by heat or cold exposure, irradiation with gamma rays etc.) on adrenocortical function as compared to that due to the stress of protein deprivation.

DEPARTMENT OF VIRAL VACCINES

Poliomyelitis**Poliomyelitis surveillance**

The Enterovirus Unit carries out every year a surveillance on paralytic poliomyelitis among the population of Greater Bombay. The results of an investigation carried out during the period January-December 1974 are presented in this report. It was second non-epidemic year after the epidemic of paralytic poliomyelitis in 1972.

A total of 811 cases of paralytic poliomyelitis were reported from Greater Bombay during this period. There was an increase of 84 cases over that in the previous year. This is in conformity with the rising incidence of paralytic poliomyelitis in Bombay City and vicinity as observed over the past 20 years.

The increase in population alone does not directly account for this rising incidence of paralytic poliomyelitis. The rise in incidence can be classified into two categories : one for the epidemic years and the other for the non-epidemic years. The rate of increase in incidence during the epidemic years is twice higher than that during non-epidemic years. The yearly rates of increase for the epidemic years are 0.08 and 0.04 per 10,000 population respectively.

The age-wise and seasonal incidence of paralytic poliomyelitis present a pattern similar to that observed in the past. Approximately, 93 per cent of the cases were reported from the age group of 3 months to 3 years.

The typical "late summer and monsoon" pattern of seasonal incidence of the disease was also maintained during 1974. About 60 per cent of the cases were reported during June and October, with a peak incidence in August.

Amongst the cases investigated, the over all fatality rate was 5-7 per cent and that amongst individuals with severe residual paralysis was 13-15 per cent.

Paralytic illness in Vaccinated Individuals

Among 811 cases of paralytic poliomyelitis reported during 1974, 131 (16 per cent) individuals had a history of receiving oral polio vaccine. The patterns of age-wise incidence and seasonal distribution were similar to those observed in the total number of vaccinated and unvaccinated cases.

However, the severity of the disease, as judged by the fatality rate and the percentage of individuals with severe residual paralysis was less in vaccinated individuals. The figures of the fatality rate and the percentage with severe residual paralysis were 3-4 per cent and less than 8 per cent respectively.

Virological studies in non-vaccinated group

The stool samples and rectal swabs from 90 cases of poliomyelitis were virologically investigated. Of the 90 cases studied, 72 (82 per cent) were positive for virus excretion. In several instances, more than one strain of enterovirus was isolated from a single individual. From the 72 individuals, who excreted virus, 97 different strains of viruses were isolated in primary monkey kidney tissue

culture and infant mice systems. Eighty per cent of the isolates belonged to enteroviruses. Among the enterovirus group, 61 per cent were polio viruses, of which 50 per cent, 21 per cent and 23 per cent of the strains belonged to polio types 1, 2 and 3 respectively.

Non-polio enteroviruses constituted about 24 per cent of the total isolates. The decreasing order of the rate of isolation of these virus types was : Cocksackie virus A4, A10, ECHO Virus 1, Cocksackie virus B1 and Cocksackie virus B5.

Virological studies in vaccinated group

Of the 131 cases reported in this group, 133 cases (94 per cent) were investigated for virological studies. Eighty per cent of the individuals investigated showed virus excretion. A total of 139 strains of viruses were isolated.

The poliovirus strains constituted 57 per cent of the isolated strains. of these, 63 per cent, 19.3 per cent and 18 per cent belonged to types 1, 2 and 3 respectively, while the non-polio enteroviruses constituted 25 per cent of the isolates. The order of isolation of these viruses with decreasing frequency was as follows : Cox A4, ECHO 1, Cox A10, ECHO 7, Cox B1, Cox B5, Cox A2, Cox A12 and ECHO 20.

Study of anti-polioviral activity in human milk and faeces

With the observed low rate of "Vaccine take" for oral polio vaccine and concomitant practice of breast feeding in India, a study to find out correlation between these two factors was undertaken. A total of 80 pairs of milk and serum samples from lactating mothers were tested for antipolio virus anti-bodies. Whereas all the serum samples showed anti-bodies against all the three types of polio virus, 80 per cent, 61 per cent and 70 per cent of the milk samples had antibodies against poliovirus type, 1, 2 and 3 respectively. The presence of antibodies in milk was related to the level of antibodies in the serum, i.e. higher the level of serum antibodies, higher was the presentage of milk samples positive for antibodies. Only few individuals excreted antibodies in their faeces. The number of individuals with copro-antibodies was higher in the vaccinated group.

Studies on stability of oral polio vaccine

The difficult task of maintaining the vaccine at low temperature in the field has been overcome by the use of divalent cations to stabilize the vaccine viruses.

A study of oral polio vaccine with 1-M, Mg C 12 at 4^o, 28^o and 37^oC was undertaken. The vaccine was comparatively stable at 4^o C.

Preparation of powdered media for tissue culture

The preparations of powdered media required for tissue culture laboratories have been standardized at the Enterovirus Unit. The use of powdered media has several advantages like storage, transportation and uniformity of preparation, over the liquid preparations. Preparations reconstituted from powdered Hank's and Earle's balanced salts have been found satisfactory for tissue culture and

virological work. Powdered media have proved useful in reconstitution of live oral polio vaccine. These media are presently being used at the Enterovirus Unit.

Oncogenicity of Simian Viruses

Monkeys used for tissue cultures often harbour Simian viruses, some of which are tumorigenic. To develop a system for such studies at the Enterovirus Unit, experiments were carried out with SV 40 in 24-48 hour old baby hamsters.

Tumours were successfully produced by SV 40 in baby hamsters. These tumours have been maintained from animal to animal and have reached the eighth passage. The incubation period for a visible tumour to appear has been shortened to as much as 7-10 days.

Rabies

Preparation of freeze-dried vaccine

Attempts to prepare freeze-dried vaccine are in progress. Ten batches of vaccine from individual sheep brain and three batches from pooled sheep brains have been prepared. All the 13 batches have been freeze-dried. Studies on titration, abnormal toxicity, infectivity and concentration of phenol have been completed. Potency test has been carried out only on one batch. Further work is in progress.

DEPARTMENT OF VIROLOGY

I. Host-Virus Interaction—Basic Studies

A. RABIES

Sarcoma blockade *in vivo*—Rabies—Rous system in chickens : further studies

Earlier experiments had been carried out in order to investigate the mechanism (s) underlying the *in vivo* blockade of Rous sarcoma virus by rabies virus. These mechanisms had not fully been elucidated.

Three different strains of rabies virus had been investigated for their capacity to produce blockade of the Bryan Master Standard strain of RSV. The data indicated a differential degree of blockade for each rabies virus strain utilized. Thus, RV (Street 2/73) produced maximal degree of blockade (54 per cent); RV (CVS) (50 per cent) blockade and RV (Paris) (20 per cent) blockade. In addition, RV (Flury, LEP) was tested and found to produce the least degree of blockade, i.e. 13 per cent.

It had been previously reported that rabies virus produces a serum factor in chickens, which blocks the Bryan Master Standard strain of RSV. In order to determine whether plasma from chicks inoculated with rabies virus would have greater anti-RSV inhibitory activity than that of the serum factor, chicks were inoculated with RV (CVS) and bled after 48 hours, the plasma having been collected in Alsever's solution. As shown in Table 1 when the plasma-RSV mixture was inoculated into chicks, all experimental and control birds developed tumours simultaneously, indicating that the plasma factor was inhibited completely by some constituent of Alsever's solution.

It was gratifying to note that when subsequent experiments, using heparin as the anti-coagulant, were carried out to test the inhibitory action of the plasma factor on the RSV, 90-100 per cent of the experimental birds were tumour-blocked, while all the controls developed tumours after the usual latent period. The tumour-blocked chicks were observed for 21 days and then re-inoculated with the plasma-RSV mixture as before, in order to determine whether any anti-plasma factor neutralizing antibody had developed in the birds. Astonishingly enough, in most of the chicks, following the second challenge (as shown in Table 2), there was total blockade of the tumour, indicating the absence of anti-plasma factor neutralizing antibody. The above results are most unusual not only from the point of view of describing a new anti-viral and or anti-tumour agent of natural origin and of great potency, but also from the point of view of the basic principles of antigen-antibody reactions which appear to be contradicted.

Intensive investigations are currently in progress to elucidate the mechanism (s) underlying these findings.

Biochemical changes in rabies

The work on biochemical changes in experimental rabies was continued using Swiss albino Kasauli strain mice and *O. cuniculus*, strain Haffkine, conventional, non-inbred. Previously, it was found that mice infected with RV (CVS) were hyperglycaemic when paralysed. Hyperglycaemia was not mediated through adrenaline as shown by reserpine treatment. Rabbits inoculated with the RV (Paris) strain at different passage levels also showed hyperglycaemia and hypokalaemia. Oral KCL, durabolin and spironolactone treatment had no effect on hypokalaemia. There was no excessive excretion of potassium through urine.

The following experiments were carried out to study the mechanisms underlying these changes :—

(i) Adrenalectomized mice were inoculated with the RV(CVS) strain. They did not show the hyperglycaemic response.

(ii) Liver glycogen was estimated from normal and paralysed mice. The increased deposition of glycogen observed in the liver is suggestive of excessive glucocorticoid release.

Thus, the simultaneous occurrence of hyperglycaemia and hypokalaemia in rabies-infected animals might be due to excessive corticoid activity.

(iii) The effect of the developing infection on the temperature regulatory mechanisms was studied in mice and rabbits. In rabbits, the temperature began to rise with the onset of illness. After about 24 hours of fever, there was initially a sharp fall in temperature, which progressed until death. In mice, there was no such hyperthermic response, but a deep hypothermia was present after paralysis.

(iv) Reserpine treatment—2.0 mg/kg, 0.3 mg/kg, intra-muscularly in rabbits and 1.5 mg/kg intra-peritoneally in mice was tried before and after inoculation of the virus. In treated animals, the course of the disease was the same as that in the untreated ones.

(v) Rabies hyperthermia in rabbits did not respond to 20 mg/kg of aspirin orally, but was brought down with 20 mg/kg of chlorpromazine orally.

(vi) Experiments to estimate noradrenaline, dopamine and serotonin from the brains of infected animals are in progress.

(vii) Free amino acids of the brain in rabies infected animals are under study.

(viii) In order to study the effect of different ambient temperatures on hypothermia in rabies infection, (a) a group of 32 mice was maintained at 37° C prior to RV inoculation and was transferred to room temperature (range 25° C—27° C) after two days; (b) the second group was kept at 37° C immediately after RV inoculation, (c) the third group was maintained at room temperature (range 25° C—27° C) and (d) the fourth group was kept at 17° C. They were inoculated intra-cerebrally with CVS (10 LD₅₀/0.03 ml). All the mice showed hypothermia (Table 3).

(ix) Mice inoculated with four different strains of RV (Street virus) isolated from (a) human brain (b) calf brain (c) cow brain and (d) dog salivary glands, also showed hypothermia.

B. WEST NILE VIRUS—RSV SYSTEM

West Nile virus was obtained from the Virus Research Centre, Poona. The strains of RSV and WNV were different from those used by Dr. Shinodkar in 1960-61. Five experiments were carried out with different virus concentrations of both WNV and RSV and with varying time intervals between the inoculation of the two viruses. Blockade was revealed to some extent, but the experiments have to be repeated, as loss of virus titre due to temperature variations in storage affected the results considerably. The work of revival of the viruses is in progress.

C. COXSACKIE VIRUS TYPE A 10—RSV

A locally isolated Coxsackie A 10 virus strain was obtained from the Enterovirus Unit. Two passages were made in suckling mice in order to enhance the titre. The identity of the virus was confirmed by a neutralization test carried out in infant mice. Two attempts were made to produce blockade of the Rous sarcoma virus by Coxsackie A 10 virus. Yet no blockade was produced.

D. VIRAL HEPATITIS

Isolation and lapinisation of hepatitis B type virus from a case of jaundice

Studies on the important virological problem posed by the global increase in viral hepatitis have, in the past, been hampered by the insusceptibility of common laboratory animals to infectious and serum hepatitis viruses.

In April 1974, active work commenced with the discovery made at our Department that a strain of hepatitis B type virus, isolated from a fulminant case of human jaundice, admitted to the Kasturba Infectious Diseases Hospital, could be serially passaged six times in the new-born (40-50g) rabbit *Oryctolagus cuniculus*, strain Haffkine, conventional non-inbred. The virus was christened HIBOMA-I—an acronym for Haffkine Institute, Bombay, Maharashtra, as well as for the first isolate of the human virus. New-born mice, as well as new born guinea pigs were found unsuitable. The route of choice was found to be the intrahepatic.

The acute syndrome produced in the rabbit, closely mimicked that seen in man in fulminant cases, leading to acute yellow atrophy of the liver—there was rapid emaciation, dehydration, ataxia, coma and death.

HIBOMA-I in the patient's serum was demonstrated both by counterimmuno electrophoresis (CIEP) and by radioimmunoassay (RIA) and in the lapinised passages by RIA only. The patient's undiluted serum (bacteria-free by routine sterility tests) was inoculated intra-hepatically (0.1 ml) and from the

moribund rabbit livers, a 10 per cent w/v homogenate was aseptically prepared in Hanks' solution with penicillin and streptomycin. After centrifugation (1500g, 10 min, 4°C), the supernatant was labelled HIBOMA-I.

HIBOMA-I passages 2-6 were similarly prepared in rabbits consecutively. Controls were appropriate virus passages inactivated by Co₆₀ irradiation (0.44—2.30 Mrad).

All materials were stored at -180°C (liquid nitrogen vapour). After weighing, experimental and control animals were inoculated in separate rooms and there these animals and the lactating mother (on a standard laboratory diet water *ad libitum*) were caged together and individually maintained. The observation period usually lasted for 144 hours.

As shown in Table 4, successful adaptation was shown by increasing virulence (shortened periods of incubation and of 100 per cent mortality). HIBOMA-I (2) produced 100 per cent mortality at 144 hours., HIBOMA-I (3) at 96 hours and HIBOMA-I (4) as early as 48 hours. Controls in the first four experiments remained healthy throughout and gained weight normally. Hence, no controls were included in the fifth experiment.

The lapinised virus is heat-resistant. Histopathology (HP) of the diseased rabbit livers closely mimics sub-massive hepatic necrosis in fulminant human serum hepatitis. Electron microscopy revealed virus like particles.

The above data are not inconsistent with the working hypothesis that isolation and lapinisation of human hepatitis B type virus or a very closely related agent, has been accomplished for the first time in a common laboratory animal, viz. the new-born rabbit. *O. cuniculus* strain Haffkine, conventional non-inbred.

Currently, intense investigational activity is in progress at this Department, which is directed towards the isolation of hepatitis-B type viruses from jaundice patients in the newly developed *in vivo* system. Furthermore, efforts are also being directed towards the possibility of cultivation of the virus in cell cultures of rabbit and human origin and towards the possibility of developing a suitable *in vivo* model for the isolation of hepatitis virus A (infectious hepatitis).

A preliminary communication has already been published in the journal entitled *International Research Communications System Medical Science (Alimentary System; Immunology, Allergy and Microbiology, Parasitology and Infectious Diseases)* as well as in the *IRCS Journal of Medical Sciences*. A full length paper is under preparation.

E. RUBELLA

Teratogenesis in heterologous hosts

The teratogenic potential of rubella virus in embryonated hen's eggs was further studied. Nine experiments were performed using the virus either from

tissue culture or chick embryo organ suspension sources. Out of 184 eggs inoculated with known rubella virus from either source, cataracts were found in 5 embryos. Similarly, 3 embryos out of 61 inoculated with UV-irradiated virus, showed cataracts. No cataract was found in eggs maintained as controls, i.e. inoculated with appropriate diluent without virus. Similarly, no cataract was present in 300 normal control eggs, kept under experimental conditions but not receiving any inoculation. Virus suspension stored for a few months did not produce any cataract on subsequent inoculation.

II. Host -Virus Interaction—Applied Studies

Epidemiology

Rubella—Congenital rubella syndrome

Thirteen suspected cases of the congenital rubella syndrome were referred for study. Five of them were negative for antibody, thus excluding the diagnosis of congenital or post-natal infection. Three adult cases of rubella showed presence of antibody. The convalescent blood samples are awaiting tests. Serological screening of 1-5 year old children showed 20 per cent positive out of 35 samples tested. Ten out of 13 contacts were also found positive.

Prophylaxis

Antirabic vaccine trial

Some results on this trial have been reported last year. The sera from volunteers receiving vaccine have been tested at the Wistar Institute, Philadelphia, U.S.A. and the results are being compiled in the form of a paper. A PL-480 project on post-exposure trials of tissue culture vaccine in man and animals is being prepared and negotiations with the Wistar Institute, U.S.A. and Dr. Balasubramanian of the Pasteur Institute Coonoor, concerning the proposed monkey trials are currently in progress.

TABLE 1

Action of forty-eight hour plasma factor on RSV as compared to that of plasma prepared in Alsever's solution.

Experiment Number	Inoculum		Number of chickens		Percentage of tumour-free chickens on 14th day	
	Experiment	Control	Experiment	Control	Experiment	Control
1	PF(H)+RSV *	NP(H)+RSV	10	13	80	0
2	PF(H)+RSV	RSV	14	6	50	0
3	PF(H)+RSV	RSV	36	12	100	0
4	PF(A)+RSV	RSV	19	11	5	0

* RSV= 10^3 PFU/ml.

PF(H)=Plasma factor in heparin.

PF(A)=Plasma factor in Alsever's solution.

NP(H)=Normal plasma in heparin.

TABLE 2

Repeated blockade of RSV by RV-induced plasma factor in chickens

Experiment	Type of chickens	Inoculum		Number of chickens		Percentage of tumour-free chickens on 14th day	
		Experiment	Control	Experiment	Control	Experiment	Control
1	(a) Normal	PF+RSV*	Normal plasma + RSV	10	13	80	0
	(b) Blocked chickens from 1 (a)	PF+RSV		8		100	
	(c) Blocked chickens from 1(b)	RV (CVS)+ RSV 48 hours later		5		40	
2	(a) Normal	PF+RSV	RSV	36	12	100	0
	(b) Blocked chickens from 2(a)	PF+RSV		10		40	
	(c) Blocked chickens from 2 (b)	RV (CVS)+ RSV 48 hours later		10		55	

* RSV=10³ PFU/ml.

PF=Plasma factor.

RV(CVS)=Challenge virus strain.

TABLE 3

Effect of different ambient temperatures on hypothermia in RV—infected mice

Group No.	Virus dose LD 50	Number of mice	Average incubation period (days)	Duration of illness (days)	Percentage mortality	Hypothermia
1.	(a) .. 100	24	6.12	3.0	100	+
	(b) .. 100	24	6.8	3.1	100	+
	(c) .. 100	24	5.9	2.0	100	+
2.	(a) .. 10	24	7.15	2.58	72.8	+
	(b) .. 10	24	6.99	3.00	80	+
	(c) .. 10	24	8.11	3.5	91.7	+
	(d) .. 10	24	7.7	3.0	78.2	—

1(a) Maintained at room temperature (range 25° C—27° C).

(b) Maintained at 20° C.

(c) Maintained at 35° C.

2(a) Maintained at 37° after RV inoculation.

(b) Maintained at 37° C before RV inoculation and transferred to room temperature (range 25° C—27° C) after 2 days.

(c) Maintained at 17° C.

(d) Maintained at room temperature (range 25° C—27° C.).

TABLE 4

Lapinisation of HIBOMA-1 : Increasing virulence with serial passage

Experiment No.	Inoculum	Mortality ratio (M.R.) with percentage-hours following inoculation											
		24		48		72		96		120		144	
		M.R.	Per-cent	M.R.	Per-cent	M.R.	Per-cent	M.R.	Per-cent	M.R.	Per-cent	M.R.	Per-cent
1	Hiboma-I(2) Control	0/6 0/4	0 0	0/6 0/4	0 0	2/6 0/4	33 0	2/6 0/4	33 0	2/6 0/4	33 0	6/6 0/4	100 0
2	Hiboma-I(4) Control	0/5 0/5	0 0	0/5 0/5	0 0	3/5 0/5	60 0	5/5 0/5	100 0	5/5 0/5	100 0	5/5 0/5	100 0
3	Hiboma-I(4) Control	0/6 0/4	0 0	4/6 0/4	67 0	6/6 0/4	100 0	6/6 0/4	100 0	6/6 0/4	100 0	6/6 0/4	100 0
4	Hiboma-I(4) Control	1/7 0/7	14 0	7/7 0/7	100 0	7/7 0/7	100 0	7/7 0/7	100 0	7/7 0/7	100 0	7/7 0/7	100 0
5	Hiboma-I(3)	0/7	0	0/7	0	4/7	57	7/7	100	7/7	100	7/7	100

DEPARTMENT OF ZOONOSIS

Microbial diseases

Plague.—Prevalence of *R. rattus*, *R. norvegicus* and *B. bengalensis* in 'D' and 'E' ward areas of Greater Bombay was confirmed. *Mus musculus* and *B. gigantea* were collected in good numbers in addition to the above three species of rodents. Seasonal variation in prevalence of various types of rodents and their significance from the plague epidemiological point of view are being studied.

In all, 718 rats from 'D' ward area and 566 from 'E' ward area were examined. *X. cheopis* was found to be prevalent on all the species of rodents examined in both 'D' and 'E' wards. Two hundred and two fleas from 'D' ward and 159 fleas from 'E' ward respectively were examined. *X. cheopis* constituted 64-73% of the flea collection in 'D' and 'E' wards.

In view of the reports of rat nuisance, rodents from Sholapur area were examined serologically for the evidence of inapparent plague infection by using FI antigen supplied by the W. H. O., Serum pools were examined. None of these pools was serologically positive.

Flea pool studies were carried out by using fleas collected from rats of various localities reporting heavy rat nuisance. No evidence of plague infection ensued from the flea pool animal inoculation tests.

Laboratory trial with RH 787

Laboratory trials with RH 787 for assessing its efficacy as a rodenticide were continued. The compound was evaluated against four species of rats, viz. *R. rattus*, *R. norvegicus*, *B. bengalensis* and *M. musculus* (albino). Four bait formulations were employed for evaluating its comparative acceptability and efficacy. These bait formulations consisted primarily of wheat flour, rice flour, corn and groundnut cake.

Susceptibility of Rats to anticoagulant rodenticides

Earlier studies had shown tolerance to warfarin in *B. bengalensis* in 'D' and 'E' ward areas. No other new areas showing resistance to warfarin were detected. The assessment of susceptibility of rats to warfarin in different areas of Greater Bombay has been undertaken.

Cholera

Cholera carrier studies.—Cholera carrier studies were continued in a non-endemic area of Thana District. The study area consisted of Bolinj, Agashi and Vatar Villages of Virar, Vasai Taluka.

Faecal samples were collected from patients admitted to the Out-patients' Department of Primary Health Centre, Virar ; Mission Hospital, Agashi, healthy population of the study area. One-thousand six-hundred and two stool samples were processed. *V. cholerae* Non-agglutinable (NAG) vibrios, *Salmonella typhi* and *Shigella* sp. were isolated.

Escherichia coli, *Proteus sp.*, *Aerobacter aerogenes* *Alkaligenes faecalis* and few NAG isolates from healthy persons were found pathogenic to infant rabbits. These studies will be continued further.

Parasitic diseases

Filariasis.—Epidemiology : Epidemiological studies in filariasis were carried out in Uttan Village near Bhayandar in Thana District. One-thousand four-hundred and thirty-two night blood samples were examined. A survey of Children's Home, Mankhurd, was also undertaken. Six-hundred and eighty-six persons from Mankhurd were screened, twenty-nine were found positive for mf. In addition to these two surveys, filariasis survey of three villages situated near Bhayandar was undertaken in order to take filariasis control trials by drug treatment alone. In this survey, 213 night blood smears were screened. One-hundred twenty-three smears proved positive for microfilaria. These microfilaria positive cases will receive diethyl carbamazine treatment once a week for twelve weeks. At the conclusion of studies, night blood survey will be repeated to assess the efficacy of the treatment.

Skin test

Skin tests were undertaken by using Prof. Sawada's FST antigen. Seven-hundred and twenty-one persons were tested from an endemic area. Two-hundred and eight-one were found to be positive to microfilaria. Out of three-hundred and sixty-six persons screened from non-endemic area, only thirteen showed positive reaction.

Immunoglobulin studies

Blood samples from 73 persons, belonging to an endemic area, were collected for studying the immunoglobulin pattern in disease positive microfilaria and microfilaria negative persons in the laboratory. This work is in progress.

Laboratory maintenance of *L. carinii*

Maintenance of *L. carinii* culture in cotton rats and white rats was continued. During the period under report, 287 animals were infected.

Transmission Studies

Infection of *Culex p. fatigans* in the laboratory with *W. bancrofti* using microfilaria carriers was continued. Infective larvae of *W. bancrofti* were dissected out from infective mosquitos and collected for the preparation of *W. bancrofti* antigen. The work is in progress.

Dracunculiasis studies

Guineaworm studies were continued in Kolaba District. Panvel area of Kolaba District was selected for study of epidemiology of guineaworm infestation in 8 villages viz. Asudgaon, Watkambe, Khanivle, Talegaon Wadi, Taloje, Dhaku was studied.

Drug evaluation

Efficacy of three newly developed antihelmintic drugs in Guinea worm infestation, viz. *Mebendazole* joint and *Levamisole* was assessed. Results obtained with the use of mebendazole were promising. Although joint showed promise in the treatment of Guinea worm infestation, the side effects with this drug posed a problem. Levamisole yielded conflicting results and suggested a need for further assessment.

Cyclops Studies

Water samples were examined for assessing the density of cyclops during different months. The work was initiated in December 1974 and is being continued.

Experimental laboratory infection

Strains of *Nematospiroides dubius* and *H. nana* are being maintained for carrying out immunological studies and drug evaluations. In addition, a strain of *Syphacia obvelata* was established in the laboratory.

Attempts to maintain *N. americanus* culture in hamsters were partially successful. It was possible to infect 30 hamsters successfully with *N. americanus*. However, the worm load as well as the egg count was poor.

Life history of *N. dubius*

Complete life cycle of *Nematospiroides dubius* was studied in commonly used non-inbred, conventional Kasauli strain of mice. Free living stages of life cycle were seen to complete within 72 to 76 hours and parasitic stages were observed in 216 hours. Total period from egg to egg cycle required about 12 to 12 $\frac{1}{2}$ days.

Studies in intestinal parasitic infestations

Investigations on prevalence and epidemiology of intestinal parasitic infestation were conducted in four districts of Maharashtra State viz. Greater Bombay, Thana, Kolaba and Parbhani districts. Two thousand seven-hundred fifty-five stool samples were examined. Sixty-nine point four per cent were found to be infected with one or more of the common intestinal parasites of man.

Generally, a high distribution of the soil transmitted nematodes and protozoal infections was observed. Among the protozoa, *E. histolytica* infestation was noted in 39.0 per cent of the subjects. Among the helminths, *Ascaris lumbricoides* was predominant followed by *Trichuris* (26.3%) and hookworm (18.7%).

Amoebiasis

The indirect haemagglutination test was evaluated for the laboratory diagnosis of amoebiasis. Sera from 427 subjects with symptomatic amoebiasis (intestinal and extra-intestinal), symptomless carriers, subjects with intestinal parasitic infestations other than *Entamoeba histolytica* and healthy control

subjects were tested. The positive results were found to range from 100 per cent in confirmed cases of amoebic liver abscess to 80.4 per cent in intestinal amoebiasis.

Insecticide resistance and vector control

A study has just been initiated to find out the prevalence of nematode pathogens of *arthropod* vectors of medical importance. Presently, *nematode* pathogens harboured by cockroaches are being studied.

DIRECTOR'S LABORATORY

During the year under review, the laboratory was actively engaged in the following studies :—

1. Pharmacological and immunological studies on Pertussis Toxin.
2. Pharmacology of scorpion venoms.
3. Detailed study of antifertility component of *Lygodium flexuosum*.
4. Biochemical studies related to the depression associated with chronic pill taking.

1. In continuation of the previous work, crude pertussis toxin as well as 22 different fractions obtained after subjecting it to starch block electrophoresis are studied individually for their pharmacological, immunological and other toxic actions. So far, very few research workers have engaged themselves in studying pharmacology of pertussis toxin. It is already reported that pertussis toxin is capable of producing β_1 adrenergic blockade histamine sensitization, hypoglycemia and some neurological disorders in children. In addition, these reported activities, we have found that pertussis toxin can also cause severe hypothermia leading to death.

When individual purified fractions (15 anionic and 7 cationic) were studied for their pharmacological, immunological and other toxic actions, it was found that any individual fraction cannot evoke any pharmacological response. But it was surprising to see that four anionic and 2 cationic fractions which were unable to cause hypoglycaemia, hypersensitivity, β_1 adrenergic blockade or hypothermia can successfully immunise the animals as in the case of pertussis vaccine. The animals were tested as per official method described in I.P.

Along with the detailed study of individual fractions, we were interested in knowing the exact mechanism of action of histamine sensitization after administration of pertussis toxin. In our investigation, the possibility of any change in the histamine levels of mast cells was ruled out by testing histamine contents of mast cells in control and pertussis toxin pretreated animals. The *in vitro* assay of histamine was performed on isolated guineapig ileum.

Further, histaminase activity in the liver and intestine of rats pretreated with pertussis toxin was studied. We have found that histaminase activity in intestine remained unchanged, whereas in liver it was significantly reduced. β_1 adrenergic blockade reported to be caused by pertussis vaccine was not demonstrated in *in vitro* model by any worker so far. This effect in isolated rabbit aortic strip and guinea-pig tracheal chain was demonstrated. In support of this theory, we also found that the cyclic AMP levels in liver of pertussis rats are significantly lowered. The cyclic AMP was estimated using radio-immunoassay technique.

2. Detailed pharmacological study on the crude venoms of red scorpion (*Buthus tamulus*) and black scorpion *Palamneus gravimanus* were carried out. The venoms were tested separately on following preparations :—

1. Guinea-pig and rabbit heart,
2. Guinea-pig and rabbit atria,

3. Rat fundus,
4. Guinea-pig Vas deferens,
5. Rat uterus,
6. Rabbit jejunum,
7. Guinea-pig ileum,
8. Rat phrenic nerve diaphragm,
9. Cat tibialis anterior muscle nerve preparation,
10. Intra-cerebral injection in mice and intracerebro ventricular injection in cat and rabbit,
11. In intact dog, cat and guinea-pig, for studying the effect on blood pressure and respiration,
12. Cat and rat hind limb perfusion,
13. Guinea-pig lung perfusion,
14. Guinea-pig chopped lung preparation and
15. Histamine sensitivity test on human volunteers.

It was interesting to note that the venom of black scorpion contains significant amount of histamine, whereas the venom of red scorpion does not possess any amount of histamine. The venoms were further purified using Sephadex column and various fractions collected were further studied individually for their pharmacological activities. So far, conclusion has been reached that the death due to scorpion bite is a result of 3 combined actions. i.e. cardiotoxicity, neurotoxicity and paralysing action on peripheral muscles. Further work is in progress.

3. The air-dried whole plant (LF1) was soxhleted successfully in petroleum-ether (60°-80°C), 95 per cent alcohol and distilled water and the solvents removed by flash evaporator. After concentration of the extracts, the petroleum ether and alcohol extracts were suspended in water with gum-acacia and the aqueous extract was used. Proven fertile male and female rats and mice were taken up for the study. The mating proportion was one male to two females. The successful mating was confirmed by daily examining morning vaginal smear and/or when thick clumps of spermatozoa were detected. The day on which thick clumps of spermatozoa were seen was considered as day 1 of pregnancy.

The mated females were separated from the males and administered the extracts orally by means of syringe and feeding needle. The mating in mice was confirmed by the appearance of a plug. The mated females were treated for three days of pregnancy. With maximum three days administration of the drug, all females remained non-pregnant. When cut open on day 10 or 11 of pregnancy, as compared to the controls which received gum acacia suspension orally, the petroleum-ether extract did not show any significant antifertility effect. The alcohol extract exhibited 100 per cent antifertility effect and aqueous extract 80 per cent effect, both in mice and rats.

The effects of active extracts in female rabbits were also tried. However, in rabbits, the extract was required to be administered for more than three days. There also, a good antifertility effect was observed. The antifertility effect was observed before mating and after mating.

Both the extracts possess the property of preventing births. The extracts either on the blastocyst nor on implantation; they do not cause either late or early abortion. However, they act during the first three days of pregnancy, i.e. the period of zygote formation. Just three doses are required for antifertility act immediately after mating. The extracts did not show either any toxic or harmful effects on the off-spring. The effect of extracts is temporary and not permanent.

4. The effect of oral contraceptive hormones in relation to the reported depression associated with the chronic pill taking, is also being studied. Clinical reports indicate that the depression induced by the pills is due to disturbed metabolism of serotonin and is primarily due to pyridoxine deficiency caused by the hormonal ingredients of the pills. But the depression as a whole involves a complex biochemical phenomena and it is sought to understand the *modus operandi* of contraceptive hormones in relation to the depression. As a first step in this direction, rats were used as experimental animals and they were treated with oral contraceptive hormones. (Ethinyl oestradiol and Norgestrel) individually and in combination. After treating the animals for a prolonged period (human dose was selected on the basis of body weight) biogenic amines (noradrenaline, dopamine and serotonin) in central nervous system were assayed. The preliminary study of the pattern of these biogenic amines in the CNS (Cerebral cortex, Cerebellum, Pons/Medulla oblongata and hypothalamic region) did not show any significant change after chronic hormonal treatment. The problem seems to be intriguing and further work to understand the interrelation between the hormonal pills and the said depression is in progress.

PART III **ROUTINE ACTIVITIES** **ANIMAL FARM**

The breeding and supply of different species of laboratory animals was carried out as usual. The farm met with the requirements of our departments during the year under report. Twelve students of Diploma in Medical Laboratory Technology Training Course (D.M.L.T.) and twelve students of Medical Laboratory Technology Training Course (M.L.T.) were trained in general handling and management of laboratory animals.

Shri Pudjoprinitno Kasmata from Indonesia, Dr. Kee Kon Teoh from Malayasia and Dr. S. A. Patil from Veterinary College, Bombay, attended short-term training course in breeding, maintenance and management of laboratory animals, during the year.

Inter-country seminar-cum-workshop, on small laboratory animal care, conducted by World Health Organization (W.H.O.), New Delhi, was held at the Institute during the year under report.

Animal House provided facilities to hold practicals during the wokshop. The staff of the Animal House took active part in demonstrating practicals during the workshop.

The figures mentioned below will show the number of different species of laboratory animals bred in Animal House and supplied to departments and surplus animals sold to the laboratories all over India during the year :

Species of animals	Production	Supplied to our departments	Supplied outside
White mice	2,54,290	1,02,245	8,354
Albino rats	15,401	6,911	1,936
Rabbits	3,024	1,134	640
Guinea Pigs	5,420	4,112	822
Hamsters	4,080	1,591	68
Cotton rats	141	3	4

In addition to the breeding of the above mentioned species of animals, sheep, monkeys, cats and fowls were also maintained at the farm as per the requirements of different departments of the Institute.

DEPARTMENT OF BIOCHEMISTRY

The department continued to render diagnostic service to city hospitals and private practitioners. The service facilities are particularly useful to M.A. Podar and Police Hospitals. Increasing number of the staff members of this Institute availed themselves of these facilities. There were more demands for special tests like Serum Transaminases, Protein-Bound Iodine (P.B.I.) and Urinary 17-Ketosteroids. Some new tests such as urinary catecholamines and 3-methoxy-4-hydroxy mandelic acid (V.M.A.) have been introduced during the course of this year. A total of 4,516 tests were carried out during the year as per Table 1 appended.

Training

The department continued to take part in the training programme for M.L.T., D.M.L.T. and Short-Term Training Courses. Shri P. S. Thombre, a D.M.L.T. trainee, stood first in the Bombay University examination held in May 1974 with 'Advanced Chemical Pathology' as his special subject.

Staff

Dr. (Smt.) S. M. Modak, Senior Scientific Officer Cl. II, who was on study leave resigned and Dr. V. P. Shinde was promoted to this post on a temporary basis. Sarvashri A. D. Kulkarni, A. V. Athavale, Syed Raziuddin, P. J. Godkar and N. M. Shivapurkar, Research Scholars and Fellows also resigned during the year. Kum. Laxmi S. Kulkarni joined the department as Junior Research Fellow of the Government of Maharashtra; Kumari N. J. Saikumar continued to work as Senior Technical Assistant on a temporary basis.

Academic

Sarvashri Syed Raziuddin, V. P. Shinde, U. J. Dumaswala and Kumari Ranjana Buit were awarded Ph. D. degrees in Biochemistry of the Bombay University. Smt. H. V. Phadnis also obtained her M. Sc. degree during the year. Sarvashri Nayak, Chatwal and Kumari P. H. Jhala submitted theses for Ph. D. degrees in Biochemistry.

Dr. V. P. Shinde, Senior Scientific Officer (Class II), was awarded a research prize for his meritorious work on "Quadruple cholera vaccine in Peanut Protein Hydrolysate" at a special function held in April 1974. The award was presented at the hands of Dr. V. T. H. Gunaratne, Regional Director, South-East Asia, World Health Organization, New Delhi.

Routine and special tests carried out during the year

Name of the test					No. of tests
<i>Blood-Serum</i>					
Icteric Index	..	..	..	..	3
Van den Bergh	..	..	..	..	2
Amylase	..	..	..	..	1

Name of the test	No. of tests
Bilirubin	317
Calcium	3
Chlorides	41
Cholesterol	108
Creatinine	7
Non-Protein Nitrogen	4
Phosphorus (Inorganic)	5
Acid Phosphatase	1
Alkaline Phosphatase	238
Proteins-Total	362
Proteins-Albumin	314
Proteins-Globulins	311
Sugar	762
Thymol Turbidity	186
Urea Nitrogen	97
Uric Acid	5
Total ..	2,836

Urine and Stool

Acetone bodies	1
Albumin	2
Bile salts and pigments	167
Calcium	1
Phosphorus	1
Sugar	188
Uric Acid	1
Total ..	361

Cerebro Spinal Fluid (C. S. F.) and other body fluids

C.S.F. Chlorides	2
C.S.F. proteins	4
C.S.F. sugar	2
S. M. test	15
Total ..	23

Special Tests (clinical)

Amino acids (Urine)	1
Faecal fat	1
Gastric analysis	3
Glucose Tolerance Test	31
Iodine (serum)	16

Name of the test					No. of test
Iron (serum)	..	..	..	..	1
Iron Binding Capacity (serum)	..	..	..	..	1
Kidney function test	..	..	..	..	1
Lange's colloidal gold	}	..	..	..	123
Curve (C.S.F.)		..	..	..	
Potassium (serum)	..	..	..	..	40
Protein-Bound Iodine (serum)	..	..	..	..	49
Proteins by paper electrophoresis (serum)	..	..	..	..	13
Sodium (serum)	..	..	..	..	44
17-Ketosteroids (urine)	..	..	..	..	41
S.G.O.T.	..	..	..	..	365
S.G.P.T.	..	..	..	..	366
Urinary stone	..	..	..	..	2
Vinyl Mandelic Acid (urine)	..	..	..	..	6
Total					1,104

Food Analysis

Moisture	..	..	..	..	11
Proteins	..	..	..	..	11
Fat	..	..	..	..	11
Ash	..	..	..	..	11
Carbohydrates	..	..	..	..	11
Calories	..	..	..	..	9
Total					64

Animal Sera

Bilirubin	..	..	..	..	2
Calcium	..	..	..	..	5
Copper	..	..	..	..	3
Iodine	..	..	..	..	1
Non-Protein Nitrogen	..	..	..	..	3
Phosphorus	..	..	..	..	5
Proteins-Total	..	..	..	..	29
Proteins-Albumine	..	..	..	..	29
Proteins-Globulins	..	..	..	..	29
S.G.O.T.	..	..	..	..	2
S.G.P.T.	..	..	..	..	2
Total					110
Vitamin D Project samples	..	..	..	..	18

Blood (serum)	..	..	..	..	..	2,836
Urine and stool	..	..	..	..	..	361
C.S.F. and other body fluids	..	..	..	..	..	23
Special tests (clinical)	..	..	..	..	..	1,104
Food analysis samples	..	..	..	..	..	64
Animal sera samples	..	..	..	..	..	110
Vitamin D project samples	..	..	..	..	..	18
					Total	4,516

DEPARTMENT OF CLINICAL PATHOLOGY

Staff

Dr. (Kum.) S. M. Bhide, Medical Officer, M.M.S.Cl. III, Typhoid Unit, resigned from the post on 7th January 1975. Dr. S. D. Telwarkar was appointed in her place as Medical Officer, M.M.S. Cl. III, Typhoid Unit, on 5th March 1975.

Dr. (Smt.) U. Srinivasan was appointed as Pool Officer on 3rd February 1975.

Shri J. B. Dhabuwala was appointed as Senior Technical Assistant *vice* Smt. A. P. Kelkar, who had resigned the post on 14th March 1974.

Shri D. S. Chitnis, Senior Technical Assistant and Kum. Smita N. Marfatia, Senior Technical Assistant, resigned their posts on 31st December 1974 and 1st January 1975 respectively. Kum. M. D. Shah and Kum. A. B. Dave were appointed in their posts on a temporary basis on 1st January 1975 and 2nd January 1975 respectively.

Kum. S. C. Gawand, Senior Technical Assistant, submitted her resignation. Thus, three trained and well-experienced Senior Technical Assistants have left the department this year.

Shri G. R. Chavan, Junior Technical Assistant, retired on 14th July 1974.

Training

Twelve students have completed the D. M. L.T. course and four students have been imparted advanced training course in Bacteriology.

Twenty short-term trainees completed the training in Clinical Pathology. In addition, sixteen M.L.T. students were given theoretical and practical training in Clinical Pathology.

Dr. S. Mukerji was declared successful and obtained his Ph.D. degree in microbiology of Bombay University.

Other activities

The department is participating in the Inter-Laboratory Evaluation Programme conducted by the Central Venereal Diseases Laboratory, Madras Medical College, Madras, under the V.D.R.L. syphilis serology programme.

Dr. (Smt.) J. K. Dave attended the Twenty-fifth All-India Conference of the Association of Pathologists and Microbiologists held in December 1974 at Delhi and read a paper entitled "Adenomyosis Uteri—A study of 150 cases".

Dr. S. Mukerji read a Paper on "A study of *Bacterium anitratum*" at the Two hundred and Fifty-fourth Meeting of Teaching Pathologists held at the Haffkine Institute.

The department extended co-operation to various other departments of the Institute in the collection of blood samples. Routing clinico-pathological investigations for the staff of the Institute, as well as the staff of various other State Government departments, were carried out. Histo-pathological study of the

tissues of the experimental animals, received from post-graduates of various departments of the Institute as well as from the Food and Drug Administration of the Government of Maharashtra were undertaken. Tissues referred by the Toxicology Unit of this Institute were studied for drug toxicity reactions.

The routing investigations carried out during the year are enumerated below :

				No. of samples
<i>A. Histo- Pathology—</i>				
Specimens received	..	..	..	2,073
Blocks prepared	..	..	..	7,998
<i>B—Haematology—</i>				
Haemoglobin estimation	..	..	..	565
Total R.B.C. Count	..	..	..	545
Total W.B.C. Count	..	..	..	581
Differential W.B.C. Count	..	..	..	592
Blood for malarial parasites	..	..	..	1
P.C.V. Estimation	..	..	..	13
E.S. R. Estimation	..	..	..	304
Prothrombin Time	..	..	..	1
Platelet count	..	..	..	3
Bleeding Time	..	..	..	6
Clotting. Time	..	..	..	6
<i>C. Clinical Microscopy—</i>				
Urine for routine examination	..	..	..	407
Stool for routine examination	..	..	..	382
Semen examination	..	..	..	27
C.S.F. for cytological examination	..	..	..	8
Other puncture fluids for cytological examination	..	..	..	12
Pregnancy Tests (Qualitative)	..	..	..	1,189
Pregnancy Tests (Quantitative)	..	..	..	159
<i>D. Bacteriology—</i>				
Blood culture, routine	..	..	..	23
Blood culture for enteric organisms	..	..	..	673
Urine culture routine	..	..	..	204
Urine culture for enteric organisms	..	..	..	222
Stool culture, routine	..	..	..	17
Stool culture for enteric organisms	..	..	..	312
Throat swab for diphtheria and other organisms	..	..	..	133
Anaerobic culture of operation theatre material	..	..	..	137
Toxin neutralisation test	..	..	..	137
Anaerobic culture of clinical material	..	..	..	175
Animal pathogenicity test	..	..	..	15
Smear for Acid Fast Bacilli	..	..	..	119

			No. of samples
Concentration of A.F.B.materials	..	..	91
Culture of specimen for tuberculosis	..	..	91
Guinea-pig inoculation test for T.B.	..	..	18
Smear for Gonorrhoea	..	..	978
Cultures for Gonorrhoea	..	..	978
Miscellaneous cultural examinations	..	..	86
Antibiotic sensitivity tests	..	..	485
Antibiotic sensitivity test for Gonorrhoea	..	..	119
Antibiotic sensitivity test against multiple drugs	..	..	710
Autovaccine	..	..	15
Autolysate	..	..	13
<i>E. Mycology—</i>			
Cultures for detection of fungi	..	..	140
<i>F. Serology—</i>			
Widal Tests	..	..	686
Weil-Felix Tests	..	..	686
Test for Brucellosis	..	..	686
Paul Bunnel Tests	..	..	22
Vi Haemagglutination Test	..	..	174
Gonococcal Haemagglutination Test	..	..	225
V.D.R.L. Test	..	..	441
Testing of V.D.R.L. antigen	..	..	108
Wassermann Test	..	..	11
Rose Waaler Test	..	..	18
Latex Fixation Test for Rheumatoid Arthritis	..	..	78
Kahn Tests	..	..	53
Testing of diagnostic reagents	..	..	90
Grand Total			21,968*

*Blocks prepared out of specimens are taken into account while calculating the total number of tests.

The following data obtained from the routine histopathology and clinical microscopy work is worthwhile recording:—

Papillary carcinoma of the nose

Adenomyosis associated with pregnancy.

Fatty infiltration in the salivary gland

Ductal carcinoma (squamous cell type) of the breast in a male patient.

Uterus unicornis Bi-Colis with evidence of adenomyosis in the accessory uterus—Rare specimen Hashimoto's Thyroiditis.

Alveolar soft part sarcoma

Adenosquamous carcinoma of uterus

Parasitic granuloma of the lung (Pneumonectomy specimen) Rare specimen

The following table shows the regional distribution of forty-eight primary malignant growths received during the year :—

Skin	..	..	..	..	7
Breast	..	..	..	..	9
Lymphatic system	..	..	..	..	3
Respiratory system	..	..	..	..	2
G. I. tract	..	..	..	..	4
Female genital organs	..	..	..	..	16
Male genital organs	..	..	..	..	5
Urinary tract	..	..	..	..	1
Eye ball	..	..	..	..	1

The fluorescent staining method for the detection of *M. tuberculosis* in tissue sections was adopted routinely.

The material received from other government institutions was also studied.

Fifty-eight specimens of tuberculous lesions were received. Their regional distribution is as follows :—

Female genital organs	..	..	..	..	18
Other organs	..	..	..	..	40

Three hundred and eighty-two stool samples were examined for parasites during the year. The following table gives the distribution of the parasitic infection in these samples :—

	Species				Nos.
<i>Ascaris lumbricoides</i>	..	..	..	..	101
<i>Trichuris trichura</i>	..	..	..	..	67
<i>Giardia lamblia</i>	..	..	..	..	34
<i>Entamoeba histolytica</i>	..	..	..	..	25
<i>Ankylostoma</i> sps.	..	..	..	..	10
<i>Enterobius vermicularis</i>	..	..	..	..	4
<i>Taenia solium</i>	..	..	..	..	2
<i>Hymenolepis nana</i>	..	..	..	..	2
<i>Strongyloides stercoralis</i>	..	..	..	..	1

DEPARTMENT OF PHARMACOLOGY

During the year under review, the work of toxicology studies was segregated from this department and a new unit was created for the purpose under the direct supervision of the Director.

Staff

Kum. N. G. Waingankar, Senior Technical Assistant, left the Institute and Kum. M. A. Miranda was appointed in her vacancy. Shri D. S. Dondé, the Pool Officer under the Government of Maharashtra Pool Officers' Scheme, was absorbed as Senior Technical Assistant in the Chemotherapy Department of this Institute.

Government Analysis Section

The Assistant Director continued to act as Government Analyst. No sample was received from Maharashtra State, but five samples were received from Gujarat State. None of the samples were found to be sub-standard.

Prohibition and Excise Section

The Prohibition and Excise Department sent two hundred and fifty-eight samples of Toddy for detection of adulteration and alcohol content. Out of these toddy samples, two were found to be adulterated with chloral hydrate and one with methylated spirit. Seven hundred and sixty-seven samples of Indian-made foreign liquors were tested for alcohol content only. Four samples of foreign liquors were tested for all the tests. The department also received 32 samples of Ganja, Bhang etc. for certification. All the samples were found to be unfit for human consumption when tested by physical and chemical methods.

Commercial samples

The department continued to extend facilities for testing samples received from commercial firms. The samples were mainly received for biological testing and infra-red spectroscopy. Two investigations were carried out for antiamoebicidal activity. Diloxanide furoate was received from Boots for determination of its potentiating effects on metranidazole when the combination of the two drugs is used. The study was carried out in rats and hamster. 'Syndys' sample was received from Lyka. It was a combination of berberis hydrochloride, tetracycline hydrochloride and chloroquine phosphate. Antiamoebic testing was carried out. The Aquaviron B₁₂ sample was received from Indian Schering for testing the local effects and acceptability of the sample.

Bioavailability studies

The department continued to undertake a large number of bioavailability studies. These included either single dose or multiple doses administered either by oral or parenteral route. Studies on Penidure LA-a Benzathine Penicillin G, Chloramphenicol, Tetracycline, Oxytetracycline, Erythromycin

Ampicillin, Cloxacillin, Gentamycin and Doxycycline were reported. Studies were also reported on Nitrofurantoin, Frusemide, Diazepam, Isonicotinic Acid hydrazide, Phenylbutazone and Oxyphenbutazone.

Methods for undertaking the studies on Rifampicin, Metranidazole, Trimethoprim, Sulphamethoxazole, Alphamethyl DOPA and Chlorpropamide were standardized. Dr. (Smt). P.K. Mehta from Harkisondas Hospital had taken up a project to correlate the blood levels of drugs with kidney damage. The blood levels of Ampicillin, Chloramphenicol, Streptomycin and Gentamycin were studied and reported to her.

Journal Club and Departmental Seminars

The department consists of scientists of different disciplines, such as Pharmacology, Chemistry, Microbiology etc. To enable them to keep pace with the recent trends in their respective fields and to encourage the habit of critical evaluation, a journal club was started last year, which meets twice a month and a seminar is arranged once a month. In addition to the staff of this department, the staff from Director's laboratory, Toxicology Unit, CCRIMH Units and ICMR unit on anti-leprosy drugs participate in the deliberations to make the activity a success.

Training

The department participated in the M.L.T. and short-term training programme of the Institute. A total of about 30 students were trained during the year. In addition to this, special training in bioavailability studies was imparted to two candidates, one Pharmacologist and the other Microbiologist from the Indian Drugs and Pharmaceuticals Ltd. Two Microbiologists from Lady Hardinge College, Delhi were trained for micro-assay of Penicillin. Shri M.A. Darshane from Aurangabad Medical College was trained in Pharmacological evaluation of bronchodilators. One candidate from Jaslok Hospital was trained in the estimation of R.W.C. test.

Post-Graduate Degrees

Sarvashri H. K. Soni and S. S. Karkhanis were awarded M. Sc. (Biochemistry) degree of the University of Bombay. Four members of the staff are preparing for their M.Sc. degree, while one is preparing for his Ph.D. degree. Two students were admitted for the first time for M. Pharm. degree in Pharmacology.

Scholarships, Fellowships and other Assistance

The department received assistance from Central Council for Research in Indian Medicine and Homoeopathy. In addition to the continuation of the inquiry on 'Anti-leprosy Drugs', the ICMR sponsored the project on "Drug Resistance in Leprosy". Unichem Trust has continued the Fellowship for studies in indigenous drugs.

Attendance at Seminars, conferences and Symposia

The department was represented at the following conference and symposia:—

(1) VIII Annual Convention of the Indian College of Allergy and Applied Immunology, and I.U.P.S. Satellite Symposium on Anaphylaxis at Manipal, October 1974. Paper presented on "The effect of Vasicine and Vasicin on histamine release during passive peritoneal anaphylaxis"—Shri R. B. Ghooi.

(2) I.U.P.S. Satellite Symposium on Histamine, Calcutta, November 1974. Paper presented "Effect of pre-treatment with Disodium chromo-glycolate on the sensitivity of guinea-pigs to histamine and sympathomimetic amines"—Shri S. S. Mahajani.

(3) VII Annual Conference of the Indian Pharmacological Society, Ahmedabad, December 1974. The following papers were presented :—

(i) "A Bradykinin potentiating substance from anaphylactic reactions in rats"—Shri R. B. Ghooi.

(ii) "Studies on the interaction of piperazine and senna on isolated rat colon"—Shri R. S. Joshi.

(4) World Congress on "Asthma Bronchitis and allied conditions", New Delhi, December 1974—Dr. M. B. Bhide.

DEPARTMENT OF QUALITY CONTROL

The Department carried on its important and varied functions of high responsibility of testing, examining and certifying the products of the Institute. Stringent check was kept with respect to purity, quality, potency and safety of drugs and other products, thus fulfilling drug control obligations and maintaining high standards of quality control.

Unification of all sections of the department at one single site was not possible owing to lack of adequate space. The rooms for experimental animals, sterility testing room and incubation rooms are located on the ground floor, whereas the main department functions on the second floor. Owing to financial stringency, physical expansion of the department could not materialise during the period under review. Only the sterility testing room was renovated completely and incorporation of suitable filters into the ducts of air-conditioning system remain pending. The plans for setting up a microbiology room, bio assay room, a sample storage room and to streamline the department had to be deferred owing to lack of adequate funds.

Raw materials that are used for production are scrupulously tested to check any impurities gaining access by chance into the finished products. Intermediaries of different drugs are also tested and necessary guidance given to the production departments.

In addition to routine work of great responsibility some important/interesting research investigations are also in progress. The department participates in other constructive activities of the Institute.

Pertussis Cell.—Collaborative work with Bacteriology Department was continued. Ceaseless interest, initiative and collaboration has successfully contributed to the production of a number of batches of triple vaccine which have passed the pharmacopoeial tests.

Monograph on D.T.P. vaccine.—After a thorough study, Dr.F.L.Saldanha, Assistant Director, I/c. of the department, prepared a Draft Monograph on 'Diphtheria-Tetanus-Pertussis' vaccine for inclusion in the next edition of the Indian Pharmacopoeia. This tentative monograph was discussed at a meeting convened with the representatives of Central Drug Control authorities, Central Drug Research Institute, Kasauli and with some of the leading manufacturers of this product in India. The monograph was approved with minor modifications.

Collaborative work on testing of diphtheria and tetanus toxoid

To standardise the methodology for testing diphtheria and tetanus components in Triple Antigen, attempts were made to initiate collaborative work with the Central Drugs Research Institute, Kasauli and some private firms. Detailed plan of work was prepared and sent to the laboratories concerned which showed interest in the collaborative work.

Some members of the staff registered for their post-graduate degrees in Microbiology, Applied Biology and Biochemistry, Chemistry. One candidate got his M.Sc. degree in Microbiology.

Dr. F.L. Saldanha, Assistant Director, was the Editor of the "Bulletin Haffkine Institute" which has a global circulation.

Shri A.K. Patil, Junior Scientific Officer of this department, went abroad on a W.H.O. training fellowship for 5 months.

Posts of Senior Scientific Officer Class I, Senior Scientific Officer Class II and Junior Scientific Officer, remained vacant during the year. Similarly, temporary appointments of Senior Technical Assistants hampered the work of the department a great deal, but this could not be remedied owing to genuine administrative difficulties.

Time and again, the trained and experienced staff rose to the occasion under different emergencies to surmount the hurdles and worked with devotion and duty, initiative and great responsibility, invariably outside office hours to cope with the sudden influx of samples of raw materials, intermediaries and finished products received for testing. This has helped in maintaining the production in full swing and expediting the release of the Institute's products promptly.

Training

The department imparted technical training to the following post-graduate candidates:—

Private firms	..	5
Short-term Trainees	..	13
M.L.T. trainees	..	14

The number of different tests and the samples analysed by the department during the year are enumerated below:—

Products	No. of samples tested			Total
<i>Sterility tests</i>				
Bacterial vaccines	..	..	6,023	
Antirabic vaccine	..	..	4,169	
Parenteral bulk solutions	..	..	11,857	
Anti-toxins sera etc.	..	..	11,731	
Miscellaneous	..	..	1,306	
				35,08
<i>Pyrogen tests</i>				
Parenteral bulk solutions	..	..	1,451	
Blood products	..	..	1	
				1,45

Products				No. of samples tested	Total
<i>Chemical and Physical Examinations</i>					
Parenteral bulk solutions	..	..	..	1,685	
Vitamin preparations	..	..	..	384	
Sulpha drugs	..	..	..	192	
Miscellaneous	..	..	..	350	
					2,611
<i>Toxicity tests</i>					
Antitoxins, Toxoids, vaccines etc.	..	..	..	618	618
<i>Absence of infective virus</i>					
Viral vaccines	..	..	..	278	278
<i>Potency tests</i>					
Antitoxins	..	..	..	27	
Antivenine	..	..	..	67	
Toxoids	..	..	..	40	
Triple antigen	..	..	..	5	139
<i>Preservatives</i>					
Vaccines and anti-toxins	..	..	..	577	577
<i>Free formaldehyde contents</i>					
Bacterial vaccines and toxoids	..	..	..	148	148
<i>Aluminium contents</i>					
Toxoids	..	..	..	32	32
<i>Microbiological Assays</i>					
Pemon	..	..	..	163	
Vitamin Preparations	..	..	..	151	314
<i>Raw materials</i>					
Vitamins	..	..	..	182	
Sulpha drugs	..	..	..	85	
Miscellaneous : fine chemicals antispetics, pharmaceu- tical necessities etc.				940	1,207
Grand Total				..	42,462

RADIATION BIOLOGY UNIT

The Radiation Biology Unit, which started functioning nearly an year ago has made significant progress in the field of public service and applied medical research.

During the current year, the unit continued to function with the same skeleton staff and was equipped further with most sophisticated instruments purchased from Electronics Corporation of India Ltd. These electronic instruments include : (a) Medical Spectrometer, (b) Gamma-ray Scintillation Counter and (c) Liquid Scintillation Counter. These instruments have been calibrated and have shown high efficiency for the respective isotopes. With these machines, the unit is now well-equipped to set up radioimmunoassay methods.

Routine :

Thyroid function studies on patients referred to the Unit were carried out as per the details given below :—

(a) ^{131}I up take studies :

(i) Total patients referred	..	..	..	91
(ii) Hyperthyroid	..	..	..	33
(iii) Hypothyroid	..	..	..	12
(iv) Euthyroid	..	..	..	46

(b) Thyroxine suppression test :

(i) Total cases diagnosed	..	..	..	16
(ii) Total patients tested	..	..	..	8
(iii) Hyperthyroid	..	..	..	2
(iv) Euthyroid	..	..	..	6

The following tests were decided to be taken up for standardisation in the interest of public and the preliminary arrangements are in progress :

- (i) Insulin Assay
- (ii) Haematological studies like vitamin B_{12} absorption and Red Blood Cell survival and
- (iii) Absorption Studies.

TOXICOLOGY RESEARCH UNIT

This unit started functioning from 1st January 1973 and is actively engaged in toxicity evaluation of various commercial samples. It is headed by Dr. B. B. Gaitonde, Director, Haffkine Institute. At present, it has the following staff :—

(1) Shri M. G. Phatak	Senior Research Fellow
(2) Shri S. S. Jadhav	Junior Research Fellow
(3) Shri V. H. Redij	Laboratory Assistant
(4) Shri S. G. Ghogle	Animal Attendant

Besides this, there is also a unit for toxicological research financed by the Central Council for Research in Indian Medicine and Homoeopathy (C.C.R.I. M.H.) under Dr. Gaitonde, in which Shri B. N. Kale and Shri G. S. Parab are working as Technician and Animal Attendant respectively. Although a post of Assistant Research Officer has been sanctioned for this C.C.R.I.M.H. Unit, it could not be filled owing to financial stringency. Hence, the Haffkine Institute Toxicological Research Unit has to assist this C.C.R.I.M.H. Unit for pharmacological and toxicological evaluation of Council's compounds under the code name ' Ayush '.

Dr S. Srinivasan joined this unit as S.S.O.C.I.—I with effect from 19th February 1975.

At present, attempts are being made to convert this unit into a full-fledged department. In spite of meagre staff, the following work has been carried out in this unit during the year under review :—

(1) Acute toxicity of about 18 commercial compounds of diverse nature, viz : Vitamin B injections, insecticides, food additives, plastics, etc.

(2) Four compounds were tested for eye irritation and three for skin irritation.

(3) About four-hundred drugs were tested for undue toxicity and presence of depressor or histamine-like substances.

The total income earned by this unit during the year under review was approximately Rs. 27,000.

DEPARTMENT OF VIROLOGY

Staff

Dr. (Smt.) Chaya H. Pandit was appointed as Medical Officer, M.M.S., Class III, following the resignation of Dr. (Kum.) C. D. Kulkarni. She took charges from 12th October, 1974.

Shri Jagadish M. Deshpande was awarded the Junior Research Fellowship of the Government of Maharashtra. He joined from 1st January, 1975.

Shri B. D. Sathe joined as Senior Technical Assistant from 6th March, 1975.

Host-Virus Interaction Applied Studies—Diagnosis.

A. Rabies

Microscopy.—A total of 19 brain specimens was received for diagnosis. The results are presented in Table 1 below:

TABLE 1
Diagnosis of rabies samples

Species and total number		Number positive for Negri bodies	Number negative for Negri bodies
Dog	9*	7	1
Human	7*	5	
Bullock	2	2	
Chimpanzee	1		1

*Two human samples and one from a dog were in putrified condition.

B. Rubella

Routine work included preparation of primary cultures of amnion at chick embryo and maintenance of VERO and BHK₂₁ cell-lines for use in the Department.

Immunisation Clinic

As a service for the staff of the Institute and for the public an Immunisation Clinic is conducted every working day between 11.00–13.00 hrs. by the Department of Virology. The following vaccines were administered during the year :

Type of immunisation	No. of persons inoculated
Oral Polio vaccine (OPV)	341
Antirabic vaccine (ARV)	385
Cholera (El Tor) vaccine	20
Anti-typhoid-para-typhoid vaccine (TABC)	10
Tetanus Toxoid (TT)	20
Triple antigen (DTP)	184
Grand Total	960

Side-effects of vaccination occurred very rarely. Only local reactions of ARV occurred in a few persons who were treated successfully with anti-allergic drugs.

The number of persons immunised at the Clinic during the year under report was nine hundred and sixty. Of these, two hundred and thirty belonged to the Institute staff or their family members and seven hundred thirty outsiders.

DEPARTMENT OF ZOONOSIS

Cholera carrier studies were continued in Virar area of Thana District. Epidemiological studies in Filariasis, Dracunculiasis and Plague were also continued

Attempts to adapt *N. americanus* to hamsters were partially successful.

Trainees from D.M.L.T. course were demonstrated different parasitological and entomological techniques. Three short-term trainees attended training course in advanced entomology and parasitology.

Dr. M. V. Sant, Deputy Director and Head of the Department of Zoonosis, retired on 28th February 1975. Dr. W. N. Gatlwar, Senior Scientific Officer, Class I, took over additional charge of the post of Assistant Director of the department.

Smt. L. V. Sarma was awarded Ph.D. degree in Microbiology and Shri S. Y. Guirgas was awarded M.Sc. degree in Microbiology.

Dr. Sant attended the 25th Annual Meeting of Indian Association of Pathologists at New Delhi and participated in various symposia sponsored by the Haffkine Institute to commemorate the Platinum Jubilee celebrations. Dr. D. M. Renapurkar was deputed to attend the 25th Annual meeting of Indian Association of Pathologists at New Delhi and present papers.

Dr. D. M. Renapurkar was recognised by the University of Bombay as post graduate teacher for M.Sc. and Ph.D. degrees in Applied Biology.

The department continued to provide expert guidance in identification and control of insect and rodent pests of public health importance as well as in the identification of venomous reptiles. The following table shows the number of snakes and other items received for identification and advice.

TABLE 1

Material received	Number
Snakes	20
Rodents, insects and other queries	6
Rodent and insect control queries	5

Besides attending to the above queries, the department also supplied insects, snakes, rodents and micro-slides of insects to various research and educational organisations. Total number of items supplied to various institutions is shown in Table 2.

TABLE 2

Material supplied	Number
Dead Cobras	27
Dead <i>Russell's vipers</i>	9
Dead Kraits	4
Dead <i>Echis carinatus</i>	9
Dead non-poisonous snakes	14
Dead scorpions	2
Snake skins	18
Cast skins	12
<i>X. cheopis</i> (fleas)	6
<i>M. domestica</i> (flies)	6
<i>Culex p. fatigans</i> (mosquitoes)	6

Maintenance of colonies of fleas (*X. cheopis* and *X. astia*), flies (*Musca domestica nebulosa*), cockroaches (*P. americana*), mosquitoes (*Culex p. fatigans*) and rodents was continued. Evaluation of two insecticide formulations, D-1 and D-2, was completed.

Visitors

Demonstration of venom extraction technique for visitors from various parts of the world proved an added attraction to the foreigners visiting the institute. Visitors were given educative information on identification of snakes and first-aid treatment of snake-bite cases etc. The total number of visiting teams and distinguished visitors, who visited this department, is shown in Table 3.

TABLE 3

Details	No. of individuals Demonstrated
Thursday demonstrations	3,577
Student from different colleges	185
Visitors from Scientific Institutions	105
Distinguished visitors	51
Grand Total	3,918

Production

During the period under report, 190.34 gms. of snake venoms were processed and 247.27 gms. of venom were supplied for the manufacture of antivenin and research purpose as shown in Table 4.

TABLE 4

Snake Venom			Quantity of venom processed	Quantity of venom Supplied
Krait	..	!..	45.17 gm.	64.25 gm.
Cobra	..	..	69.70 gm.	70.76 gm.
Russell's viper	..	..	52.31 gm.	60.34 gm.
Echis	..	..	23.16 gm.	51.92 gm.
Grand Total			190.34 gms.	247.27 gms.

The quantity of scorpion venoms processed and supplied is shown below

TABLE 5

Scorpion Venom			Quantity of venom processed	Quantity of venom supplied
Black Scorpion	..		8.74 gms.	1.31 gms.
(<i>Palamneus gravinanus</i>)	..			
Red scorpion	..		9.57 gms.	5.74 gms.
(<i>Buthus tamulus</i>)	..			
Grand Total			18.31 gms.	7.05 gms.

MAINTENANCE ENGINEERING SECTION

The year under review kept the section fully busy with varied activities due to the over all increase in the activities of this Institute. The Engineers and Technicians of the Section carried out routine maintenance work as well as executed a number of erection and fabrication jobs. Constant attention to Desivac Plant of the Immunohaematology Department has resulted in the smooth working of that department.

It is gratifying to note that the Mechanical Section carried out a number of jobs this year departmentally, viz. complete fabrication and erection of the 15 ton capacity cooling tower for the airconditioning plant of Chemotherapy Department and 7.5 ton capacity cooling tower for the airconditioning plant of Quality Control Department. Further, this Section has executed complete erection of power laundry equipment. In addition to this a large number of additions and alterations to the various pharmaceutical machineries were carried out as per the requirements of the departments. Such types of jobs are highly quoted for by outside agencies. This has also resulted in saving loss of production hours.

The Electrical Section played a major role in the repair and maintenance work. The staff undertook a number of electrical wiring jobs for various machineries and also undertook maintenance of ovens, incubator-rooms, centrifuge machines, air compressors, vacuum pumps, shaking machines, stirrers and various types of electrical motors etc., on a regular basis. In addition to this, the Section is maintaining and servicing various electronic instruments which were so far being maintained and serviced by outside agencies.

During the year, this section has introduced a system of "Planned preventive maintenance" in order to prevent the major breakdowns and also for the smooth workings of the various equipments located at the Institute.

The Refrigeration Unit also played a significant role both in the fields of production and research. A large number of refrigeration plants was maintained i.e. deep-freeze, refrigerators, airconditioning plants, coolers, walk-in-coolers, water coolers etc. It is gratifying to note that during this year, the Section has installed two more walk-in-coolers (one at the Sales Counter and the other at Pimpri Stud Farm), thus providing increased cold room facilities at the Institute.

The Carpentry Section also contributed substantially to the carpentry jobs undertaken at the Institute, both for production and research departments and also for other services, sections and administrative departments.

In short, this section played quite a significant role in the total output of the Institute.

PART IV

PRODUCTION ACTIVITIES

DEPARTMENT OF ANTITOXINS AND SERA

Staff

During the year under review, more changes took place in the staff distribution as well as in the activities of the department.

Staff of Filling and Packing Section was placed under Centralized Filling and Packing Section with Shri Patki as Officer-in-charge. This resulted in corresponding reduction in the staff of the Department of Antitoxins and Sera.

Dr. (Smt.) Sulabha Kulkarni, who joined the Department of Immunology (Research) last year as Sr. Scientific Officer, Class I, resigned and went abroad for better prospects. Shri A. A. Parelkar from Immunohaematology Department, joined the Department of Antitoxins and Sera (Production) as Junior Scientific Officer.

Dr. D. P. Buduk was awarded M.Sc. degree of the Bombay University for his thesis on "Plasmapheresis in horses". Smt. A. A. Gandhi was awarded M.Sc. degree for her work on Studies in Tetanus Toxin Production.

Shri G. S. Chhatwal, Sr. Research Fellow in the Department of Immunology (Research) under Diamond Jubilee Fellowship, completed his exhaustive and very good work for Ph.D. and submitted his thesis for the same to the Bombay University.

Dr. (Smt.) Sharda R. Kankonkar, Ph. D., started work on irradiated Cobra venom. Shri R. C. Kankonkar, M.Sc., completed his work on snake venoms and submitted his thesis for Ph. D. degree to the Bombay University.

Dr. B. A. Kulkarni from the Bombay Veterinary College, working in the Department of Immunology, was awarded Ph.D. degree by the Bombay University.

Part of the Stables Section of the Department of Anti-toxins and Sera, which was converted into Stores and Bleeding Chamber, is now being used for production of Pemon by the Department of Pharmaceuticals.

Pimpri Development

A circular road is being constructed to connect most the stable areas, newly built as per plan. Tube light arrangement was made on the main pathway. The ditch between two stable, which was dangerous for horses, was filled up.

Lucerne grass project gave good dividends in terms of crop as compared to the average yield, as stated in Agricultural Hand Book.

Arrangements were made for temporary additional water supply to the Lucerne grass crop from the well near E.S.I.S. Unit in the compound of the Pimpri Farm. Permanent arrangement has been deferred to the next financial year as a result of stringent economy measures.

Plan was chalked out and submitted to Government for increasing bleeding chamber area, but this could not be constructed during current year. However, provision has been made to aircondition the additional area. It is proposed to provide a larger sterilizing unit in the Pimpri laboratory to sterilize 20 and 40 litre bottles and to increase capacity to sterilize 10 litre bottles to cope up with the increased work load of sterilization.

Strength of the horses, as it stood at the close of the year was 233, which included 199 horses under bleeding, 23 pre-bleeding, and eleven on rest, out of which ten are foals. Strength of the horses for anti-snake venom was increased to 110 due to increase in the demands for anti-snake venom serum. Some of the demands were from countries like Africa, Philipines, Ceylon and Bangla Desh. Corresponding reduction was made in number of horses for tetanus in view of the reduced demands.

The area under cultivation of Lucerne grass was extended to provide for larger quantities in order to become self-sufficient in this respect in view of the fluctuating supply and fluctuating rates quoted by the contractors from time to time.

However, problems were faced regarding water supply to the area and these are being gradually solved. It is planned to have two boring wells near the Lucerne grass area to provide water in addition to available water from sewage store and E.S.I.S. well. The present output of Lucerne grass is 800-1000 kgs/day, which is double that of last year (400 kg./day).

The department produced over Rs. 50,000 worth of hay to sustain horses for two months in Pimpri. Also, in Bombay, sufficient hay was produced and no money was spent for supply of hay to horses in Bombay during the period July to April.

Fresh plantation of Madras thorn was made to protect Lucerne grass where it did not grow.

Experiments were made to improve the yield, weight and quality of Lucerne grass by providing waste products from Department of Anti-toxins and Sera (Bombay Unit), which proved successful. Also bacteriological manure was tried.

More trees were planted during this year to improve the perspective ; the gardens were also improved. No large replenishment was made in the number of horses in order to keep the maintenance cost low.

'Cast' horses were procured free of cost from the Military authorities. More horses were tried for the production of gas gangrene antisera. Two horses showed good response. Two horses, which gave marginal response a few months ago, dropped in their response. In spite of this, the department could meet most of the demands. Further efforts are being made to improve Gas gangrene antitoxin stock.

Slight improvement in tetanus response and antivenin responses was observed.

Maximum economy was implemented as in the previous years.

The production and sales figures are as follows :—

TABLE 1

Production.—

(1)	Average Number horses during the year	239
(2)	Total Plasma from horses available during the year from these horses.	2,44,11,000 ml.
(3)	Amount of plasma processed	2,40,66,000 ml.
(4)	Amount of final serum prepared	12,01,000 ml.
	Tetanus antitoxin	582,500 ml.
	Diphtheria antitoxin	159,600 ml.
	Gas gangrene anti-toxin	31,600 ml.
	Anti-snake-venom serum	427,300 ml.

TABLE 2

Production of individual antisera supplied to pharmacist

	No of ampoules
A. (i) Anti-Tetanus Serum 1500 I.U. in 1 ml.	3,62,301
(ii) Anti-Tetanus Serum 10,000 I.U. in 3.4 ml.	15,480
(iii) Anti-Tetanus Serum 20,000 I.U. in 5 ml.	11,181
(iv) Anti-Tetanus Serum 15,000 I.U. in 10 ml. vials	6,498
B. (i) Anti-Diphtheria Serum 1,000 I.U. in 1 ml.	251
(ii) Anti-Diphtheria Serum 10,000 I.U. in 5 ml.	1,027
(iii) Anti-Diphtheria Serum 10,000 I.U. in 10 ml.	25,267
C. Anti-snake Venom Serum	70,534

Sales of Individual Products through Pharmacist

A. (i) Anti-Tetanus Serum 1,500 I.U. in 1 ml.	3,46,010
(ii) Anti-Tetanus Serum 10,000 I.U. in 3.4 ml.	15,706
(iii) Anti-Tetanus Serum 20,000 I.U. in 5 ml.	11,294
(iv) Anti-Tetanus Serum 15,000 I.U. in 10 ml. vials	5,690
B. (i) Anti-Diphtheria Serum 1,000 I.U. in 1 ml.	214
(ii) Anti-Diphtheria Serum 10,000 I.U. in 5 ml.	1,122
(iii) Anti-Diphtheria Serum 10,000 I.U. in 10 ml.	26,275
C. Anti-Snake Venom serum 10 ml.	67,497
D. Anti-Gas Gangrene Serum	215

DEPARTMENT OF BACTERIOLOGY

During the year, the department continued its usual activity of production of Cholera *El tor* and T.A.B. Vaccines, Diphtheria and Tetanus Toxins and Toxoids and Triple Antigen. As the demands for Plague Vaccine were very scanty during the year, production of this vaccine was not undertaken. The following quantities of these different products were produced and supplied during the period under review:—

Product	Quantity Produced (litres)	Quantity Supplied (litres)
Cholera <i>El tor</i> vaccine	9,203	10,534
T.A.B. Vaccine I.P.	297	391
Plague Vaccine I.P.		0.560

The department successfully met the increasing demands for tetanus toxoid from different parts of the Maharashtra State as well as from outside the State.

The following quantities of toxins and toxoids were produced during the year

Product	Quantity (litres)
Tetanus toxin and toxoid—	
Peptic-digest medium toxin	136
Protein-free medium toxin	2,365
Protein-free medium toxoid	2,196
Tetanus toxoid, I.P. (in simple solution)	1,960
Tetanus vaccine, B.P. (adsorbed)	1,350
<i>Welchi</i> toxin	84
Diphtheria toxin and toxoid—	
Papain-digest medium toxin	121
Papain-digest medium toxoid	35
Protein-free medium toxin	1,977
Protein-free medium toxoid	1977
Diphtheria-Tetanus vaccine B.P. (absorbed)	71
Diphtheria vaccine (PTAP)	11

The production of Triple Antigen was undertaken by the department on a regular schedule basis. During the year, sixty-one litres were produced and supplied. It is planned to produce and supply about three million doses of triple antigen during the year 1975-76.

The department also examined forty-two samples of water for their suitability for human consumption, five samples of frog-legs for freedom from *Salmonella* contamination and two samples of water filters for their efficiency to render water bacteria-free.

During the year, twenty-six persons, including M. L. T. and Short-term trainees, were trained in the production and testing of bacterial vaccines.

MEDIA SECTION

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

The manufacture of parenteral solutions also continued in full swing. In addition to the above, the department provided general facilities for sterilization and cleaning of glassware etc.

The Ampoule Cutting Section supplied cut and sterilized ampoules and vials to the Central Filling and Packing Section and other production departments, whenever necessary, for special purposes.

The production figures of the different items are presented below:—

Product	Quantity (litres)
<i>Culture Media</i>	
Case in-hydrolysate medium (cholera)	12,995.800
Meat-digest agar (typhoid)	1,304.800
<i>Sterility Test Media</i>	
Aerobic medium	4,561.920
Anaerobic medium	4,717.440
Sabouraud medium	3,925.440
<i>Parenteral Solutions</i>	
Dextrose and Sodium Chloride Injection, U.S.P.	40,332.500
Dextrose injection, I.P. 5 per cent	93,175.500
Sodium Chloride Injection, I.P.	55,739.000
Water for injection, I.P.	10,870.000
Water for injection (Blood Bank)	43,435.200
Sodium lactate, B.P.	5,620.000
A.C.D. Solution 'A'	724.500
Sodium Chloride Injection, 20 per cent	184.400
Peritoneal solution 'A'	135.000
Peritoneal solution 'B'	6.000
<i>Other Various Types of Media and Solutions</i>	
Acid-digest of meat agar	257.000
Nutrient agar	744.500
MacConkey's agar	213.000
Sabouraud's agar	57.400
D.E.C. agar	5.400
Löffler's medium	23.290
Lowenstein Jensen's medium	5.500
Peptone water	212.900
Ox-bile medium	22,900
MacConkey's broth	19.500
Hartley's broth	11.300
Koser's citrate	1.000

Product				Quantity (litres)
Glucose phosphate	..	..	..	3.700
2 per cent Citrate saline	..	..	..	13.700
Glucose broth	..	..	..	37.800
Nutrient broth	..	..	..	128.800
Alkaline peptone water	..	..	..	12.400
Lab-lemco broth	..	..	..	3.800
Selenite broth	..	..	..	11.400
Thioglycollate medium	..	..	..	32.900
Christensen's medium	..	..	..	3.200
Standard Staphylococcal broth	..	..	..	0.500
T.C.B.S. agar	..	..	..	13.500
Hoyle's base	..	..	..	3.500
Potato agar	..	..	..	15.000
Normal saline	..	..	..	11,335.000
Muller and Hinton medium	..	..	..	9.000
Buffer saline	..	..	..	12,546.000
Sabouraud's broth	..	..	..	73.800
<i>Sugars for Biochemical Tests</i>				
Glucose	..	..	..	15.000
Lactose	..	..	..	13.000
Mannitol	..	..	..	13.000
Sucrose	..	..	..	13.900
Dulcitol	..	..	..	5.500
Maltose	..	..	..	4.800
Arabinose	..	..	..	0.200
Xylose	..	..	..	1.500

Diagnostic Reagents Section

Stains—

Product	Quantity (litres)
Ziehl Neelsen's Carbol Fuchsin	233.000
Methylene blue	152.000
Gram stain No. 1	44.000
Gram stain No. 2	44.000
Albert stain No. 1	20.000
Albert stain No. 2	24.000
Löffler's Methylene blue	4.000
Safranine	29.000
J.S.B. stain No. 1	26.7000
J.S.B. stain No. 2	32.000
Gentian violet	12.000

Diagnostic Reagents

Benedict's solution	720.000
Preservative fluid for R.B.C.	24.000
Preservative fluid for W.B.C.	24.000
5 per cent Carbolic acid solution	12.000

During the year under review, the diagnostic reagents prepared and supplied were as follows:—

Reagents	Prepared, ml.	Supplied, ml
Dead emulsions	4,04,430	4,02,375
High titre sera	5,738	5,947
Kahn antigen	3,960	5,640
Wassermann antigen	580	1,240
Casonils antigen	533	365
E. coli sera	572	463
R.V. Venom solution	460	371
Cobra Venom Solution	314	479
Stains	20,69,300	20,36,600
Total	24,85,887	24,54,480

DEPARTMENT OF CHEMOTHERAPY

General

The synthesis and studies of newer compounds for possible activity against tuberculosis, leprosy, protozoal infections like amoebiasis, filariasis and helminthic infestations, were continued and about two hundred and twenty-compounds were synthesized, characterized, most of which were subjected to preliminary screening.

As in the previous year, this year too, stress was laid on synthesis of compound for possible anthelmintic or antiamoebic activity. Accordingly, synthesis and study of bicyclic compounds as well as other piperazine derivatives were continued.

The DADDS depot injections, taken up last year, were given a clinical trial, which is in progress at Acworth Leprosy Hospital, Wadala, Bombay, and Richardson Leprosy Hospital, Miraj. Results will be evaluated after some months.

Standardization work on PVC containers was taken up and the monograph of necessary tests has been prepared which has been sent to the Food and Drug Administration of the State Government for acceptance.

The Pilot Plant Committee held meetings at regular intervals and laboratory investigations of some drugs were taken in hand. Styrene oxide, which is the important ingredient in the preparation of tetramisole, has been fruitfully investigated using locally available materials.

Staff

Two Senior Research Fellows and two Junior Research Fellows continued to work on their research problems. Similarly, Government of India Scholar and Government of Maharashtra Scholar continued to work on their problems.

During the year under review, one student obtained M. Sc. degree and two were awarded Ph.D. degree of the Bombay University in Organic Chemistry.

Production

The production and supply of all the products manufactured by the department for the use of Government Hospitals, E.S.I.S., and Public Health Organizations was continued. The output was slightly higher than that of the previous year. The list of items and their production are given below :

Name of the products					No of tablets manufactured.
Vitamin A-D(P)	..	..	..	..	77,68,600
Vitamin A-D(S)	..	..	..	..	38,68,400
Vitamin B ₁	..	..	..	..	7,74,200
Vitamin B ₂ (5 mgs.)	..	..	..	..	1,95,100

Name of the Products					No of tablets manufactured
Vitamin B ₂ (2 mgs.)	..	..	..	..	1,97,358
Vitamin C	..	..	..	..	53,44,100
Thiacetazone and Isoniazid (S)	..	..	..	..	13,90,000
Vitamin C (100 mg)	..	..	..	..	26,81,400
Vitamin C (500 mg)	..	..	..	..	40,72,316
Nicotinic acid	..	..	..	..	7,92,800
Vitamin BC ₃	..	..	..	..	10,21,100
Polyvitamin	..	..	..	..	2,28,40,400
Vitamin B-Complex	..	..	..	..	1,21,43,700
Di-iodohydroxyquinoline	..	..	..	..	48,22,000
Calcium with vitamin D	..	..	..	..	16,18,880
Isoniazid	..	..	..	..	59,40,800
Isoniazid (strong)	..	..	..	..	14,85,000
Thiacetazone and Isoniazid	..	..	..	..	47,63,500
Aspirin	..	..	..	..	2,61,38,000
Sulphadimidine	..	..	..	..	2,28,07,000
Analgin	..	..	..	..	24,92,600
Phthalyl Sulphathiazole	..	..	..	..	26,90,100
Vitamin K	..	..	..	..	2,25,800
Sodium aminosalicylate	..	..	..	..	67,46,600
Chlorpropamide (S 250 mg)	..	..	..	..	3,43,100
Chlorpropamide (M 100 mg)	..	..	..	..	10,84,800
Berberine hydrochloride	..	..	..	..	4,36,000
Tolbutamide	..	..	..	..	16,98,900
Vitamin B-C	..	..	..	..	1,57,29,500
Grand Total					16,21,11,094
Sodium Pas-granules	..	..	..	..	11,78,900 kgs.
Pemon	..	..	..	..	71,280 litres
Sulpha paste	..	..	..	..	38,590 kgs.

DEPARTMENT OF IMMUNOHAEMATOLOGY

General

The routine production activities of the department were continued.

In addition, laboratory testing facilities for specialised laboratory tests like Rh antibody, Australia antigen red-cell enzyme, were offered to the medical profession.

Samples of human sera for export were tested for the Custom's authorities. The State Government has taken keen interest in promoting the voluntary blood donation movement and has issued instructions to the district authorities to help the mobile blood collection drive.

This has helped the Public Relations Officer of this department in expanding his activities further. It is encouraging to note that the number of prisoners donating blood has been on the increase.

Staff

Dr. K. V. Kulkarni, Assistant Director, proceeded on leave prior to retirement with effect from 1st April 1975.

Dr. (Smt.) J. K. Dave, took over the additional charge of the Department as Assistant Director, with effect from 1st April 1975.

Smt. J. M. Gokarn (Nee J. A. Bellare), Senior Technical Assistant, was awarded M.Sc. degree for her thesis entitled "Studies on hepatitis and other diseases with special reference to Australia antigen (Hepatitis β Antigen, HB-Ag)" by University of Bombay.

Routine

The following figures give the account of the routine activities of the department :—

Number of institutions colleges and factories etc. contacted ..	71
Number of institutions/colleges and factories etc. where blood collection sessions wereheld.	37
Number of voluntary donors enrolled	3,384
Blood donors—	
(i) Voluntary	3,041
(ii) Paid	3,106
(iii) Prisoners	4,525
(iv) Hospitals	6,328
Grand Total ..	17,000

Production

The production and supply figures are as follows :—

Name of product	Packing	Product	Supplied
Dried plasma	250	2,192 bottles ..	1,885 bottles
Human fibrinogen	150	524 bottles ..	519 bottles
Human fibrinogen... ..	10	185 ampoules ..	215 ampoules
Human gamma globulin	1	2,520 ampoules ..	2,813 ampoules
Cryoprecipitate	100	164 bottles ..	161 bottles
Fresh plasma	250	6 bottles ..	6 bottles
Packed cells	150	812 bottles ..	812 bottles
Fresh plasma	1	510 ampoules ..	500 ampoules
Fresh plasma	125	2 bottles ..	2 bottles

Production and supply of blood grouping reagents

Name of Product	Packing	Product	Supplied
Anti-A	1	15,200 Ampoules	14,517 ampoules
Anti-A	5	3,320 ..	3,135 ..
Anti-B	1	14,364 ..	14,674 ..
Anti-B	5	3,067 ..	3,030 ..
Anti-A+B	1	1,888 ..	2,312 ..
Anti- A+B	5	210 ..	318 ..
Coomb's reagent	1	900 ..	995 ..
Anti-D (Rho)	1	4 084 ..	4,513 ..
Bovine albumin	1	600 ..	550 ..
Bovine albumin(in powder form)	gm	25 gms.	25 ms.
Anti-A1(Human)	1	230 ampoules	235 ampoules
Anti-H (Lectin)	1		101 ..

Test carried out—

Rh tests	256
Paternity tests	2
Coomb's tests	8
Cross-matching tests	6
Tests on samples exported	57
Number of charges of anti-venin dried	68

DEPARTMENT OF VIRAL VACCINES

Rabies

During the year under review, the following vaccines and sera were prepared and supplied:—

Name	Prepared (ml.)	Supplied (ml.)
Sheep bran carbolised vaccine for human use ..	37,42,769	37,42,796
Canine prophylactic vaccine ..	41,538	41,538
Oral Polio vaccine ..	19,92,095	19,92,095
Hyperimmune rabies serum (freeze-dried) ..	1,765	1,765

Immune serum amounting to 45.5 litres was collected from immunised horses. This has been concentrated to 5 litres by ammonium sulphate precipitation, lyophilised in 250 ml. aliquots for future use.

PART V **ADMINISTRATION AND FINANCIAL REPORT** **FINANCIAL SUMMARY**

During the year under review, the Institute continued its traditional activities of production, research and other activities in the cause of public health of the State and the country. As compared to the sales of Rs.123.95 lakhs (inclusive of sales worth Rs.29.62 lakhs to the Employees' State Insurance Scheme) during the last year, those during the year under review showed an increase of Rs.106 lakhs. The sale of the year under review is Rs.163.01 (inclusive of sales worth Rs.17.93 lakhs to the Employees State Insurance Scheme). In spite of difficulties encountered in procurement of raw materials and the overall increase in the cost of raw materials, packing materials etc., the Institute has been able to increase its sales during the year under review by Rs.39.06 lakhs.

The trifurcated accounts started in 1966-67 were drawn up for the year under review as well. The preliminary outstandings against sales as on 31st March 1975 stood at Rs.65.27 lakhs as against the final figure of Rs.6.77 lakhs for the last year. The financial picture as on 31st March 1975 is reproduced below:—

Our position as on 31st March 1974

	1973-74 Rs	1972-73 Rs
What we have (Assets)—		
Land and buildings	1,15,24,970	1,13,416
Plant and Machinery	58,87,324	57,105
Furniture and fixtures and equipments	16,62,408	16,181
Motor vehicles	1,74,720	1,720
Library books	11,83,927	10,498
Live stock (excluding gifts)	2,43,228	3,750
Stocks and stores	1,19,89,267	94,531
Amount to be received	44,69,679	32,465
Cash transit remittances etc	13,18,618	13,723
H.I. Depreciation Fund (Investment accounts with Government).	21,50,000	19,000
	4,06,04,141	3,62,139
What we owe (Liabilities)—		
Depreciation fund	37,18,255	34,571
Amount to be paid	20,58,955	14,240
Miscellaneous deposits	4,30,781	2,175
H.I. depreciation fund with Government	21,50,000	19,000
	83,57,991	71,986

ACADEMIC COURSES AND TRAINING OF HEALTH PERSONNEL

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

Post-Graduate Degrees

M.Sc. Microbiology, Biochemistry, Zoology, Organic Chemistry, Applied Biology and Pharmacology
Ph.D. Microbiology, Biochemistry, Zoology, Organic Chemistry, Applied Biology and Pharmacology.

Diploma

DNMLT. Diploma in Medical Laboratory Technology conferred by the University of Bombay.

During the year, the Institute was recognised for M.D. course in Pathology and Pharmacology.

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

Dr. B. B. Gaitonde	..	..	Dr. C. V. Deliwala
Dr. M. V. Sant	..	..	Dr. P. Y. Dighe
Dr. K. W. Kulkarni	..	..	Dr. Y. S. Nimbkar
Dr. M. B. Bhide	..	..	Dr. K. H. Dave
Dr. F. L. Saldanha	..	..	Dr. S. S. Sabnis
Dr. A. J. Baxi			
Dr. D. M. Benapurkar			

During the year, three more post-graduate teachers were recognised by the University of Bombay:

Dr. (Smt) J. K. Dave
 Dr. M. V. Shirodkar
 Dr. N. E. Vad

Dr. M. V. Sant and Dr. (Smt.) J. K. Dave of this Institute are recognised by the College of Physicians and Surgeons of Bombay, for training candidates for: (a) F.C.P.S. examination in Pathology, Bacteriology and (b) D.P.B. (Diploma in Pathology and Bacteriology).

The Institute is also affiliated to the Poona, Nagpur and Baroda Universities for post-graduate studies, viz M.Sc. and Ph.D. by research in various subjects. It is also recognised by the National Council of Educational Research and Training, New Delhi, for guiding National Talent Scholars for M.Sc. studies.

During the year, the Institute was also recognised by the Kokan Krishi Vidyapeeth in the faculty of Veterinary Science, Marathwada University has also recognised this Institute as research centre for guiding students for research leading to Ph.D. degree in Biochemistry, Applied Biology, Organic Chemistry, Zoology and Pharmacology.

Trainees admitted to :—

Number of research fellowships and scholarships :—

Government of India Research Training Scholarships	..	4
Government of Maharashtra Senior Research Fellowships	..	6
Government of Maharashtra Junior Research Fellowships	..	5
Government of Maharashtra Senior Research Scholarship	..	1
Government of Maharashtra Research Scholarships	..	3
Haffkine Institute Diamond Jubilee Research Fellowships	..	3

DEGREE AWARDS DURING THE YEAR

Name of the candidate 1	Degree and Subject 2	Topic of Thesis 3
Shri S. R. Prabhu ..	M.Sc. (Organic Chemistry).	Synthesis and study of Lere-phthalic acid and Homophthalic acid as possible antitubercular agents.
Shri D. T. Chaudhari	Ph. D. (Organic Chemistry).	Chemotherapy of Cancer
Shri B. G. Khadse ..	Ph.D. (Organic Chemistry).	.. Synthesis of heterocyclic compounds as possible anthelmintic agents.
Shri D. G. Bapat	Ph. D. (Organic Chemistry).	Chemotherapy of cancer synthesis of nitrogen mustard derivatives of 5-Aminobenzimidazoles and 1-(aminoaryl) imidazoles as possible anticancer agents.
Shri D. S. Chitnis ..	M.Sc. (Microbiology)	Studies on R-factors.
Shri A. K. Patil ..	M.Sc.(Microbiology)	Studies on <i>Vibrio parahaemolyticus</i> in Bombay.
Kum. M. N. Bablad ..	M.Sc.(Microbiology)	Studies in the antigens secreted by <i>Clostridium perfringens</i> type A.
Shri Sami Yousuf Guirgas	M.Sc. (Microbiology).	A survey of intestinal parasites in Maharashtra State with special reference to amoebiasis and its haemagglutination test.
Kum. L. S. Iyer ..	Ph.D.(Microbiology)	Studies in Leptospirosis.
Shri K. N. Joshi ..	Ph.D.(Microbiology)	Drug resistance in Mycobacteria : role of cell wall, cell membrane and mycobacteriophages in acquiring drug resistance by Mycobacterial.
Kum. L.S. Shirsat ..	M.Sc.(Biochemistry)	Study of blood levels of 5-Hydroxy tryptamine Cholinesterase and heat stable alkaline phosphatase in different stages of pregnancy.

1	2	3
Kum. J. A. Bellare ..	M.Sc.(Biochemistry)	Studies on hepatitis and other diseases with special reference to Australia antigen (Hepatitis B antigen, HB—Ag).
Shri R. B. Pathare ..	M.Sc.(Biochemistry)	Studies in tetanus toxin production.
Shri S. S. Karkhanis ..	M.Sc.(Biochemistry)	Biochemical studies on protozoan <i>Tetrahymena</i> Pyri-formis "W".
Shri H. K. Soni ..	M.Sc.(Biochemistry)	Effects of drugs on red cell metabolism, <i>in vitro</i> studies.
Smt. H. V. Phadnis ..	M.Sc.(Biochemistry)	Biochemical studies on <i>Vibrio</i> and <i>El Tor cholerae</i> with reference to Ampicillin, Chloramphenicol and Berberine.
Kum. R. S. Buit ..	Ph.D.(Biochemistry)	Biochemical studies on the action of Furazolidone on cholera organisms.
Shri V. P. Shinde ..	Ph.D.(Biochemistry)	Studies on nutritional requirements of cholera organisms.
Shri Syed Raziuddin ..	Ph.D.(Biochemistry)	Biochemical studies on <i>Vibrio</i> and <i>El Tor cholerae</i> .
Shri U. J. Dumaswala	Ph.D.(Biochemistry)	Studies on the effect of dietary carbohydrates (starch / sucrose) on lipid metabolism in the albino rat.
Shri S. N. Joglekar ..	Ph. D. (Applied Biology).	Central integrated mechanism of vomiting and neurotoxic effects after administration of cardiaglycosides in cats.

Trainees admitted to :—

Diploma in Medical Laboratory Technology (DMLT)	..	14
Medical Laboratory Technology Course (MLT)	..	12
Short-Term Training Course (STT)	..	82

Number of research fellowships and scholarships :—

Government of India Research Training Scholarships	..	4
Government of Maharashtra Senior Research Fellowships	..	6
Government of Maharashtra Junior Research Fellowships	..	5
Government of Maharashtra Senior Research Scholarship	..	1
Government of Maharashtra Research Scholarships	..	3
Haffkine Institute Diamond Jubilee Research Fellowships	..	3

FELLOWSHIPS AND SCHOLARSHIPS ATTACHED TO THE INSTITUTE DURING THE YEAR

Name	Fellowship or Scholarship	Subject	Title of Project
Shri A. V. Athawale	Government of India Research Training Scholarship.	Biochemistry	Amoebiasis
Kum. R. M. Baride	Government of India Research Training Scholarship.	Biochemistry	Biochemical changes in experimental rabies.
Shri B. G. Khadse ..	Government of Maharashtra Senior Research Fellowship.	Organic Chemistry.	Synthesis of heterocyclic compounds as possible anthelmintic agents.
Shri D. T. Chaudhari	Government of Maharashtra Senior Research Fellowship.	Organic Chemistry.	" Chemotherapy of Cancer ".
Shri M. G. Phatak ..	Government of Maharashtra Senior Research Fellowship.	Pharmacology.	Toxicity studies of indigenous drugs and commercial products.
Dr. S. V. Gadre ..	Government of Maharashtra Senior Research Fellowship.	Microbiology	Diphtheria toxin production in surface and submerged culture.
Shri A. D. Kulkarni	Government of Maharashtra Senior Research Fellowship.	Biochemistry	Studies on immunological aspects of helminthic infections.
Dr.(Smt.) S.R. Kankonkar.	Government of Maharashtra Senior Research Fellowship.	Biochemistry	(1) Cobra venom toxoid (2) Biochemical and immunological studies of scorpion venom (Black and Red).
Shri S. S. Jadhav	Government of Maharashtra Junior Research Fellowship.	Pharmacology	Pharmacology of Pertussis and Diphtheria toxins.
Kum. S. W. Awachat	Government of Maharashtra Junior Research Fellowship.	Biochemistry	Immunological studies on leprosy.
Shri H. S. Ved ..	Government of Maharashtra Senior Research Scholarship.	Biochemistry	Studies on Rickets
Shri B. R. Colah ..	Government of Maharashtra Research Scholarship.	Organic Chemistry	Synthesis of thiazoly derivatives of possible therapeutic value.

Name	Fellowship or Scholarship	Subject	Title of Project
Kum. S. M. Joshi ..	Government of Maharashtra Research Scholarship.	Biochemistry	Biochemical studies on the venom of <i>Echis Carinata</i> .
Shri S. P. Chitnis	Unichem Fellowship.	Research Pharmacology.	Pharmacology of scorpion venom.
Shri S. C. Talwalkar	Unichem Fellowship.	Research Biochemistry	Studies on malnutrition stress effects on adrenal function.
Shri R. S. Joshi ..	Unichem Fellowship.	Research Pharmacology.	Pharmacological studies on the venoms of Indian elapid snakes.
Shri G. S. Chhatwal	Diamond Jubilee Senior Research Fellowship.	Biochemistry	Biochemical studies on the venoms of Indian elapid snakes.
Shri K. T. Shetty ..	Diamond Jubilee Junior Research Fellowship.	Biochemistry	Effect of oral contraceptive on brain neuro-humoral pattern in experimental animals.
Shri V. B. Babne ..	Diamond Jubilee Junior Research Fellowship.	Biochemistry	Antifertility agents of plant origin.
Shri N. T. Joshi ..	Government of Maharashtra Research Scholarship.	Microbiology	(1) Sensitivity of <i>N. gonorrhoea</i> to antimicrobial agents.

PART IV ACADEMIC ACTIVITIES

CRITICS' CLUB

The club continued its activities under Dr. H. J. Mansharamani as its secretary.

The activities during the year under report included thirteen lectures by the institute scientists and seven lectures by guest scientists from outside. In addition, there were seven other meetings for other purposes. They are listed below :—

Institute Lectures

Speaker	Topic
Dr. D. M. Renapurkar	.. Laboratory trials of a new rodenticide RH 787.
Dr. B. G. Khadse	.. Approach to synthetic anthelmintics and mode of action of different anthelmintic agents.
Shri S. N. Joglekar	.. Mechanism of vomiting and neurotoxicity of cardiac glycosides.
Dr. (Smt.) S. A. Kulkarni	.. Homo-reactant, a naturally occurring auto-antibody in rabbits.
Kum. Shaila M. Rao	.. Sarcoma blockade <i>in vivo</i> : Rabies-Rous and other systems.
Shri R. B. Pathare	.. Tetanus toxin production in tryptic digest of skim-milk powder.
Dr. S. Mukerji	.. A bacteriological study of <i>Acinetobacter anitratum</i>
Dr. (Smt.) N. Sagheer	.. Mechanism of action of Anthelmintics—A review
Dr. V. H. Apte	.. Life cycle of <i>Nematospiroides dubius</i> (Baylist, 1926).
Shri A. V. Muthe	.. The effects of <i>Naja naja</i> venom on blood clotting factors.
Shri B. R. Colah	.. Synthesis of thiazolyl derivatives of possible therapeutic value.
Dr. S. Mukerji	.. Scope of radio-labelled antigen of <i>S. typhi</i> in serology of typhoid fever.
Shri G. S. Chhatwal	.. Purification and characterization of some biologically active components from <i>Bungarus caeruleus</i> (Common Krait).

Guest Speakers

Speaker	Topic
Dr. V. T. H. Gunaratne, F.R.C.P., D.P.H., Regional Director, World Health Organisation, New Delhi.	
Dr. C. V. Bapat, M.Sc., Ph.D., Senior Scientific Officer, Bio- logy, Biology Division, Cancer Research Institute, Parel, Bombay.	Problems in cultivation of <i>M. leprae in vitro</i>
Dr. Paul Boquet, Head of the Department of Venom Research, Pasteur Institute, Paris.	.. Some immunological aspects of snake ven- oms.
Dr. S. S. Kelkar, M.D., Professor of Microbiology, Medical College, Aurangabad.	Hepatitis ' B ' antigen.
Mr. J. Mcl. Philp, Environmental Safety Officer, Unilever Limited, London.	
Dr. D. A. Hemns, M.B.B.S., Ph.D., Lecturer, Department of Biochemistry, Imperial College of Science and Technology, London.	.. Hormonal control of hepatic metabolism.
Shri A. Y. Nimbkar	.. Evaluation of the nature of the autonomic transmission in the isolated rat stomach fundal strip preparation.

Other Meetings

26th April 1974	.. Lecture demonstration on "Advances in Filtration Technology" by the courtesy of M/s. Sartorius Membran Filters GmbH, Germany.
26th April 1974	.. Screening of a film entitled "Life of M. Pasteur"
27th April 1974	.. Screening of a film entitled "Rabies Vaccine and Pasteur Institute."
7th September 1974	.. Screening of a film entitled "New face of Rabies".
7th October 1974	.. Send-off to Dr. C. V. Deliwalla on his retirement as Deputy Director (Research), Haffkine Institute.

OBITUARIES

- 7th June 1974 .. Condolence meeting to mourn the sad demise of Dr. Karl F. Meyer, Director Emeritus, The George Hooper Foundation, University of California, U.S.A. and Shri B. V. Patel, *ex*-Assistant Director, Department of Pharmacology, Haffkine Institute, Bombay.
- 19th June 1974 Condolence meeting to mourn the sad demise of Dr. A. K. Hazra, *ex*-Assistant Director, Department of Anti-toxins and Sera, Haffkine Institute.

BULLETIN OF HAFFKINE INSTITUTE

Three issues of ' Bulletin of Haffkine Institute ' were published during the period under review.

The bulletin projected the research work done at the Institute. Each issue carried an article by an eminent scientist. This bulletin is covered by the following international abstracting journals :—

1. Biosciences Informations Services of Biological Abstracts, U.S.A
2. Chemical Abstracts Services, U.S.A.
3. Current Contents, U.S.A.
4. Tropical Diseases Bulletin, England
5. International Pharmaceutical Abstracts, U.S.A.

LIBRARY

Reorganization

The work of collecting references and reprints from 1968 onwards on communicable diseases except on cholera, is still in progress. Over 1,800 references and 400 reprints on cholera have been collected so far. Presently, current, references are being added to the list and the reprints are being asked for. Collection of references and reprints on plague has already been taken up and so far about 100 reprints and 400 references have been collected from different sources.

For the 'Haffkine Biography Division', which was introduced last year, 23 off-prints of the papers about Haffkine, photo-copies of two letters written by Haffkine, a photograph of his tombstone and a copy of inscription at the bottom of tombstone and many other interesting publications have been collected so far. Dr. (Mrs.) Lutzkar has helped immensely in this work by supplying many interesting off-prints. Recently, a complete list of Haffkine archives has been received from the Jewish National and University Library, Jerusalem.

The Xerox Copying Service started last year, is getting response from Research Institutes, private firms, Colleges etc. in Bombay and from outside also.

General

In October 1974, the Library Committee was reconstituted. During the year, six meetings of the Library Committee were held in which reorganization of library and other related matters were discussed. The committee members made useful suggestions for improvement of the library. The library continued to participate in the international exchange programme sponsored by W.H.O. and obtained 44 issues of different journals to complete the volumes of these journals. During the year, 22 books were donated by World Health Organization and others. CIBA of India has kindly donated 20 back volumes of "Experientia" and Bull. de L'Institut Pasteur.

From January 1975 the library started subscribing to the following new journals :—

- A.S. M. News
- Current Labour Laws
- Clinica Chimica Acta
- International Journal of Systematic Bacteriology
- Bulletin of the Chemical Society (Japan)
- Pharmaceutical Bulletin (Japan)
- Bulletin of Medical Library Association

This year, owing to financial stringency, the library could purchase only a smaller number of books as compared to those in the last year. The library continued to participate in the inter-library loan service. The extension of working hours from 5 p.m. upto 7 p.m. was continued during the year.

Working of the Library

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

Number of outside readers visiting the library	..	5,833
Number of books and periodicals issued to our staff	..	1,561
Number of periodicals issued under inter-library loan service		240
Books and periodicals obtained for our staff from other libraries.		50

Additions to library

Number of books added during the year	..	107
Number of volumes bound	..	464
Number of current periodicals subscribed	..	195
Current periodicals received free of charge	..	120
Number of reports received from scientific institutions	..	40

LIST OF PAPERS PUBLISHED DURING THE YEAR

- Baxi, A. J. and Kulkarni, K. V. :
Haptoglobins in hemolytic anaemias (1974)
Bulletin of Haffkine Institute, 1, 79-81
- Bellare, Jyoti A., Kulkarni, K. V. and Baxi, A. J. :
Liver function tests in relation to hepatitis-B antigen in hepatitis, leukaemia and leprosy (1974)
Bulletin of Haffkine Institute, 2, 120-122
- Bhide, M. B. :
Animal models : Their utility and potentialities in the study of anti-leprosy drugs (1974)
Brochure of the Indian Association of Leprologists Seminar on Leprosy, 23-25.
- Bhide, M. B.; Naik, P. Y.; Mahajani, S. S.; Ghooi, R. B. and Joshi, R. S. :
Pharmacological evaluation of 'Vasicinone' as an anti-asthmatic drug (1974)
Bulletin of Haffkine Institute, 2, 6-11
- Bhide, M. B.; Naik, P. Y.; Mahajani, S. S.; Ghooi, R. B. and Joshi, R. S. :
Studies on the anti-asthmatic effects of 'Tylophora indica' (1974)
Bulletin of Haffkine Institute, 2, 82-85
- Bhide, M. B., Nikam, S. T.; and Chavan, S. R. :
Bioavailability of nitrofurantoin from commercially available nitrofurantoin tablets (1974)
Bulletin of Haffkine Institute, 2, 32-36
- Dave, J. K. and Chitnis, D. S. :
Urinary Tract infections : Part-I-Multiple drug resistance in pathogens isolated from urinary tract infection in hospital and general practice (1974)
Bulletin of Haffkine Institute, 2, 103-107
- Divakaran, P.; Rao, N. R. and Venkataraman, A. :
Serum cholesterol levels in a typical urban group of population using coconut oil as the chief dietary fat (1973)
Bulletin of Haffkine Institute, 1, 43-45
- Gaitonde, B. B.; Kulkarni, H. J.; Nabar, S. D. and Joglekar, S. N. :
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Part I—Mathematical nature and measurement (1974)
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Transmission Dynamics of malaria—quantitative studies :
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Laboratory trials of a new rodenticide : RH 787 (1974)
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Studies on ecology and symbiosis of *V. cholerae* and *E. coli* (1974)
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Studies on antagonism between *V. cholerae* and *Sh. dys. Flexneri* (1974)
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Shinde, V. P. and Ambegaonkar, S. D. :

Studies on the effect of vitamin B₁₂ on nitrogen metabolism in albino rat (1974)

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Venkatraman, A. :

A critical study of the conditions for the colorimetric estimation of serum transaminases. Part II : The need for following a uniform procedure for the colorimetric estimation of S.G.O.T. in the clinical laboratories (1973)

Bulletin of Haffkine Institute, 1, 28-34.

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Baxi, A.J.; Bapat, J. P. and Kulkarni, K.V.;
Immunoglobulin levels in hepatitis—B antigenaemia.
Bulletin of Haffkine Institute

Bhide, M.B. and Mahajani, S.S.:
Effect of pre-treatment with di-sodium chromoglycate and vasicinone on
sensitivity of guinea-pigs to histamine and sympathomimetic amines:
A correlation with anti-anaphylactic activity.
Bulletin of Haffkine Institute

Chaudhari, D.T.; Sabnis, S. S. and Deliwalla, C.V.:
Potential anti-cancer agents I: Schiff bases from benzaldehyde dimoethene-
sulphonates.
Bulletin of Haffkine Institute

Chitnis, D.S.; Dave, J.K. and Sant, M.V.:
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Bulletin of Haffkine Institute

Dumaswala, U.J. and Venkatraman., A. :
The relative effect of dietary fats and carbohydrates on lipid metabolism in
the albino rate.
Bulletin of Haffkine Institute

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Effect of dietary carbohydrates (Starch/sucrose) on hepatic enzyme levels in
the albino rat.
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Gaitonde, B.B.; Joglekar, S.N.; Nabar, S.D. and Kulkarni, H.J.:
Oestrogenic activity of *Pueraria tuberosa* (Vidharikand)
Bulletin of Haffkine Institute

Gaitonde, B.B. and Kankonkar, Sharda, R.:
Preparation of Cobra (*Naja naja*) venom toxoid using gamma-radiations (1)
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Gaitonde, B.B. and Mahajani, S.S.:
Studies on the mode of anthelmintic action of tetramisole
Bulletin of Haffkine Institute

Gaitonde, B.B. and Renapurkar, D.M.:
Helminthiasis in India I: Prevalence and geographical distribution; Soil
transmitted nematodes.
Bulletin of Haffkine Institute

Gaitonde, B.B. and Talwalkar S.C.:
A study of thyrotropin stimulated ^{131}I release *in vitro* by thyroid glands
obtained from normal and protein-deficient rats.
Biochemical Medicine

Unit Level Class of the Worker's Education Scheme

With the cooperation of the Central Board for workers' Education, the first Unit Level Class was conducted at this Institute from 8th July 1974 to 14th October 1974 by Shri P.G. Patil of this Institute. Twenty employees of this Institute received training. Three employees obtained first grade in the examination, while the remaining seventeen were declared successful. Encouraged by the performance of the first Unit Level Class, the second Unit Level Class was conducted from 20th January 1975 up to the middle of April 1975. Twenty-one employees attended this class. The result of the examination is awaited.

Other activities

Chawl Committee.—There is a Chawl Committee with Dr. K.H. Dave, Assistant Director I/c Department of Viral Vaccines, as President and Shri V.G. Bavaskar, Laboratory Attendant, Animal House, as Secretary. This Committee attends to grievances of the chawl residents and looks after the management and maintains discipline in general.

(b) *Union activities.*—During the period under report, one meeting of Class III Employees' Union and four meetings of the Class IV Employee's Union were convened for discussing their demands, besides other meetings arranged with the office-bearers of both the Unions for discussion on specific subjects.

Labour Welfare Committee

In April 1972, the Director constituted, for the first time, a Labour Welfare Committee. The functions of this Committee are (i) to deal with the cultural and social activities that the management may like to undertake (ii) to evolve, from time to time, labour welfare programmes for the general uplift of the workers (iii) to dispose of various matters, such as individual complaints and other day-to-day problems, in consultation with the nominated representatives (iv) to obtain advice from the Presidents of both the Unions etc. The present Committee consists of the Director as Chairman and the Deputy Director (Production), Deputy Director (Research), Assistant Director (Administration), Presidents of the Class III and Class IV Employees' Union, Exhibition Officer, Assistant Engineer (Maintenance), Deputy Engineers of both Divisions of Buildings and Communication Department as members, and the Labour Welfare Officer as the Convener. This Committee published four issues of the 'Haffkine Vrutra', house journal, during the year under report. This journal is distributed to all the employees free of charge. It serves as a news bulletin covering a gist of the current activities of the Institute and its employees, such as social and cultural life/office life, new appointments, retirements etc. It contains social and semi-scientific articles and poems contributed by the staff members. It is expected that this will help to develop the latent writing faculties present in the various classes of employees.

Sewing Class

As proposed by the Labour Welfare Committee, the Sewing Class, which started from 15th August 1973, continued functioning during this year also. This class is held thrice a week in the Gymkhana Hall, in which the lady members of the employee's families get coaching facilities. About 25 lady members took advantage of this class during the year.

Balwadi

A ' Balwadi ', which was started from 15th August 1973, was also continued during the year. Nearly 50 children of the employees of this Institute took advantage of the classes conducted in this ' Balwadi '.

Miscellaneous

(1) " Ambedkar Jayanti ", " Buddha Jayanti ", " Shiv Jayanti " and " Maharashtra Day " were celebrated by the Haffkine Institute Chawl Committee. " Ganesh Utsav " festival was celebrated by the employees of this Institute this year also.

(2) Collection for the Flag Day on 7th December 1974 was arranged for the benefits of the *Ex-servicemen* and their families.

Gatlewar, W.N. and Sant, M.V.:

Evaluation of Sawada's filarial antigen

Bulletin of Haffkine Institute

Iyer, S. Natarajan and Gaitonde, B.B.:

(1) *Giardia lamblia*—A simple method of cultivation.

(2) Effects of environmental conditions on the culture of *Giardia lamblia*

(3) Studies on the cultivation of *G.lamblia*—possible role of bacterial associates and amino acids.

Bulletin of Haffkine Institute

Kekatpuray, S.V.; Gadgil, S.D.; Dattani, K.K.; Rao, N.R. and Gaitonde, B.B.:

Pharmacokinetic study of a new iron preparation, ferro-glycinesulphate complex

Bulletin of Haffkine Institute

Khadse, B.G.; Shah, M.H. and Deliwala, C.V.:

Synthesis and study of 2-(4'-substituted 1'-piperazinyl) pyrido (3,2-*d*) thiazoles, 5-nitro-2-(4'-substituted-1'-piperazinyl) benz-thiazoles and allied compounds as possible anthelmintic agents.

Bulletin of Haffkine Institute

Kulkarni, K.V.; Baxi, A.J.; Kulkarni, S.K.; Bapat, J.P.; Chanderkar, N.G. and Gaitonde, B.B.:

Evaluation of radio-immune assay for the detection of Australia antigen

Bulletin of Haffkine Institute

Muthe, A.V.; Kulkarni, S.K.; Bapat, J. P.; Baxi, A.J.; Kulkarni, K.V.; Chanderkar, N.G. and Gaitonde, B.B.;

Detection of hepatitis-B (Australia) antigen in human plasma protein fractions using radioimmune assay.

Bulletin of Haffkine Institute

Nerurkar, C.B. and Chaudhari, D.T.:

Synthesis of 2-(*p*-aminophenyl) thiazoles as useful chemotherapeutic intermediates.

Bulletin of Haffkine Institute

Rao, N.R.; Vig, O.P.; Agarwal, S.N.; Sabnis, S. S; and Rao, V.N.:

Transmission dynamic of malaria-quantitative studies III: A computer simulation approach to measurement

Sent for presentation to the 10th Annual Convention of the Computer Society of India.

Saldanha, F.L.; Sant, M.V.; Jhala, H.I. and Nimbkar, Y.S.:

Ecological studies on *V. cholerae* and *P. vulgare*

Bulletin of Haffkine Institute

PART VII
CULTURAL ACTIVITIES
LABOUR WELFARE

General

Welfare activities at the Institute were not only maintained, but also given further impetus during the period under report. Though the main activities of the Institute are research, training and production of drugs and vitamins, the welfare activities of the workers received increased attention of the management.

Sports Club

The Sports Club has facilities for all the Indian and foreign games. The Kabaddi team of this Institute participated in the All-India Kabaddi tournament held at (Badnagar) Ujjain (M.P.) between 31st October 1974 and 3rd November 1974.

It is gratifying to mention here that Shri Salim Abbas, Shri K.S.Tambe, Shri D.M. Waghmare and Shri Shankar Tandel of this Institute were deputed to participate in the Ninth All-India Civil Services Foot-ball tournaments held at Chandigarh from 21st October 1974 to 27th October 1974. Shri Tukaram S. Kadam participated in the Ninth All-India Civil Services Kabaddi tournament held at Bombay from 30th December 1974 to 1st January 1975.

Book Bank

The 'Book Bank' Section started in the year 1970, is now becoming increasingly popular and more than 60 employees of this Institute regularly avail of this facility for college books, S.S.C. magazines, guides, etc. Two hundred and seventy-seven new college books were purchased during the year under report.

Training

(a) *Civil Defence Unit*.—The fourth batch of the Civil Defence Course of House Fire Party received training from 5th August 1974 to 16th August 1974. Twenty-one employees of this Institute attended the class and all of them were declared successful. At a function specially arranged on 30th October 1974, Shri Y.S. Nimbkar, Deputy Director (Production), gave away Civil Defence Course Certificates to the successful candidates,

(b) *Civil Defence Basic Course*.—The fifth batch of the Civil Defence Basic Course, under the auspices of the Civil Defence Organisation was conducted from 18th November 1974 to 7th December 1974 and fifteen employees received training and all of them were declared successful.

(c) *Civil Defence Special First-Aid Course*.—Smt. V. D. Talwalkar and Shri P. P. Mavlankar underwent training from 12th December 1974 to 23rd December 1974 under the Civil Defence First Aid Course No. 40, sponsored by the Civil Defence Staff College, Bombay.

PART VIII
MISCELLANEOUS
DISTINGUISHED VISITORS

Mr. Popav	..	..	Deputy Mayor, Leningrad City.
Mr. Nefedov	..	..	Party Leader (C.P.S.U.), Leningrad City.
Mr. Shetav	..	..	Worker Hero of the Socialist Labour, Leningrad City.
Prof. Dr. A. G. Spais	..	..	Veterinary Faculty, Aristotelian University of the Ssaloniki, Greece.
Mrs. Spais	..	..	" " "
Dr. V.T.H. Gunaratne	..	..	Regional Director for South-East Asia, World Health Organisation, New Delhi.
Dr. Michael Subourdy	..	..	Director, French National Institute.
Dr. W. Rupp.	..	..	Head, Clinical Pharmacology, Medical Department, Farbwerke, Heochst, Ger- many.
Major D. Bandopadhyay	..	..	Armed Forces Medical College, Poona.
Dr. Tibu Foncsi	..	..	University of Veterinary Science, Budapest, Hungary.
Shri J. L. Hathi	..	..	M.P. and Chairman, Drugs and Pharmaceu- ticals Committee, Government of India.
Shri K. S. Chavada	..	..	M. P. and Member, Drugs and Pharmaceu- ticals Committee, Government of India.
Shri Ranen Sen	..	..	M. P. and Member, Drugs and Pharmaceu- ticals Committee, Government of India.
Shri Sharad S. Marathe	..	..	Chairman, Bureau of Industrial Costs and Prices, Ministry of Industrial Develop- ment, New Delhi.
Dr. B. D. Tilak	..	..	Director, National Chemical Laboratory, Pune.
Shri P. R. Gupta	..	..	Adviser (Drugs), Ministry of Petroleum and Chemicals, New Delhi.
Dr. P. S. Ramchandran	..	..	Drugs Controller (India), New Delhi.
Dr. M. L. Dhar	..	..	Director, Central Drug Research Institute, Lucknow.
Shri Vinod Kumar	..	..	Joint Secretary, Ministry of Petroleum and Chemicals, New Delhi.
Dr. O. P. Grover	..	..	Section Officer, Ministry of Petroleum and Chemicals, New Delhi.
Shri Vinod Sathe	..	..	M. P. and Member, Drugs and Pharmaceu- ticals Committee, New Delhi.
Shri S. H. J. Pratt	..	..	Assistant Director, National Police Aca- demy, Abu.
Mr. Alexander V. Bolshakov	..	..	Vice-Consul of General Consulate of U. S. S. R. in Bombay (Science and Technology).

Mr. Valdimir I. Yurin	..	Vice-Consul of General Consulate of U.S. S.R. in Bombay.
Shri P.V.G. Raju	..	M. P. and Chairman, Committee for the purpose of Controlling and Supervising Experiments on Animals, Government of India.
Dr. G. S. Tawil	..	World Health Organisation, South-East Asia Regional Office, New Delhi.
Dr. Abdul Latif Miah	..	Deputy Director, Biological Production Laboratory, Institute of Public Health, Dacca
Dr. Akhlaqur Rahman	..	Superintendent, Pasteur-cum-Vaccine Institute of Public Health, Mohakhali, Dacca.
Mr. Md. Wazed Ali	..	Research Officer, Rabies Vaccine Section, Livestock Research Institute, Mohakali Dacca.
Dr. A. N. Rai Chowdhuri	..	Deputy Director, Vaccine Production and Assay, Central Research Institute, Kasauli.
Dr. A. Balasubramanian	..	Director, Pasteur Institute, Coonor, The Nilgiris (South India).
Dr. J. C. Suri	..	Deputy Director, National Institute of Communicable Diseases, Delhi.
Dr. F. N. Kharkongor	..	Deputy Director, Pasteur Institute, Shillong, Assam.
Dr. N. K. Dutta	..	Special Director, Vaccine Institute, Baroda.
Major S. K. Chatterjee	..	Reader, Department of Pathology, Armed Forces Medical College, Poona.
Dr. M. Pyati	..	Virologist, Division of Biological Products, Indian Veterinary Research Institute Izatnagar (Uttar Pradesh).
Dr. M. S. Nasution	..	Director, Bio-Pharma (Pasteur Institute), Bandung.
Dr. Abdulkadir Madiadipura	..	Head of Rabies Department, Bio-Pharma (Pasteur Institute), Bandung.
Dr. P. N. Sharma	..	Assistant Director, Rabies Vaccines, Central Veterinary Laboratory, Kathmandu.
Dr. P. D. P. Gunatillake	..	Bacteriologist, I/c. Rabies Laboratory, Medical Research Institute Colombo, Shri Lanka.
Miss. C. L. Barsenback	..	Veterinarian, Veterinary Research Institute Gannaoruwa, Peradeniya.
Dr. (Mrs.) Nonglak Asavachinda	..	Medical Officer, Virus Research Institute, Department of Medical Science, Bangkok, Thailand.
Dr. Vara Meesomboon	..	Division of Communicable Diseases Control Department of Communicable Disease Control, Ministry of Public Health, Bangkok, Thailand.

DISPENSARY

During the year, the dispensary was located in the new premises. It continued to serve the needs of its staff and their dependants as hitherto.

A total of about 21,445 patients attended the dispensary for treatment during the year.

About 195 cases of pulmonary tuberculosis were reported. Fourteen new cases were diagnosed from the staff members and 25 new cases were diagnosed from the family members. It was observed that half the number of patients did not take regular treatment. The old cases were sent for regular check ups at J. J. Group of Hospitals, Bombay.

The children are immunised with polio and triple antigen vaccine at the Immunization Clinic.

Family planning programme has been carried out by giving contraceptive tablets. About 45 women are taking these tablets regularly. Ten women have undergone sterilization operation.

Cases requiring expert guidance were referred to the teaching hospitals.

The Departments of Immunohaematology, Clinical Pathology and Biochemistry co-operated in rendering facilities for routine investigations required to be carried out.

HAFFKINE INSTITUTE CO-OPERATIVE CREDIT SOCIETY LTD.

The Society continued satisfactory progress during the year.

					Rs.
Membership	..	..	..	..	1005
Loans advanced	..	..	..	..	10,07,736.00
Share Capital	..	..	..	..	8,50,414.00
Net profit	..	..	..	..	66,195.00

The Society is doing the following types of business :—

1. Advancing loans to members for various purposes including insurance premia.
2. Running a Grain Shop.
3. Running a Staff Canteen.

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