



सत्यमेव जयते

GOVERNMENT OF MAHARASHTRA

ANNUAL REPORT
OF THE
HAFFKINE INSTITUTE
FOR THE YEAR
1968-69



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PART I
GENERAL REVIEW

REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1968-69

GENERAL REVIEW

Research work in Microbiology, Biological Chemistry, Immunology, Virology, Pharmacology, Chemotherapy, Medicinal Chemistry, Haematology continued throughout the year with significant progress being achieved in some areas. During the year, Haffkine Institute staff collaborated with scientists of many laboratories and outside organisations (National or International) to further research in many fields of Institute's activities. Some representative projects are reviewed briefly in this and following pages.

The main effort of the Haffkine Institute is focussed in finding out better remedies or to improving those already existing for the prevention and treatment of diseases and at the same time providing the public with life saving biologicals. Epidemiological studies are a special feature. Training of personnel in scientific research and pharmaceutical manufacture is, in no way, less important. When measured by the yardsticks of qualitative achievements and effectiveness in bringing about significant progress through constructive work, the Institute's record of accomplishment is evident.

The research programme designed to develop an anticholera drug, about which a reference was made in the last report, has been particularly rewarding. After seven years of work, berberine, an alkaloid isolated from an Indian medicinal plant, was introduced in the market as a remedy against acute cholera and diarrhoea. The reports that are coming from the market studies show that this drug is potent and appears to have gained a foothold in the treatment of diarrhoeal diseases including cholera.

The mode of action of berberine was a subject for investigation for the last few years. No satisfactory solution was forthcoming. Last year's study has perhaps given a glimpse of its possible action. It was observed that both classical *V. cholerae* and *Vibrio El tor* assimilate berberine when they come in contact with the drug. Seventy per cent of the assimilated berberine was found to be associated with lipid fraction of the cell, while the rest was located in cellular proteins. The finding of the drug in lipid indicates the possibility of the cholera-genic toxin being denatured. Experimentally, berberine inactivated or detoxicated the cholera-genic toxin to make it harmless.

There was an universal demand for development of a drug against small-pox. Earlier a simple home remedy, powdered seed of wild *banana*, turned out to be an effective drug against both small-pox and chicken-pox in laboratory

models. One of the chemically isolated fractions from the powdered seed was endowed with these properties. This active constituent was studied pharmacologically and toxicologically. It appears that it has no adverse effects on the different systems of the experimental animals which would preclude its use clinically. Further work on the seed of *Banakaddali* has shown that there are more than one active principles.

The Institute has given thought to one of the most important problems of the country—the control of population. The Government of Maharashtra has given special emphasis on family planning. The Institute, therefore, embarked upon a programme of examining a series of indigenous drugs reputed to be useful as an antifertility agent by modern pharmacological methods. One of the fractions obtained possesses marked antifertility action in laboratory animals like white mice, white rats, guineapigs, hamsters and in the rabbits. This active constituent could be administered in large doses to laboratory animals without producing ill-effects. The substance is sufficiently promising for pursuing further investigation.

Institute manufactures several antisera. Horse sera are enzymatically digested by pepsin in the preparation of antitoxins. Several attempts to prepare insoluble pepsin which is enzymatically active have been going on. Success is at last achieved after repeated failures. The use of insoluble pepsin to degrade proteins has a number of advantages over soluble pepsin. The reaction can be stopped at will by just centrifuging and removing insoluble enzyme. The insoluble enzyme does not also contaminate the product of reaction. It is hoped that the insoluble pepsin could be used with great advantage to process horse sera for the manufacture of antitoxins.

In the past decade synthetic drugs belonging to the class of alkalating agents were prepared and found to be useful in treating cancer. Unfortunately, the main drawback of these agents are that they are relatively toxic and have low margin of safety. The Institute undertook synthesis of newer compounds containing nitrogen mustard grouping with a view to find compounds which would be more active and less toxic. The testing of these compounds have been going on in co-operation with the Cancer Chemotherapy National Service Centre, National Institute of Health, U.S.A. Out of the 300 compounds so far prepared, 88 have undergone preliminary tests for anti-tumour activity and toxicity and 7 of these have shown high order of activity against different tumours.

An interesting work on the aetiological role of enteroviruses and its relation to serotype of *E. coli* in juvenile diarrhoea or non-dysenteric nature has been carried out. Gastro-enteritis cases yielded a large variety of strains pathogenic to Monkey Kidney Tissue Culture (MKTC) and a few pathogenic to mice alone. Largest single groups were E 7 (72 isolation) and Cox-

sakie B₃ (27 isolation). These cases occur throughout the year with slight increase in number during October-December quarter. This contrast with the greatest incidence of paralytic enterovirus infections during the monsoon period. No correlation of the enteroviruses could be found with the presence of *E. coli*.

There was greater incidence of poliomyelitis during the year under review. There was unusually high incidence of paralytic poliomyelitis in Greater Bombay. One thousand and thirty cases were reported. Paralytic poliomyelitis is a crippling disease and the persons afflicted with it remains a burden to the society as long as he lives. The paralytic disease was mainly restricted to the children below three years old.

Introduction of oral polio vaccine for immunization has brought to light two problems, viz., problem of future of the vaccine to protect certain individuals and incidence of paralytic illness amongst some children who were successfully vaccinated with oral polio-vaccine. Both these problems have occurred in other countries. Failure of immunization as demonstrated by sero-conversion studies has been noted in tropical countries. The investigations are underway to ascertain the probable causes of such failures following vaccination.

The field trials of cholera vaccines carried out at different places indicate that the vaccines containing 8 billions vibrio did not give adequate protection. Studies at this Institute in which larger number of organisms was used have demonstrated that the vaccine containing more than 12 billions vibrios gave rise to severe reactions in human beings so as to preclude its usage. If the number of bacteria has to be increased in order to make it an effective antigen then it should not be more than 12 billions in number.

In our earlier report it was mentioned that human gamma-globulin has been introduced for the first time in our country. The demand has been steadily increasing. It is now available in greater quantities. Reports from the clinicians on its effectiveness are now being received. The reports stress the good preventive value of our gamma-globulin against infective hepatitis, serum jaundice (post transfusion cases) and measles.

In order to try the effectiveness of a new oral typhoid vaccine, a preliminary study with a view to determine the incidence of typhoid fever in a selected locality in the city of Bombay was undertaken. A survey of a population of about 14,000 people was done most systematically to find out the incidence of enteric fever. This group of population was followed up twice weekly to record and diagnose each person suffering from fever (children below 5 years were excluded). Laboratory tests were carried out to prove the occurrence of typhoid fever in individual suffered from fever. The results show that the incidence of typhoid fever in the group of population under study was between 2.9 and 4.7 per 10,000 of the general population. The incidence of the disease was not sufficiently high so that a true field trial of the oral vaccine could be recommended in the succeeding year.

For the last few years several drugs have been investigated for their efficacy in experimental amoebiasis in rats and golden hamsters. It is now possible to make a comparative statement on their usefulness. All the drugs, i.e. emetins, chloroquine, camoquine, vioform, dehydroemetine, metranidazole and berberine are effective remedies against amoebic infection. Metranidazole is very potent. Berberine hydrochloride is as effective as emetine hydrochloride. The latter results have also been confirmed elsewhere. Now that berberine is available in the market, it would be possible to know its value in clinical cases in course of time.

The menace of rats is another national problem which is being attacked by the Institute. No doubt that the plague has disappeared from the State of Maharashtra but no one knows when it will flare up again. One has to be vigilant in detecting the possibility of the persistence of infection amongst wild rodents (rat is one of them). A systematic screening of rat population in the different districts of Maharashtra has been started some years back. In the year under review 171 villages of Panvel Taluka were taken up. As a result of trapping and baiting in these villages 20,476 rats were destroyed. Susceptibility of some of these rats to plague infection varied from place to place even among villages situated within range of two miles. There was no evidence to show the existence of plague among the rats of these localities.

Laboratory tests have shown that live vaccines using *V. cholerae* 569B Inaba offer a high protection against the disease. When attempt was made to attenuate this strain in order to make it fit for live vibrio vaccine by repeated subcultures it had resulted in its loss of immunogenicity. Two new strains were examined then. $C_{14}S_5$ was a mutant strain developed by Dr. Bhaskaran and *El tor* Water vibrio 6 of Dr. Mukherjee. Between the two, $C_{14}S_5$ was less virulent. Live vaccine prepared from $C_{14}S_5$ or *El tor* water was 6 to 19 times more potent than the killed conventional vaccine. But the live vaccines were more toxic to mice and guineapigs than the killed ones. Whether these (live vaccines) will give greater reactions in human being is yet to be seen.

Several members of the staff went abroad for different assignments. Dr. N. K. Dutta, the Director, attended the Eighth International Congress of Tropical Medicine and Malaria held in Teheran (Iran) from the 7th to 15th September 1968. As a member of the W.H.O. Expert Committee on Biological Standardisation Dr. Dutta attended the 21st meeting held in Geneva from 30th September to 5th October 1968. Dr. Dutta inaugurated the Indian Pharmaceutical Congress held in Ahmedabad in January 1969. He is elected as a Member of the Indian Academy of Medical Sciences.

Dr. C. V. Deliwala, Assistant Director, Department of Chemotherapy, was deputed to U.S.S.R. under Cultural Exchange Programme. He had spent two weeks in U.S.S.R.

Dr. M. V. Sant, Assistant Director, Department of Entomology, participated in the Third Joint Meeting of Veterinary and Medical Research Workers on Brucellosis in New Delhi.

Dr. S. S. Rao, Assistant Director, Immunology, was deputed by W.H.O. for a course of study in Immunology in New Delhi.

Dr. V. N. Jai, Senior Scientific Officer, Department of Nutrition and Biochemistry, presided over the Section of Nutrition at the Fifth Asian Conference on Occupational Health held in Bombay.

Kum. M. S. Kotnis, Factory Canteen Adviser, returned to Bombay after 18 months assignment as Nutrition Officer in Malawi, Africa. Dr. Kum. S. Arora, Senior Lady Nutrition Officer, rejoined the Institute in February 1969 after completing 14 months assignment as Nutrition Officer in Sudan, Africa. Both of them worked under F.A.O.

A number of guest workers and foreign students came to work at this Institute. They are Mohd. Nabi Sifai, Head of the Biological Section at the Public Health Institute, Kabul ; Dr. V. A. Lashkivitch from the Institute of Poliomyelitis and Viral Encephalitis, Moscow ; Dr. Myint To, Manager, Burma Pharmaceutical Industry, Rangoon and Shri Georgi D. Dimitrov, Institute of Technology, Sofia, Bulgaria.

Sixty-four post-graduate students have been continuing their studies for M.Sc. or Ph.D. under sixteen University teachers at this Institute. The subjects covered by them are : Organic Chemistry, Biochemistry, Microbiology, Zoology and Applied Biology. Much of the Institute's research work is being carried out by these students.

In 1968-69, four students qualified for Ph.D. and five for M.Sc., of the Bombay University. Seven students for M.Sc. and six for Ph.D. were new entrants. Three medical graduates qualified in Diploma in Pathology and Bacteriology.

The training courses conducted by the Institute continued to be very popular. Seventeen people underwent short term training course. Eleven students successfully completed Diploma in Medical Laboratory Course (D.M.L.T.) and received their Diplomas from the Bombay University. In the Technicians Training Course eight science graduates were admitted. All of them passed.

Individual scientists of the Institute continued to attract grants from C.S.I.R., I.C.M.R., Defence Department, University Grants Commission, World Health Organization, Government of India, Government of Maharashtra, Private Trusts. Two new schemes were sanctioned by the I.C.M.R. and two by the C.S.I.R. One new scheme was financed by the Atomic Energy Commission. The total grant received from all these sources amounted to Rs. 3,26,342.

The Institute is the centre of attraction for many well-known scientists and dignitaries. Seventy people visited the Institute and some of them gave lectures or seminars. Twenty-seven lectures were arranged under the auspices of the Critics Club. Nine eminent foreign scientists addressed the scientific members of the staff.

The Analytical and Control Department continued its vigilance over the products manufactured by the Institute. New methods were developed to test tablets of (i) Polyvitamins (ii) Sodium aminosalicylate (iii) Phthalylsulphathiazole and (iv) diiodohydroxyquinolin. The Department carried out 44,093 tests in one year.

In co-operation with the Defence Department, the scientists of the Department of Bacteriology have undertaken study of some of the problems connected with the stability of vaccines at high altitude. Under the auspices of the W.H.O., the department participated in a collaborative assay of a new cholera vaccine which is proposed to be recommended as the International Standard.

The Department of Blood Bank ensured the most efficient utilisation of human blood supplied by donors. Plasma is processed into several useful products. The growth of the requirement of the gamma globulin has increased as a result of which its production is stepped up.

On the superannuation of Dr. P. J. Deoras, Assistant Director of the Department of Entomology, Dr. M. V. Sant took over the charge from him. This Department will shortly be converted into the Department of Zoonosis. The Entomology will be a section of the new Department then.

With more emphasis being given to the applied aspect of the nutrition, it is felt that the time has come when the Nutrition Section of the Department of Nutrition and Biochemistry should serve the rural people more than in urban areas. A proposal has been made to the Government to transfer this activity under Director of Public Health to utilise the full benefit of the applied nutrition programme for the village people.

The Maharashtra Government has decided to transfer the Government Analyst Section of the Pharmacology Department to a separate place under the control of the Director, Drug Control Administration. Only the Chemical Section of the Government Analyst's Laboratory has been shifted from the Institute to the Government Dental College. The remaining sections will be transferred as soon as more space becomes available elsewhere. The total number of drug samples tested by the Pharmacology Department amounted to 2,619. About 29 per cent of them were of non-standard quality.

The Institute participated in the laboratory investigations of the cases of encephalitis occurring in Nagpur area. About 57 cases were reported. Complete autopsies were carried out on 9 cases, while partial post-mortem examination was possible in 6 cases. A total of 618 samples were collected for virological studies. The Epidemiologist also collected 403 samples from contacts of clinical cases and apparently healthy population. Virological investigations are in progress.

The Institute was called upon to investigate cases of paralytic "poliomyelitis" in Akola District. Four adults and one boy were admitted to the Civil Hospital with a history of inability to move both the lower limbs and retention of urine.

Four more cases were admitted to the hospital later. These cases were finally diagnosed as not of paralytic poliomyelitis. These appeared to be cases of transverse myelitis. However, materials, such as C.S.F., rectal swabs, throat swabs, blood samples, were collected for virological studies.

Haffkine Institute distributes oral polio vaccines not only to our State but also to other States whoever indents it. During the year, 1.9 million doses were distributed which were 300 per cent higher than the last year.

Influenza broke out in an epidemic form in the months of September and October 1968. Fortunately, it was short-lived. Vaccines incorporating the A2/Hong Kong/68 and A2/India/57 antigens were prepared to meet the emergency condition. Influenza vaccine is normally required for immunizing key personnel in hospitals, factories, Red Cross etc.

It is a matter of great satisfaction that after years of persistent effort, the Government has now sanctioned the construction of the much-needed staff quarters within the compound of the Institute. A grant of 5 lakhs has been made for the 1969-70 as the first instalment. Many of the Institute's staff work round the clock and it is essential to provide them with quarters. These quarters are meant for lower category of workers.

The construction of the Gymnasium for the Institute's staff is in full swing. It is hoped that this building should be completed within 6 months. This will be a very valuable addition to the welfare activities of the staff members.

The Institute possesses one of the finest snake-farms in the country. It harbours nearly 1800 snakes and scorpions of different varieties. With the addition of one more floor over the existing snake-farm building, the shortage of space has disappeared.

Dr. H. I. Jhala relinquished the post of the Director from the 5th May 1968 after serving for 10 years at this Institute. The Director and his staff record their appreciation of the services rendered by him during the tenure of his office.

Dr. P. J. Deoras, Assistant Director, Department of Entomology, was superannuated on the 4th January 1969. He has served the Institute for nearly 19 years. The Institute records the meritorious services rendered by him.

The Institute records the sad demise of Dr. D. C. Lahiri, formerly the Assistant Director, Department of Antitoxins and Sera (now Department of Immunology). Dr. Lahiri was the main architect in establishing the Department. He was a scientist of repute.

The Director and the members of the staff were greatly benefited by the advice and guidance given by the Haffkine Institute State Advisory Board and the Haffkine Institute Research Advisory Board. They wish to thank the members of these two Boards for taking keen interest in the affairs of the Institute.

In carrying out Institute's function of deciding the overall policies of the laboratories, the Director is very conscious of the fact that success of such policies depends ultimately on the integrity, conscientiousness and technical skill of the individual staff members.

The Director, therefore, wishes to express his appreciation of the existence of these qualities amongst the staff at all levels, and to express also his appreciation of continuing support of the administrative, scientific and non-scientific staff of the Institute during the past year.

PART II
PROJECTS

RESEARCH PROJECTS

ANTI-CANCER DRUGS PROJECT

(DEPARTMENT OF CHEMOTHERAPY AND DEPARTMENT OF PHARMACOLOGY)

In the past decade synthetic drugs belonging to the class of alkylating agents were prepared and found to be useful in treating cancer. Unfortunately, the main drawback of the agents was the relative high toxicity and low margin of safety.

The chemotherapy department undertook synthesis of newer types of compounds containing nitrogen mustard grouping with a view to synthesise a compound which would be more active and less toxic. The screening of these compounds for their anti-tumor activity was undertaken by Cancer Chemotherapy, National Service Centre, of National Institute of Health, U. S. A. (C. C. N. S. C.).

Out of the 300 compounds prepared in the department 88 have undergone preliminary tests for anti-tumor activity and toxicity and seven of these have shown a high order of activity against different tumors.

Chemo R-1238 was chosen for studying its pharmacological activity. It was shown that the drug did not cause any appreciable changes in blood-pressure and respiration of an anaesthetised animal. It did not exhibit any anti-acetylcholine, anti-histamine and anti-adrenaline activity. It had no local anesthetic or analgesic action.

BANAKADALI PROJECT

(DIRECTOR'S LABORATORY, DEPARTMENT OF CHEMOTHERAPY, DEPARTMENT OF PHARMACOLOGY AND DEPARTMENT OF VIROLOGY.)

Pharmacological observations on the fractions isolated from seeds of banakadali (*Ensete superbum*, Cheesm, N. O. musaceae.

Seeds of Banakadali are being used in the treatment of small pox and measles by physicians practising in the indigenous system of therapy. Three fractions have been isolated and tested for anti-variola and anti-vaccinia effect and have shown encouraging results. One of the fractions VIDR-2GD was studied in details.

A dose of 0.5 mg. to 7.5 mg. did not produce any marked effect on the isolated perfused heart of frog. VIDR-2GD caused hypotension in cats. Topical (5 mg/ml.) and intravenous administration of the drug (250 mg./kg.) produced congestion of the mesenteric blood vessels of the rat and mice. The stasis of circulation due to topical application was restored to normal after washing with normal saline. Perfusion of the frog's vessels with the drug
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(2.5 mg.) showed dilatory effect with increase in outflow. The drug VIDR-2GD did not have any effect on the bronchial musculature in a dose of 250 mg./kg. It neither enhanced nor antagonised the effect of histamine induced broncho-constriction. It did not produce any appreciable action on the tracheal chain of guinea-pig in concentrations between 20 to 100 mcg./ml.

In a dose of 30 mg/ml, it caused immediate contraction of guinea-pig ileum but after atropine the drug effect was antagonized showing cholinergic like effect. The amplitude of peristaltic movement of guinea-pig was increased with 5 mcg/ml of the drug and adrenaline in a large dose removed the tonic. The tonic of the uterus of guinea-pig and rat was increased in a concentration of 20-40 mcg/ml. and adrenaline checked the tonic. It has shown slight diuretic effect in rats with a dose of 2.5 mg/kg. The drug potentiated the pentobarbitone induced sleep in mice and rats in a dose of 180 mg/kg but without any sedative effect when given alone. The drug could not check metrazol induced convulsions in rats and mice in a dose of 180 mg/kg given intraperitoneally half an hour and one hour before metrazol. But the animals were protected for further half an hour before death than the metrazol treated control animals. In the dog and rabbit the drug was able to reduce whole blood clotting time, recalcified plasma clotting time and bleeding time.

The drug caused hypoglycemia by 15 per cent at the end of the 5th hour with a dose of 100 mg/kg. in the rabbit. After 24 hours the blood sugar remained low by 8.5 per cent. It did not have any inhibitory effect on the growth of *E. histolytica* upto a concentration of 50 mg/ml. Acute toxicity studies showed that a dose upto 500 mg./kg. orally, 2,500 mg./kg. intraperitoneally and 1,000 mg/kg. intravenously did not produce any deleterious effect during 6 days' observation.

Active fraction (VIDR-2T) from Banakadali was taken for further fractionation, identification and characterisation of the molecules responsible for the activity. During purification, a number of fractions were isolated, out of which two fractions P_1 and P_5 were obtained in a crystalline form of m.p. 105-06° C and 205-06° C (decom) respectively. These two fractions are being studied pharmacologically.

BERBERINE PROJECT

(DIRECTOR'S LABORATORY, DEPARTMENT OF NUTRITION AND BIOCHEMISTRY
AND DEPARTMENT OF PHARMACOLOGY)

Berberine has been found to be effective in experimental and clinical cases of cholera and acute diarrhoea. It was shown to be as effective as chloramphenicol and tetracycline. The pharmacological studies of the same are reported here.

(A) The absorption, distribution and excretion of berberine in rabbits

The absorption, distribution and excretion of berberine in infant rabbits aged 10 days were studied and reported here.

Berberine was estimated fluorometrically by a modification of the method described by Schein and Hanna. Tissues were removed and homogenised. The proteins were precipitated by 20 per cent trichloroacetic acid and centrifuged. The supernatant fluid was transferred to another flask and sodium bicarbonate added to it. Berberine was extracted from it by adding and shaking with ethylene-dichloride. The ethylene-dichloride was separated and read in fluorophotometer using a primary filter passing 230-270 m μ . and secondary filter which cuts off below 530 m μ .

It was found that berberine was absorbed when given orally. It persisted in blood even after 72 hrs. It was excreted through urine. Tissue values were highest in heart, followed by pancreas and liver. On intramuscular injection it was found that berberine was excreted through stools in small amounts. Tissue levels were higher in small intestine, kidney and liver.

(B) Mechanism of action of berberine

(a) *Vibriocidal effect of berberine sulphate, chloramphenicol chlortetracycline hydrochloride and oxytetracycline hydrochloride.*—Berberine prevents diarrhoea in infected infant rabbits. One of the factors may be the bactericidal action of the drug. The vibriocidal action was compared with other antibiotics.

Berberine sulphate	..	200	μ g/ml.
Chloramphenicol	..	1	μ g/ml.
Oxytetracycline	..	10	μ g/ml.
Chlortetracycline	..	10	μ g/ml.
Inoculum size	..	10 ³	viable orgs/ml.
			(<i>V. cholerae</i> 569 B Inaba.)

(b) *Effect of berberine on toxin induced cholera in infant rabbits.*—Cholera toxin can produce disease very similar to the one produced by the administration of *V. cholerae*. The effect of berberine was, therefore, studied on toxin induced diarrhoea to see whether it possesses any action other than shown above.

Toxin induced cholera in infant rabbits was treated with berberine orally. It was found that 18-24 hours pre-treatment of the animals with berberine reduced the intensity of the diarrhoea and prolonged the survival time of the treated rabbits as compared to untreated controls (infant rabbits).

(C) Effect on capillary permeability

The syncase cholera toxin causes increased capillary permeability in the skin of rabbit. This was noticed as a zone of blueing around the site of injection of the toxin when trypan blue was administered intravenously. The effect of berberine was studied on this system.

Berberine itself caused increase in permeability when injected intradermally. Syncase toxin was injected intradermally on the left hand side of the back of the rabbit, Trypan blue was injected and the zone of blueing was noticed. Berberine was then administered intravenously and after half an hour the toxin was injected on the other side of the back and zone of blueing compared with the first. The zone of blueing was less when berberine was injected, indicating that berberine reduced the capillary permeability increased by the toxin. Further, six different dilutions of the toxins were injected intradermally in rabbits and the resulting patches of blueing measured. Another group of rabbits was given berberine intravenously. The zone of blueing in berberine treated animals was noted. In normal rabbits a 32 fold dilution was inactive while in berberine treated ones a 16 fold dilution was inactive. This further indicated that berberine reduced the capillary permeability induced by cholera toxin. These experiments show that berberine may be acting in cholera by reducing increased capillary permeability of the intestinal vessels.

Effect of berberine on increased skin permeability of V. cholerae toxin.—Syncase toxin caused increased vascular permeability on intracutaneous injection into the skin of adult rabbits, as detected by localised blueing after 24 hrs following intravenous injection of Evan's Blue. 0.05 ml. of a solution of 20 mg./ml. of lyophilised syncase toxin with an equivalent amount of a solution of 2 mg./ml. of berberine base, caused a skin lesion identical to that produced by the same amount of toxin alone. The lesion, however, became diffused by 48 to 64 hrs and then disappeared completely. The lesion produced by the toxin alone remained localised within a 1 cm. area and persisted after the above lesion disappeared, i.e. beyond 72 hrs and then faded away. The lesion produced by berberine alone also diffused by 48 hrs and blueing was erased before either of the above two reactions, approximately by 55 hrs.

(D) Effect of berberine on the motility of g.i. tract.

Cholera is essentially a disease of the gastro-intestinal tract. So the effects of berberine on the motility of the gastro-intestinal tract were studied.

Berberine failed to show any antidiarrhoeal effect in infant rabbits when diarrhoea was introduced either by magnesium sulphate or by castor oil. In anaesthetised cat it stimulated peristalsis.

Biochemical studies on Vibrio and El-Tor cholera with special reference to berberine action.—Earlier studies had indicated that berberine possesses vibriostatic effect only during the early period of the growth. This effect was observed to be a true static effect and during this period there was no 'enzyme induction'. The effect of the drug has also been proved to be due to the result of the cell membrane damage which causes the leakage of synthesized macromolecular pool. The drug was also found to inhibit the incorporation of macromolecular precursors.

Assimilation of berberine and toxin inactivation.—Studies on the assimilation of berberine by *V. cholera* and *El-Tor cholera* revealed that *V. cholera* assimilated more of berberine compared to *El-Tor cholera*. However, in both the

organisms 75 per cent of the assimilated berberine was found to be associated with the lipid fraction, while the rest was located in cellular proteins. This binding of the drug to lipid indicates the possibility of toxin denaturation and hence the treated cells were tested for the toxicity in young infant rabbits. The results show that berberine inactivated or detoxicated the cholera toxin.

Studies on the effect of berberine on various biosynthetic mechanisms.—Berberine initially inhibits growth of *V. Cholerae* but later on the growth resumes though the cells are devoid of toxin (either deformed or inactivated). This was indicative of a defective generation (since the drug was present while the growth resumed) and hence the metabolism and incorporation of various nucleic acids and protein precursors in the presence of berberine were studied. These studies could throw some light on the fundamental differences between the two organisms—particularly the difference in the synthetic abilities of the two strains. Following sets of experiments are included in this study.

(a) *Metabolism of glycine and aspartic acid.*—Glycine contributed mostly to proteins and nucleic acids in these organisms, while the major portion of aspartic acid was incorporated into the residual proteins. *El-Tor cholera* showed higher specific activities of RNA, DNA and proteins with both the amino acids. This indicated that it was metabolically more active than *V. cholerae*. It seems that though higher biosynthetic activity in the initial stages of the growth was shown by *V. cholerae* (higher incorporation of precursors) during the early log phase, *El-Tor cholera* overtook *V. cholerae* in the biosynthetic processes during the later stages.

Berberine did not alter the general metabolic pattern of these amino acids. However, inhibition in the synthesis of all the macromolecules except the proteins was observed in the berberine treated cells. Protein fraction was found to possess very high specific activity (2-3 times higher than the control).

(b) *Synthesis of various RNAs and their nucleotide composition.*—The ratios of different RNAs were not found to vary significantly in the two organisms, although the amount of individual nucleotide differed slightly. *El-Tor cholera* showed higher synthetic ability than *V. cholerae*. The specific activity of various RNAs from the former (guanine-8-C-14 incorporation in various RNAs) was higher than that from the latter.

Berberine inhibited the synthesis of all the RNAs in an identical manner. However, the alkaloid was not found to alter the nucleotide composition of the RNAs.

(c) *Effect of berberine on the synthesis of different macromolecules.*—During the static period, there was an inhibition in the synthesis of all the macromolecules, (viz: macromolecular pool, nucleic acids, proteins and lipids, during the growth cycle using glycine-1-C-14). As the growth resumed, the synthesis of the different components started. By the end of the growth period, the nucleic acid synthesis reached to that of normal. But the lipid and protein synthesis seemed to be stimulated (the specific activity is 2-3 times higher than the control).

The higher specific activity of protein suggests that this protein was an abnormal protein which could be utilised by the cell. The higher specific incorporation of lipid was an indication, that lipid synthesis was also affected by the drug. This was further investigated by studying the fatty acid spectrum. Berberine treated cells showed an altered fatty acid composition.

CHOLERA PROJECT

(DIRECTOR'S LABORATORY, DEPARTMENT OF BACTERIOLOGY, DEPARTMENT OF IMMUNOLOGY, DEPARTMENT OF NUTRITION AND BIOCHEMISTRY AND DEPARTMENT OF PHARMATOLOGY)

Preparation of live cholera vaccine

Laboratory tests using infant rabbit test and the mouse protection test have shown that live vaccines using one of our virulent strains, 569/B Inaba, offered very high protection. Attempts were made in the past to attenuate the strain 569/B Inaba by repeated subcultures in Mc Conkey's medium. It was observed that the strain attenuated by this method though lost its virulence its immunogenicity was also considerably reduced. By this time evolution of an avirulent strain $C_{14}S_5$ was reported by Bhaskaran. The use of strain, Water vibrio 6, used by Mukerjee for live oral vaccine has also been reported. It was, therefore, considered desirable to prepare live parenteral vaccines from these two strains and test them by mouse protection as well as by infant rabbit techniques. For mouse protection test the Feeley—Pitman technique was used.

The two strains $C_{14}S_5$ and El tor Water 6, were tested initially for virulence. The virulence status of $C_{14}S_5$ was also reported earlier, LD_{50} being 16×10^8 organisms. The results of further virulence test along with other standard virulent strains are given below.

TABLE 1
Results of virulence tests

Strain	LD ₅₀ in no. of organisms	
	Test No. 1	Test No. 2.
1. $C_{44}S_5$	—	6.1×10^8
2. El tor Water 6	1.2×10^4	2.4×10^4
3. 5321 Ogawa	—	2.8
4. El tor Ogawa (Philippines)	0.7	—

The above results show that $C_{14}S_5$ had the least virulence while El tor Water 6 was at least 200 to 300 times more virulent than $C_{14}S_5$. The strains 5321 Ogawa and El tor Ogawa (Philippines) were virulent. The results also showed that the mouse strain was highly susceptible to the virulent strains.

Live vaccines were prepared from both the strains by growing them on casein hydrolysate agar for about 20 hours at 37°C . It was observed that $C_{14}S_5$ was a very slow growing organism and its colonies were also very small almost pin point in size. The Water vibrio El tor 6 showed normal growth and the sizes of the colonies were as those of our usual vaccine strains of *V. cholerae*. Growth was harvested in physiological normal saline. The opacity of the harvest was adjusted to contain 400×10^6 organisms per ml. in normal saline 24 hours after harvesting and after confirming the results of the purity test. The vaccines were immediately put up for a potency test in NIH mice. One of our routine killed Ogawa vaccine—Batch No. 147—was also included in the test. The viable counts of the two live vaccines were determined on the same day as the potency test was commenced. The $C_{14}S_5$ vaccine was found to contain only 3×10^6 organisms per ml. while the Water vibrio 6 vaccine contained 7×10^6 organisms per ml. One more batch of each of the two vaccines was also prepared and tested for potency as above along with a batch of routine killed Ogawa vaccine 151. The procedure for preparation of the live vaccines was exactly the same as in the case of previous batches, however at this time instead of normal saline 0.02 M. phosphate buffer pH 7.2 was used for harvesting the growth and for dilution to the required opacity. The viable counts were found to be 340×10^6 organisms per ml. for the $C_{14}S_5$ vaccine and 370×10^6 organisms per ml. for the El tor Water 6 vaccine. The results of the potency tests are given below.

TABLE 2
Results of potency tests

Vaccine	ED ₅₀	Relative potency
Live $C_{14}S_5$	.. 0.00005	5.6
Live El tor Water 6	.. 0.00005	5.6
Killed Ogawa B. No. 147	.. 0.00028	1
Live $C_{14}S_5$	.. 0.000022	19.1
Killed Ogawa B. No. 151	.. 0.00042	1

It is apparent that the two live vaccines in series 1 were at least 5·6 times or higher in potency than the killed vaccine and in series 2 the live vaccine $C_{14}S_5$ was about 19 times more potent than the killed vaccine. In series 1, the survival rates in mice inoculated with the two live vaccines were more than 50 per cent at the lowest dose level of 0·00005. Hence the exact ED_{50} could not be determined. In series 2 of the experiment, the surviving mice in case of live El tor water 6 vaccine did not show a graded response to the doses used. Hence the ED_{50} could not be determined.

Toxicity test of live vaccines

The two live vaccines $C_{14}S_5$ and El tor water 6 were then tested for freedom from abnormal toxicity by injecting 5 ml. of the vaccine intraperitoneally to each of the two guinea pigs weighing 350 to 400 g. and 0·5 ml. subcutaneously to each of the four mice weighing 18 to 20 g. Routine killed vaccine Ogawa 151 was also used as control.

All the four guinea pigs injected with the two live vaccines died within 48 hours. Cholera cultures were isolated from the heart blood of all the guinea pigs. The guinea pigs injected with the killed vaccine did not show any signs of sickness during the observation period of 8 days. Two of the four mice injected with live El tor water 6 vaccine also died within 96 hours. Rest of the mice were all right and survived for at least 8 days.

As both the live vaccines showed high toxicity when tested by the routine prescribed test, a quantitative toxicity test was carried out in the following way.

TABLE 3

A quantitative toxicity test

Vaccine	Animals used	Dose ml.	Route of injection	Result
Live $C_{14}S_5$	.. 2 guinea pigs	3	Intraperitoneally	Both died. One within 24 hours. The other within 48 hours.
Do.	.. Do. ..	1	Do. ..	Survived for more than 8 days.
Do.	.. Do. ..	0·5	Do. ..	
Do.	.. 4 mice	.. 0·5	Subcutaneously	Did not show any signs of sickness.
Live El tor water 6	2 guinea pigs	3	Intraperitoneally	Both died within 24 hours.
Do.	.. Do. ..	1	Do. ..	One was slightly sluggish for first 3 days. Both survived for more than 8 days.
Do.	.. Do. ..	0·5	Do. ..	Survived for more than 8 days. Did not show any signs of sickness.
Do.	.. 4 mice	.. 0·5	Do. ..	

It may be seen from the above experiment that though the live vaccines are more potent when tested by the mouse protection test they are relatively toxic as compared to the routine killed vaccine. Toxicity tests show that whereas 5ml. of the killed vaccine was very safely tolerated by guinea pigs, only 0.5 ml. of the live vaccines could be used without any adverse effects at the level of the bacterial density used.

Release of active substances by cholera toxin

Panse and Dutta have shown that cholera toxin releases histamine in patients and in infant rabbits made choleraic by infection or toxin administration. The work was extended further to study the role of 5-hydroxytryptamine and bradykinin.

It was shown that the blood levels of 5-hydroxytryptamine were significantly higher in choleraic rabbits than those in normal rabbits. By using an indirect method of assay of bradykinin it was shown that bradykinin was released in infected rabbits. When toxin was injected intradermally in rats, the skin showed more amount of 5HT than in untreated rats. From these results it was suggestive that 5HT and bradykinin may be playing a role in the pathogenesis of diarrhoea.

Study of the protective power of sera raised against live vibrio strains C₁₄S₅ and El Tor Water 6, in comparison with the serum raised against the standard pathogenic vaccine strain (formalin killed).

Sera raised against Water vibrio El Tor 6, C₁₄ S₅ (Ogawa attenuated strain) and formalin treated vaccine of Ogawa 5321, have been tested in infant rabbits for passive protectivity (results have to be evaluated statistically).

Suitable media for *V. cholerae*

The work on this project was mainly undertaken to achieve the following three objectives :—

- (i) To find out a suitable indigenous protein to substitute imported casein for the preparation of vaccines.
- (ii) To simplify the present method for the preparation of acid hydrolysate i. e., using enzyme hydrolysis.
- (iii) To work out a simple medium containing glucose and salts for growth of *V. cholerae*.

It was reported previously that peanut protein hydrolysate prepared by acid digestion was found to be as efficient as casein hydrolysate for the growth of the two strains of *V. cholerae*.

Casein and peanut hydrolysates were prepared by enzyme digestion with papain (pH 5.0 for 24 hours) and trypsin (pH 8.4 for 72 hours). Under these conditions about 60 per cent hydrolysis could be achieved. The total N was adjusted to 270 mg./100ml. Both casein and peanut protein hydrolysate contained about 67 per cent amino nitrogen (Cu phosphate procedure)/100 mg. and about 25 per cent peptide N/100 mg. of total N (Biuret).

The two strains of *V. cholerae* could grow fairly well on both the casein and peanut protein digest at an N concentration of 135 and 270 mg. per cent. The comparative figures are given in Table in terms of optical density.

TABLE 4.

Growth of V. cholerae (Ogawa and Inaba) in terms of optical density

Medium	Ogawa			Inaba		
	24 hrs.	48 hrs.	72 hrs.	25 hrs.	48 hrs.	72 hrs.
Casein hydrolysate (acid) 135 mg. N/ 100 ml.	0.35	0.36	0.49	0.18	0.26	0.54
Casein hydrolysate (enzyme) ..	0.36	0.35	0.47	0.19	0.23	0.30
Peanut protein hydrolysate (acid) ..	0.34	0.52	0.60	0.26	0.45	0.66
Peanut protein hydrolysate (enzyme)	0.26	0.42	0.52	0.21	0.39	0.46

The two strains of *V. cholerae* did not give satisfactory growth in the simple medium containing glucose, salts and supplements of glutamic acid, methionine, tryptophane and histidine.

DIPHTHERIA PROJECT

(DEPARTMENT OF IMMUNOLOGY, DEPARTMENT OF NUTRITION AND
BIOCHEMISTRY AND DEPARTMENT OF CLINICAL PATHOLOGY)

The nutritional requirements of *C. Diphtheriae* (Weissensee strain) for toxin production has been worked out. The optimum conditions for the production of toxin in acid hydrolysate of casein medium and a completely synthetic medium have been worked out.

Studies in diphtheria toxin production in acid hydrolysed casein medium

The carbohydrate requirements of *C. diphtheriae* (Weissensee strain) were first of all investigated. Glucose, sucrose, acetate, lactate and citrate were tested for their ability to replace part or whole of maltose. It was found that the best source of carbohydrate for toxin production was maltose. The next best source was acetate followed by sucrose. About a fourth part of maltose could be replaced by acetate with no decrease in toxin production. This modification has now been incorporated in the medium used for routine production of toxin on a large scale.

The vitamin requirements were then investigated for toxin production. Nicotinic acid was essential for growth and toxin production. Thiamine and βalanine were stimulatory. Pimelic acid, pyridoxin, biotin, PABA, riboflavin, folic acid and pantothenic acid were not necessary. With these supplements, the Lf of toxin produced in casein hydrolysate medium was over 100 Lf/ml in 5 days.

Casamino acids could not be replaced even partially by urea or ammonium sulphate. Among requirements of inorganic ions, Fe, Ca, Cu, Zn and Mn were not necessary. Mg and phosphate were essential. The role of iron or calcium in decreasing toxin production is now under investigation.

The replacement of cystine by other simpler sources containing sulphur were investigated. Sodium sulphide, sodium sulphate, sodium thiosulphate and sodium thioglycollate were tested. Cystine could be replaced partially or fully with thioglycollate, sodium sulphide or sodium thiosulphate. The other sources of sulphur were not utilized and toxin production was low.

Evaluation of a simple synthetic medium for diphtheria toxin production

A purely synthetic medium capable of giving 60 to 70 Lf/ml of diphtheria toxin in 5 days was evolved. The strain of *C. Diphtheriae* used was 'Weissensee G5'. The medium consisted of 5 amino acids, 4 vitamins, salts, acetate and maltose. This medium was formulated using the "all minus one" technique. Using this technique it was possible to get clear-cut results on the nutrients which were "essential" without which the Weissensee strain would not grow and those which stimulate the production of toxin. This medium will now be used to study the metabolism of the organism and the role of iron in secretion of toxin.

Toxigeny of *C. diphtheriae*

The conversion of three toxinogenic strains PW8 (Weissensee), PW8 (Massachusetts) and CN 2000 into non-toxinogenic strains by treatment with acriflavine was tried. The Weissensee strain could be easily converted into a non-toxinogenic (T^-) strain. It was not possible to convert the Massachusetts strain or the CN 2000 strain into a non-toxinogenic strain even with 50 µg of acriflavine. Whether this was due to impermeability of the cell wall of these organisms to acriflavine or otherwise is yet to be determined.

Phage W obtained from Dr. Alice Saragea of Rumania produced plaques on strain PW8T⁺ Weissensee strain or its non-toxinogenic T⁻ variant obtained by acriflavine treatment. The lysogenised strain was resistant to phage P. This indicated that phage W occupies the same or similar site in the host. However unlike phage P, the W phage was incapable of transducing the T⁺ factor from PW8T⁺ (Weissensee) to PW8T⁻ (Weissensee).

Cellular Composition of *C. diphtheriae*

Earlier studies on the cellular constituents of the 7-day old culture had given varying analytical data from batch to batch. Therefore, it was felt necessary to study systematically 1-7 day old cultures.

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

mg. per cent on dry weight basis

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

The growth of the organism could not be followed by the usual turbidimetric method as the organism grew only on the surface of the medium. However, the growth could be evaluated by estimating the RNA and proteins formed at various intervals during the growth phase of the organism. Synthesis of toxin was mainly based on the formation of RNA in the cells eventually leading to the toxin proteins. It was observed that maximum RNA content was present during the 4th and 5th day old cultures. As the protein was released, as toxin, the RNA content was seen to decrease on the 6th and 7th day.

Fatty acid composition

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

The following studies were carried out:

Total lipid content of 1-7 day old cultures.

Separation of total lipids into neutral lipids and phospholipids by silicic acid column chromatography.

Fatty acids pattern of the total lipid, phospholipid and neutral lipid by gas chromatography.

Separation of neutral and phospholipids into different fractions by thin layer chromatography.

The detailed study of the fatty acid spectrum of *C. Diphtheriae* WG-5 (the standard strain used for toxin production) at intervals revealed that the total lipids of the organism contained 11 fatty acids, the palmitic (C 16) and palmitic (C16:1) being predominant. Oleic acid (C18:1) was next in abundance. The proportion of low molecular weight fatty acids was much less as compared to the higher ones.

The neutral and phospholipid fractions also exhibited a similar trend particularly in the latter, palmitic and palmitoic were the only constituents without any significant amount of the lower fatty acids.

Phage typing of *C. diphtheriae*

About 200 strains of *C. diphtheriae* were isolated from suspected patients. They were tested biochemically and also for toxigenicity. *C. diphtheriae* *Mitis* were 60 per cent, *gravis* 35 per cent and *intermedius* 4 per cent and the others diphtheroids. Eighty-five per cent strains were found to be toxigenic. They were subjected to phage typing. About 80 per cent strains belonged to type no 19 only, and 6 per cent showed phage pattern while the remaining strains were untypable. Thus No. 19 phage type *C. diphtheriae* was found to be the predominant one.

ENTEROVIRUS PROJECT

(DEPARTMENT OF VIROLOGY AND DEPARTMENT OF CLINICAL PATHOLOGY)

The object of the study was to investigate the aetiological role of enteroviruses and of serotypes of *E. coli*, in the causation of juvenile diarrhoeas of non-dysenteric nature.

The project was undertaken in collaboration with the Department of Clinical Pathology, which isolated and studied *E. coli* serotypes in the stools of these patients while the Department of Virology studied the enteroviruses in stools and/or rectal swabs of the same patients.

Patients were drawn from admissions to the L.T.M.G. Hospital at Sion, Bombay. Cases were personally screened by a Senior Paediatric Physician, Dr. Athawale, and stool, rectal swab, blood etc., were collected from these cases as required for our studies. Simultaneously, control samples were collected from non-diarrhoea cases of the same age group admitted to the same hospital, and were subjected to similar studies in the laboratory, for comparison with the diarrhoea cases.

It was also decided to carry out enteroviruses studies, on similar lines, for cases of aseptic meningitis, of which a large number are admitted to the paediatric wards of the L.T. M.G. Hospital. The cases were similarly screened, and samples, including for these cases, cerebrospinal fluids, were collected and sent to the laboratory. Control cases, for this group had perforce to be such cases in which cerebrospinal fluids were collected, and control samples were collected from cases of tuberculous or pyogenic meningitis in the same hospitals.

The enterovirus project was accordingly divided into two studies:—

- (i) A study of gastroenteritis cases.
- (ii) A study of aseptic meningitis cases.

Gastroenteritis study

A collaborative study of the first 150 cases showed that no correlation could be established between the *E. coli* serotypes isolated and the clinical picture, or

between enterovirus isolation and *E. coli* serotypes isolated from the same patients. Subsequent studies were, therefore, restricted to the enterovirus aspect alone.

This study was very fruitful. Thanks to the personal attention and care exercised by Dr. Athawale in selection of cases, a very high rate of isolation was obtained, and a number and variety of enteroviruses could be isolated.

However, after three years of study, covering 177 cases and 128 controls, it was seen that while certain limited conclusions could be drawn from these findings, prolonging the study would not yield any further information, as there were certain lacunae which could not be covered without augmenting the staff available for this work. The conclusions which could be drawn were as follows:—

(i) There was a significant difference, both quantitative and qualitative, between enterovirus isolations from clinical cases and from controls. Enteroviruses were isolated from 72·3 per cent of cases and from 28 per cent of controls.

(ii) Gastroenteritis cases yielded a large variety and number of strains pathogenic to MKTC and few pathogenic to mice alone; isolations were often multiple (2 or 3 viruses from a single specimen); the largest single groups were E7 (72 isolations from cases, 4 from controls) and Cocksackie B3 (27 from cases and 1 isolation from controls). In controls the isolations were fewer, and a small proportion of these were pathogenic to MKTC alone.

(iii) There was no significant difference due to age in the groups under study (age 1 month to 5 years), when compared to the general incidence of hospital admissions.

(iv) Isolations and cases occurred throughout the year, with slight increase in number during the October-December quarter. This contrasts with the greatest incidence of paralytic enterovirus infections in the monsoon period.

(v) No correlation with *E. coli* serotypes could be established.

These conclusions are highly suggestive of correlation between enteroviruses, especially E7 and Cocksackie B3, and diarrhoeas in infants and young children. We are unable, however, to establish whether the role of the enterovirus is causative or incidental (i.e. it finds more ready acceptance in an already inflamed intestinal mucosa), because of the lacunae which cannot be covered with our present organization. These lacunae are as follows:—

(i) *Serology*.—Confirmation by serology, of actual infection with rise in antibody titres, would be further suggestive though not conclusive, of the causative role of the isolated enterovirus (or of one of the isolated enteroviruses). This is not obtainable, since these patients generally recover and are discharged from hospital between the third and fifth day after admission. A field staff for follow up of patients to their homes for collection of convalescent blood samples, is not available at the present time.

(ii) *Contact studies*.—It would be strong supporting evidence of the causative role of these enteroviruses, if it could be shown, in a substantial proportion of cases, that other children in the same family who are unaffected have been protected by presence of antibodies. Such household or family studies require a field staff for house to house collection which is not available at the present time.

Aseptic meningitis

This study also yielded a large number of virus strains, showing significant quantitative and qualitative differences between cases and "controls", a very high rate of isolation from cases being obtained.

In these cases a definite causative role can be assigned to viruses isolated from CSF (no isolations were made from CSF of controls, whereas the cases yielded 40 isolations from 197 CSF tested). The isolation rate was very high and for this we must thank the pediatrician for his careful selection of the cases.

However, in these cases also, it was not possible to obtain convalescent blood samples after a sufficient interval of time and a rise of antibody titre could not be demonstrated.

It was felt that this project also would be better studied after a suitable field staff became available. In last year of the study, therefore, no further work was undertaken on this project.

PLAGUE PROJECT

(DEPARTMENT OF ENTOMOLOGY, DEPARTMENT OF BACTERIOLOGY
AND DEPARTMENT OF BIOCHEMISTRY)

Plague epidemiology

Plague epidemiological studies were continued in Western Maharashtra region and are being followed up in Thana district. A rat control campaign was organised in 171 villages situated in Panvel block in the year under review.

Western Maharashtra.—Plague epidemiological studies were carried out at Satara, Radhanagri, Kudal, Sawantwadi, and nearby villages. Rats were collected from houses, outskirts of the town as well as from the forest locations by trapping. In all 229 rats were trapped, out of which 221 were *R. rattus*, 5 were *Leggada budooga*, 2 were *G. ellioti* and one *Mus musculus*. The rat density was 6.0 at Satara, 15.78 at Radhanagri and 8.8 at Sawantwadi. *R. rattus* predominated in the total rat infestation being 95 per cent of the total rat catch.

Seven hundred and thirty five fleas were recovered from these rats. *X. cheopis* was observed to be the most predominant flea species. In the course of our plague epidemiological studies, for the first time, *X. brasiliensis* were recovered in good number at Radhanagri.

Rats from Radhanagari area of Kolhapur district showed high degree of resistance to plague infection (70 per cent.) whereas rats from Sawantwadi area showed 44 per cent. resistance.

Panvel rat elimination project.—Rat control campaign was taken up in 171 villages of Panvel block on 9th June 1968 in collaboration with the Kolaba Zilla Parishad, Public Health and Agriculture Departments of Government of Maharashtra and was concluded on 27th December 1968.

The work primarily constituted the extension of the work done last year in four villages of Panvel Taluka. It involved trapping of rodents with the help of wonder traps followed up by baiting with zinc phosphide. Two bordering villages outside Panvel block were taken as control to evaluate the results. The initial rat densities in the control villages outside Panvel block, viz. Ris and Jasai, were (20.0) and (69.0) respectively on 16th June 1968. Two villages were similarly taken from experimental group and studied on a comparative basis for rat densities. The rat densities at Dapoli and Pargaon were 44.0 and 130.8 respectively on 10th June 1968. The densities at the conclusion of campaign in the control villages were observed as 56.0 and 59.0 whereas in the experimental villages selected for comparison, viz. Dapoli, Pargaon, these were observed to be 5.0 and 18.0 respectively, showing reduction in experimental villages.

As a result of trapping and baiting in 171 villages, 20,476 rats were destroyed. Out of these 18,705 belonged to the species *R. rattus*, 320 *B. bengalensis*, 348 *B. gigantea*, 1937 *Suncus caeruleus*, 8 *Leggada budoo8a*, and 58 *Gohunda ellioti* and *M. musculus*. Rats collected from Morbe area from Panvel block were challenged with a standard dose of *P. pestis*. It was significant that susceptibility varied from place to place even among the villages situated within a range of two miles.

Plague studies in Thana district.—Plague epidemiological studies were initiated in Thana district in 1969. Studies carried out in 6 places till 31st March 1969 yielded 1003 rats, 1407 fleas were collected from these rats. *R. rattus* and *X. cheopis* were the most predominant rat and flea species. These studies are in progress at other places in Thana district.

Susceptibility of rats (*Rattus rattus*) to *P. pestis*

The work of determination of susceptibility status of rats in different parts of Maharashtra State commenced in 1965, was continued during the period under review. Rats collected from South Ratnagiri, Kolhapur, Kolaba and Thana districts were challenged with a fixed dose of approximately, 5,000 organisms of the plague strain 195/P. The results of susceptibility studies are recorded in the Table 4. The results show that rats from Kolhapur district were highly resistant to plague infection while those in Panvel area of Kolaba district were more susceptible. Rats from South Ratnagiri and Poorna village of Thana district showed a susceptibility of about 50 per cent while the rats from Bhivandi town were more resistant. Further work in Thana district is in progress.

With a view to ascertain the presence of antibodies against *P. pestis* as a result of latent infection among the rats serum samples were collected from number of rats and serum pools so prepared were tested by haemagglutination test using Fraction 1 as antigen. The results of these studies are recorded in Table 5.

The results show that one serum pool out of six collected from South Ratnagiri gave a positive reaction while 13 out of 41 pools from Marathwada showed a positive or weakly positive reaction. One of the serum samples from this area tested earlier had also given a highly positive reaction. Therefore, large number of serum pools were tested again during the year under review and about 30 per cent of the sample pools gave a weakly positive or positive reaction indicating that there was a possibility of existence of a latent focus of infection in this area and therefore this area should be watched carefully for evidence of plague infection. All the serum samples collected from adjoining areas of Sholapur district were negative. Similarly, the samples collected from Thana district were negative and showed no indication of any latent plague focus.

Development of a standard vaccine for assay of plague vaccine

A mouse protection test based on the absolute value of ED^{50} of a plague vaccine without a reference vaccine for comparison is incorporated in the monograph of Indian Pharmacopoeia and therefore, the test is unsatisfactory for control purposes. For this purpose, development of a suitable standard vaccine is necessary. It was reported earlier that some batches of vaccines were tested with this end in view. However, the batch to be selected for this purpose was found to be in 1968 and was tested only once as mentioned in the previous report. It has inadequate for lyophilized as a standard. Another batch was lyophilized now been tested six times during the period of report along with a batch of vaccine lyophilized in 1949 for comparison. In addition to these two batches, one more batch of vaccine which was in liquid form still was also tested. The results showing the ED^{50} values obtained for all these three batches are given in the Table below:—

TABLE 1
ED⁵⁰ values obtained for three batches of plague vaccine

Plague Vaccine	ED ⁵⁰ in ml.						
	1	2	3	4	5	6	7
Lyophilized in 1949 (B. 140)	0.0065	0.012	0.011	0.0074	0.0071	0.0067	0.0062
Lyophilized in 1968 (B. 35)	0.0046	0.011	0.0096	0.0072	..	0.0087	0.0072
B. 35-5 liquid	..	..	..	0.0125	0.013	0.018	0.013

The results show that the batch No. 35 lyophilized in 1968 was almost equal in potency to the batch No. 140 lyophilised in 1949. This batch No. 140 has been tested before lyophilisation and also after lyophilisation over a number of years along with other vaccine batches which were routinely prepared. This batch was found to be 2 to 2.5 times more potent than the routine batches. Batch No. 35 specially prepared in 1968 also showed the same potency. If this is adopted as a standard the routine batches would be half as potent as this. Therefore, another batch of vaccine (35-5) prepared during this period has been subjected to the tests and it showed almost half the potency as much batch Nos. 140 or 35 and this might favourably compare with routine batches. Therefore, it was decided to lyophilize this batch and test it again along with some freshly prepared routine batches. If these routine batches prepared at different times gave comparable results, this lyophilised batch may be recommended for adoption as reference vaccine after collaborative assays in different laboratories.

A complete characterization of the batch to be recommended as a reference vaccine is desirable and will be undertaken.

Media for plague vaccine preparation

The work on this project was mainly undertaken to find out a suitable indigenous protein to substitute imported casein for the preparation of vaccine and to simplify the present method of acid hydrolysis of casein by enzymic hydrolysis.

Enzyme hydrolysate.—Casein and peanut enzyme hydrolysates were prepared by enzyme digestion with papain (pH 5.0 for 24 hrs.) and trypsin (pH 8.4 for 72 hrs.). Sixty per cent of the protein could be hydrolysed by this procedure. The total N was adjusted to 270 mg. 100 ml. Both casein and peanut protein hydrolysates contained about 67 per cent amino nitrogen (Cu phosphate procedure) and about 25 per cent peptide N per 100 mg. of total nitrogen.

The hydrolysates were supplemented with some amino acids to see whether the growth of *P. pestis* could be enhanced. Supplementation of the acid hydrolysate with either phenylalanine or tryptophane could enhance the growth by 50 per cent. As seen in the Table the organism could not grow in both peanut protein acid and enzyme digests. Supplementation with phenylalanine, tryptophane and methionine had no effect.

Microbiological assay of peanut protein showed that it was somewhat deficient in amino acids particularly sulphur containing amino acids as compared to casein.

Cellular composition of *P. pestis*

Cellular composition of *P. pestis* grown on casein hydrolysate supplemented with phenylalanine and tryptophane is given in Table 2.

TABLE 2

Growth of P. pestis(195/P) in terms of optical density

Medium	No supplement	Phenylalanine	Tryptophane	Ph. alanine-tryptophane.	Methionine	
Casein hydrolysate (acid)	..	0.31	0.56	0.62	0.57	..
Casein hydrolysate (enzyme)	..	0.51	..	..	..	..
Peanut prote in hydrolysate (acid)	Practically		no	growth		
Peanut protein hydrolysate (enzyme)	Practically		no	growth		

TABLE 3

Cellular composition per 100 mg. of dry cells

Medium	Casein acid hydrolysate	C. hydrolysate phenylalanine	C. hydrolysate tryptophane	C. hydrolysate Ph. alanine tryptophane	Casein enzyme digest	Casein digest
Optical density	..	0.31	0.56	0.62	0.57	0.51
Total nitrogen	..	8.8	8.8	8.9	8.9	11.0
Protein nitrogen	..	8.5	8.5	8.5	8.7	10.6
RNA	..	9.0	8.3	8.7	7.9	10.8
DNA	..	2.3	2.6	2.7	2.1	3.6
Total phosphorus	..	1.02	0.93	1.08	0.89	1.45
Total fat	..	5.5	4.0	3.9	5.1	5.3

TABLE 4

Susceptibility of rats (Rattus rattus) to P. pestis (strain 195/p)

Place of collection				No. of rats inoculated			No. of rats inoculated.	Per cent susceptible	Per cent Resistant
				Date of inoculation	Male	Female Total			
Radhanagri (Kolhapur) ..	..	..	11-5-1968	29	33	62	62	30.6	69.4
Kudal Borgaon Harakul (Ratnagiri) ..	..	..	11-5-1968	21	20	41	41	56.1	43.9
Kurvai Pisara Morbe (Kolaba) ..	..	..	13-8-1968	16	60	76	76	73.7	26.3
Poorna ..	..	..	15-3-1969	35	40	75	75	45.3	54.7
Bhiwandi ..	..	..	22-3-1969	36	39	75	75	30.7	69.3

TABLE 5

Results of haecmagglutination test on pooled samples of rat sera (Rattus rattus)

Place and district	No. of pools tested.	No. of pools +ve, at titres		Total No. positive	Per cent positive
		1/10	1/20		
Sawantwadi (Ratnagiri) ..	6	1	..	1	16.6
Murud and Harangul (Osmanabad)	19	6	2	8	42.1
Dhak-Yedsi (Osmanabad) ..	22	2	3	5	22.7
Sholapur Tabavali (Sholapur) ..	21	Nil.	Nil.	Nil.	0
Poorna (Thana) ..	8	Nil.	Nil.	Nil.	0
Bhiwandi (Thana) ..	13	Nil.	Nil.	Nil.	0

PART III
OTHER DEPARTMENTAL RESEARCH

DEPARTMENT OF BACTERIOLOGY

Studies to determine the optimum conditions of aeration and agitation for submerged growth of *S. typhi* using a fortified casein hydrolysate medium showed that a good yield of 'O' and 'VI' antigens could be obtained under certain conditions. However, under the same conditions production of 'H' antigen was not satisfactory. A simple laboratory model of continuous culture apparatus was locally fabricated which could maintain *v. cholerae* in a steady state culture for five to seven days. An increased rate of survival of *P. pestis* after lyophilisation was obtained when different concentrations of lactose were used as suspending media. T.A.B. vaccine did not show any deterioration in potency as tested by mouse protection and agglutinin production tests on being kept in a frozen condition up to two months.

Studies in dynamics of disinfection

The rates of inactivation of different bacterial species by different concentrations of bactericidal agents vary considerably. Other factors also affect the rate of inactivation. The concentration co-efficient of these bactericids also differs widely. With a view to study some of the above mentioned aspects in case of some common bactericidal agents such as formalin, phenol and mercurial compounds with reference to *S. typhi* and *S. paratyphi* B, the investigations mentioned below were undertaken. The inactivation of *S. paratyphi* B at different concentrations of formalin at three different pH levels, viz., 5.5, 7.5 and 9 was studied. It was observed that at pH 5.5 there was an initial lag in the disinfection process while, at higher pH levels, there was no lag. The time required for complete sterilization of the culture at 0.2 per cent concentration of formalin varied from 125 mts at pH 5.5 to 45 mts at pH 9. The concentration co-efficient at pH 5.5 is approximately 2, while at pH 9 it is about 1. With *S. typhi* at pH 5.5, though there was no initial lag in the rate of inactivation, complete inactivation required 405 minutes which was a much longer period than that required for *S. paratyphi* B. However, at pH 9, the time required for inactivation was only 37 minutes. At lower concentrations of formalin, the inactivation time with *S. paratyphi* B was much higher than that required for killing *S. typhi*. The concentration co-efficient of formalin for *S. typhi* at different pH levels was round about 1.

Growth of *S. typhi* by submerged culture method

The application of submerged culture techniques as used for production of some biologicals is an economical and more convenient method for the large-scale cultivation of micro-organisms for vaccine production. This work was undertaken to study the application of this technique in typhoid vaccine production. The results of the work carried out using a fortified casein-hydrolysate medium, showed that the growth obtained in the fermentor was four to five-fold of that obtained in a stationary culture using the same medium. With

the help of a locally fabricated glass fermenter, the use of some substances as anti-foam agents was investigated. Pure groundnut oil was found to exhibit no inhibitory effect on growth but reduced frothing. However, it was emulsified during growth which interfered with growth measurements. Two different types of air spargers were tried. Fountain type air sparger with an airflow rate of 8 litres per hour and an agitation rate of 500-700 r.p.m. gave fairly good growth with production of 'O' and 'Vi' antigens. Under these conditions, the production of 'H' antigen was poor. Studies to work out optimum conditions for better growth with higher yield of the major antigens are in progress.

Studies on growth of *V. cholerae* in continuous culture

Continuous culture techniques are being used for growth of various micro-organisms and preparation of various biological products. With a view to study their application for growth of pathogenic micro-organisms, this work was undertaken.

A simple laboratory model of a continuous culture apparatus, depending upon the flow rate of medium, was locally fabricated. By using this apparatus and by incorporation of a suitable buffer system in the growth medium a steady state culture of *V. cholerae* Ogawa, lasting for five to seven days, was obtained. The results indicated the need for a more precise apparatus. Attempts to design and fabricate such an apparatus are being made.

Studies in lyophilization of *P. pestis*

The most essential prerequisite for production of live bacterial vaccines is the method of preservation of the organisms in live condition over long periods, under adverse conditions of temperature etc. in the field or during transport. The commonly used method of lyophilisation of bacterial cultures suffers from one drawback that during the process of lyophilization, more than 99.9 per cent organisms are killed. Therefore, it is necessary to work out optimum conditions for enhancing this survival rate. The investigations mentioned below were undertaken to work out optimum conditions for the survival of *P. pestis* which could facilitate production of live plague vaccine. Various factors affect the survival rate during lyophilization, out of which the nature of medium used for suspending the organisms before lyophilization plays an important role. Therefore, three suspending media, viz. gum acacia-nutrient broth, different concentrations of lactose varying from 1 per cent to 12 per cent and 1 per cent sucrose were tried. Out of these 3 per cent, 6 per cent and 12 per cent lactose gave a maximal rate of survival of 0.8 per cent while gum acacia nutrient broth showed a survival rate of 0.0001 per cent. Sucrose also showed a poor rate of survival.

Stability of typhoid vaccine in frozen condition

The storage conditions prescribed for vaccines state that they should be stored at temperatures between 2-10° C and should not be allowed to freeze. However, under certain natural conditions such as high altitudes, the vaccines

will freeze due to low temperatures. It was, therefore, thought necessary to investigate the effect of freezing on the potency of typhoid vaccine. For this purpose, a batch of T. A. B. vaccine was frozen in a deep freeze at 30°C for different periods varying from 2 days to 2 months. A sample of the same was also stored at 4°C and both the samples were tested by mouse protection tests using an acetone-dried vaccine as a reference. Rabbits also were immunized with the three vaccines including the reference and agglutinin production was studied. It was observed that there was no significant fall in potency of the frozen vaccine by both mouse protection test as well as by agglutinin production.

Preparation of Cholerae-El tor Vaccines of higher concentrations

Field trials of cholera vaccine carried out in different parts of the world indicate that cholera vaccine of normal strength containing eight billion organisms per ml. does not offer adequate protection. Therefore, it has become necessary to prepare vaccines with higher bacterial concentration. The following work was undertaken to see if a vaccine containing higher bacterial concentration can be prepared by a suitable method. For this purpose, two sets of *V. cholerae-El tor* cultures containing all the four standard vaccine strains with a bacterial density of (i) 24 billion, (ii) 20 billion, (iii) 16 billion and (iv) 12 billion organisms per ml. were prepared. One of these sets was inactivated, using 1 per cent phenol and the other was inactivated with 0.15 per cent formalin. The toxicity of the first two vaccines was tested in guinea pigs by injecting the vaccines both by subcutaneous as well as intraperitoneal route. Either one or both guinea pigs succumbed to the vaccine dose excepting in case of phenolized vaccine containing 20 billion organisms administered subcutaneously. The results showed that vaccines prepared with a strength of 24 billion and 20 billion organisms were toxic and hence were not suitable for human use. Experiments on the other two vaccines are in progress.

DEPARTMENT OF BLOOD BANK

Research projects on the study of genetical characters in some more endogamous populations in India were continued. Studies on characterisation of and antibody pattern of immunoglobulins were undertaken. Some projects for improvement in the routine procedures of the departments were also undertaken.

Studies on genetical characters in some endogamous groups in Indian population

This work was undertaken to study the distribution of genetical characters in the endogamous groups of population in our country. The data, so collected, will be a useful addition to ethnical data of the population in the country. As compared to the data available in other countries, the data available in this country are very scarce so far.

The present study was intended to cover some groups of muslim populations in this part of the country.

A study of blood and serum groups was undertaken on some muslim endogamous groups of Bombay together with other genetic markers, viz. colour blindness, ability to taste phenylthiocarbamide, abnormal haemoglobulins and G-6 PD deficiency. The groups under study at present are the Bohoras and Khojas. The blood groups studied are the ABO, Rh (C, c, D, E, e), MNSs, Duffy, Kidd, Diego and Kell system (Cellano, Benny, Stutler). The serum groups studied are the haptoglobins and transferrins.

So far two groups of muslims have been studied viz. Khojas and Boharas (about 400 samples) and it has been observed that (i) 2 males were G-6-PD deficient, (ii) 1 male showed colour blindness for red and green, (iii) 1 male had a variant of the N antigen.

The families of all the above cases are under study. At present, the study of 3 endogamous groups of muslims is under consideration.

One hundred and forty blood samples were collected from sewage workers in Bombay and were screened for their blood groups, red cells and serum, proteins. The results are as under (i) no abnormal haemoglobin was detected, (ii) G-6-PD deficiency ranged from 3-4 per cent, (iii) haptoglobinHp² frequency was high (—0.85), (vi) all samples showed a transferrin type CC.

In addition, over 60 serum samples from this group are also being analysed for their serum proteins with an idea of studying gamma globulin level in this population, which is handling infective material in the course of their duties. It is interesting to note that the level of gamma globulins in this section of population is much higher than in the normal population.

Studies on the distribution of normal haemoglobins, glucose-6-phosphated hydrogenase (G-6-PD) deficiency, haptoglobins and transferrin in some populations.

Andhra Pradesh Group (Kapu) —Ninety-three blood samples have been analysed so far for the above parameters. No abnormal haemoglobins were found. G-6-PD deficiency ranged from 3-4 per cent. While haptoglobins showed a predominantly high Hp² frequently (0·85), all transferrins were of CC type.

Bacterial contamination and haemolysis of 'banked' blood and method for reducing the same

This work was undertaken to minimise the wastage of blood collected by the blood banks. This posed a big problem during the last two national emergencies when considerable quantities of blood collected by mobile units by outside agencies were found to be contaminated. The blood collected in institutional centres is satisfactory whereas that collected in mobile sessions especially at long distances was affected. This is due to the unsatisfactory premises for collection of blood and transport to long distances over rough roads.

This study was continued further. The major contaminants isolated in chance contamination of blood were *Staphylococci* and *E. coli*. Antibiotic sensitivity of the organisms isolated was carried out and it was found that chloromycetin was the most effective antibiotic. Chloromycetin was added to A.C.D. in a strength of 1 mg. per ml. before autoclaving. It was found that 30 per cent of the antibiotic was lost in autoclaving. The blood was collected in this solution at outstation centres. Sterility tests were carried out on all the blood bottles and they were found to be sterile. The plasma was separated and dried and amount of antibiotic therein was estimated before and after drying. There was no loss on drying.

Studies on normal human immunoglobulins

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

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

Ten batches of the gamma globulin were referred to World Health Organization International Reference Centre for Immunoglobulins at Lausanne. The report from that laboratory confirmed our findings.

The gamma globulin is further purified by preparative agar electro-phoresis to prepare pure IgG. Its purity was tested by immunoelectrophoresis and gel diffusion techniques. The IgG so prepared was quite homogenous.

The gamma globulins are also being studied as regards the antibody level against some of the common infective agents. So far they have been screened for presence of anti-polio, anti-diphtheria, anti-tetanus, anti-streptolysin 'O' small-pox, measles and typhoid antibodies. They showed good concentration of polio antibodies and anti-streptolysin (ten times concentrated than original plasma pools).

Clinical trial reports are being collected from the clinicians. The reports so far received show prophylactic and preventive value of our gamma globulin in cases of infective hepatitis and serum hepatitis in post-transfusion cases and measles.

Anti-Rh (Anti-D) Immunoglobulins (IgG)

Recent development in the field of prevention of Rh isoimmunisation of women is the administration of Rh immunoglobulin IgG (Anti-D) to the vulnerable women before isoimmunisation takes place. These immunoglobulins are prepared from high-titre Anti-D sera obtained from naturally immunised donors and deliberately hyper-immunised donors. In our country, high-titre sera from naturally immunised donors is not available in large quantity. So, we had to use sera from the second source. Here also, pure Anti-D sera is not available; so mixed sera like Anti C×D has to be utilised.

A batch of Rh immunoglobulins IgG (Anti D) has been processed from Anti-Rh sera obtained from deliberately hyper-immunised blood donors. This sample shows pure IgG.

The product was sent to Dr. N. C. Hughes-Jones, M. R. C., Experimental Haematology Research unit, London, for testing. According to his report the product is of lower titre than needed. Another batch is being processed to get the desired titre. This product will be very valuable in the treatment of Rh sensitized women.

Cryoprecipitates from plasma for the treatment of haemophilia

Modern treatment of haemophilia is by the use of "Cryoprecipitates" prepared from the fresh human plasma. They show greater concentration of A.H.G. than fibrinogen (Cohn fraction I) prepared from fresh human plasma and do not contain any other contaminants. They can also be prepared in ordinary blood bank set-up.

Cryoprecipitates are being prepared in Haffkine Institute blood bank. But unfortunately all the facilities needed for the process are not available in the country.

Cryoprecipitates from fresh human plasma, which are of great value in the treatment of haemophilia are being processed from freshly collected plasma. The method described by D. L. Brown is followed, but siliconised bottles are used in place of plastic bags, as the latter are not yet available in our country.

Cryoprecipitates have been clinically used in one case at the K. E.M. Hospital. In this case, there was a very good response (anti-haemophilic globulin level rose from 15 per cent to 85 per cent of normal on administration of these cryoprecipitates.).

Tissue Banking

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

Surgeons were interested in preserving the ossicles (middle ear bones) removed from cadavers, for use in replacement surgery. The ossicles were removed from the cadavers and preserved in antibiotic fluid and taken up for freeze-drying. They were freeze-dried under varying conditions and lengths of time, to find out the optimum conditions of drying these bones. Each charge was subjected to different conditions of freeze-drying. The samples were studied histologically for the structural variation in bones. So far, 5 batches have been processed on these lines. A satisfactory method of preservation is still to be arrived at.

Blood group substances form different sources

Blood group substances are needed in the blood bank operation. They were being imported all these years. There are different sources of blood group substances, which were being processed from other sources.

From Hog Mucosa.—Five mucosal linings of hog stomach were digested at an acid pH. 2.0. After the digestion, the substances were precipitated by adding ethyl alcohol (5 volumes at pH at 5.5 in presence of sodium acetate. Each lining yielded 4-5 gms. of A and H substances. They were active and suitable for immunising donors.

DEPARTMENT OF CHEMOTHERAPY

Schiff bases from 3, 5-Dichloro or Dibromo 4-Substituted Anilines with different nitrogen mustard aldehydes as potential anticancer agents.

In recent years, synthetic drugs like Chlorambucil, Sarcolysin, Endoxan etc. have found a place in the clinical management of some forms of cancer. However, their lower margin of safety prompted attempts at the synthesis and study of newer derivatives with better therapeutic index. In continuation of the previous work (J. Med. Chem. 11,154-156, 1968) nearly 40 new Schiff bases have been synthesized from 3,5-dichloro or 3,5 dibromo-4 substituted anilines and 19 prepared in larger amounts and were sent to Cancer Chemotherapy National Service Centre (C.C.N.S.C.). Bethesda, U.S.A. Out of these, 8 compounds have been found to be active in lymphoid leukemia L-1210 (mouse), (3-LE) and 3 compounds in Walker-256 (rat), (5-WM) and have passed sequential and confirmation steps I and II respectively.

Schiff bases from 4-(p-amino-phenyl)-5-methyl-2 substituted thiazoles and from 4 (p-amino-2-chlorophenyl)-2-substituted thiazoles as potential anticancer agents.

Nearly 25 new compounds in this series were synthesized by the condensation of 4-(p-amino phenyl)-5-methyl-2-substituted thiazoles with various N, N¹-bis-2-(chloroethyl) aminobenzaldehydes.

Additional 15 Schiff bases were also prepared from 4-(p-amino-2-chlorophenyl)-2-substituted thiazoles with different mustard aldehydes with a view to see if the introduction of chlorine in the meta position would enhance the activity.

Five representative compounds from among these were prepared in larger amount and sent to C.C.N.S.C. for the evaluation of their anticancer activity against different systems. The rest of the compounds are now being screened for anticancer activity by *in vitro* (tissue culture) techniques in collaboration with Cancer Research Institute, Bombay.

Twenty-seven reports from C.C.N.S.C. on some of the compounds in these series, prepared and sent in the previous year, were received. These reports show NSC-115488 and NSC-113449 to possess a high order of activity against 3LE (lymphoid leukemia) and against Walker-256 (5WM).

Synthesis and antitubercular activity of N.N.¹-substituted ethylenediamine derivatives.

In the last decade, a number of ethylenediamine derivatives have been studied for possible antitubercular activity and N.N¹-(ethylenediamine)-di-1-butanol di-hydrochloride (ETHABUTOL) has been clinically used as a secondary drug against resistant cases.

The present work comprises of synthesis and *in-vitro* tuberculostatic evaluation of 50 N-(p-chloro-phenyl-benzyl)-N¹-(substituted piperazine) ethylenediamines and N-(1,2-diphenylethyl)-N¹-(substituted piperazine) ethylenediamines.

Almost all these compounds showed varying degrees of *in-vitro* antitubercular activity against H₃₇ Rv strain. Eight were found active at 5 µg/ml and 9 at 20 µg/ml.

Synthesis of N¹-(phenyl)-(p-substituted benzoyl) ethyl]-N⁴-(substituted) piperazines.

In continuation of our previous work, new Mannich bases containing substituted piperazines have been prepared and studied for possible antitubercular and antifungal activity. Out of the 40 compounds when tested *in vitro* for their antitubercular activity, five compounds displayed activity at a concentration of 2 µg/ml., five at 4 µg/ml. and 18 compounds manifested antifungal activity at 10 µg/ml. against *Trichophyton rudrum* and *T. mentagrophytes*.

Synthesis of quinoline-4-carboxylic acid derivatives and amidino-carboxamides.

In further continuation of work in the synthesis of quinoline-4-carboxylic acid derivatives, compounds having chloro and sulphanilamido groups in the carbocyclic part of the quinoline ring have been synthesized by condensing 6-chloro-5-sulphonamido isatin with various substituted acetophenones by Pfitzinger reaction. Some of the quinoline-4-carboxylic acid derivatives were further converted into N-amidino carboxamides. Most of the compounds were found inactive when fed to dogs orally. However, these compounds, on broad pharmacological screening, have shown anti-inflammatory activity.

Exposure hazards in parathion formulation Industry

In collaboration with Dr. S. K. Chatterjee of Central Labour Institute, a study was made in two factories in Bombay where parathion concentrate is made into 50 per cent emulsifiable spray fluid and packed into aluminium containers. The exposure risk to workers was studied by (a) medical examination (b) blood or plasma and erythrocyte cholinesterase activity and (c) urinary *p*-nitrophenol concentration determination, both prior to exposure and during exposure at different intervals. The items (b) and (c) were done at this Institute. More than 50 workers were studied. It was seen that there were no statistically significant changes in cholinesterase activity between pre-exposure and post exposure. However, it was seen that the urinary *p*-nitrophenol concentration was a very sensitive method of exposure detection. It clearly showed the difference in the pre and post exposure values and showed the gradual increase in concentration of *p*-nitrophenol with daily exposure. From this study, it was concluded that the urinary *p*-nitrophenol determination would greatly help in detecting the exposure levels in a factory or the working condition of the different factories or individual workers at different jobs in hazardous locations.

Synthesis and study of compounds for possible use in CNS and cardiovascular disorders.

In further continuation of the work published by us in the Journal of Medicinal Chemistry, 11 (1968) pp. 332-336, several N¹, N⁴-disubstituted piperazine derivatives, in which N¹-substituents are 3, 4, 5-trimethoxy benzoyl acetyl, 3, 4, 5-trimethoxy cinnamoyl or hydrocinnamoyl, 3, 4, 5-trimethoxyphenyl

propyl and 3,4, 5-trimethoxybenzoyl alkyl, whereas N⁴-substituents are benzyl, *m*-methyl-or *p*-*t*-butylbenzyl, *p*-chlorophenyl benzyl, phenyl, chloro-fluoro or methoxyphenyl, tolyl, α , α , α -trifluorotolyl, 2-pyridyl, 2-pyrimidyl, or 2-thiazolyl groups were screened for CNS activity. A few of these compounds exhibited significant C.N.S. depressant activity. The 3,4,5-Trimethoxyphenyl moiety was found to be the most essential for CNS activity as stepwise omission of the methoxy groups from the highly active compounds resulted in loss of activity.

Cardiovascular actions of a new piperazine derivative : N¹-(3, 4, 5-Trimethoxybenzoyl) ethyl-N⁴-(*o*-tolyl) piperazine (R-1361).

A series of newly synthesized piperazine derivatives was observed to possess interesting psychosedative properties in laboratory animals. One of the compounds, designated R-1361, was evaluated in detail to determine the pharmacological profile. In anaesthetised cats and dogs, R-1361 produced a dose dependent hypotension. The hypotensive action was accompanied by depression of centrally mediated vasomotor reflexes. Spinalisation and decerebration demonstrated the brainstem to be the probable site of hypotensive action. Local administration of R-1361 to CNS and studies of medullary stimulation responses confirmed the central origin of the hypotensive action. In addition, R-1361 exhibited adrenolytic action *in vitro* as well as *in-vivo*. The antagonism was found to be competitive in nature.

DEPARTMENT OF CLINICAL PATHOLOGY

Studies on detection of chorionic gonadotrophic hormone levels in urine

These studies were undertaken with the object of evaluation of the Gravindex Test with the biological rat test for the detection of chorionic gonadotrophic hormone in urine.

Seventy-five cases of normal pregnancy, 50 cases of abnormal pregnancy, 15 cases of normal non-pregnant females, and 10 cases of normal males were studied.

Gravindex Test (Ortho-Diagnostic) was found to be very accurate in the detection of normal pregnancy and was more sensitive in cases of ectopic gestation.

Quantitative estimation of chorionic gonadotrophin (H. C. G.) in urine

The prepuerin-haemagglutination inhibition test reagent was provided by Welome Research Laboratories.

Quantitative estimation of the hormone (H.C.G.) is being studied in normal as well as abnormal pregnancies. This quantitative assay has proved very helpful in the prognosis of threatened abortions.

Bacteriological Analysis of food products

Bacteriological analysis of 100 samples of icecream, 100 samples of sweetmeats and 50 samples of sugar cane juice was done. The predominant organisms isolated were—*E. coli*, *Strep. faecalis*, *Staph. aureus* and *Strep. haemolyticus*.—The results in testing had indicated that it did not meet the I.S.I., requirements (I.S., 2802, July 22, 1964) in some cases. Similarly, the results obtained in sweetmeats and sugar cane juice also indicated the poor quality in a few samples.

Studies on incidence of typhoid fever in a closed community in Bombay

These studies were undertaken with a view to determine the incidence of typhoid fever in a specified area in Bombay. The work included a survey of a population of about 14,000 done with the object of finding out the incidence of enteric fever. This group of population was followed up twice weekly to record each and every case of fever. Patients with fever suspected to have enteric fever were investigated to determine the diagnosis by cultural examination of blood. On the basis of this study and other available data it was concluded that the annual incidence of enteric fever in Bombay was between 2.9 and 4.7 per 10,000 of general population, which can be considered as low.

Studies on β . haemolytic Streptococci of Group A

These studies were undertaken to determine the incidence of Streptococci of Group A amongst the β -haemolytic streptococcal infections.

To study these, 965 samples of throat swabs, pus and sputum were collected. Throat swabs of the patients suffering from tonsillitis were also collected. Patients clinically diagnosed as suffering from diphtheria but bacteriologically negative for *C. diphtheriae* were also included.

These samples were studied on sheep blood agar for the presence of β -haemolysis. Out of the total 965 samples 129 (13.3 per cent) were found to be β -haemolytic streptococci. These β -haemolytic streptococci were grouped by Lancefield's grouping method and also by the bacitracin disc method. Forty-one (31.7 per cent) were found to be of Group A. Further study of typing of Group A is in progress.

Serological Survey of leptospirosis

At present work on leptospirosis is carried out from a zoonosis point of view. A total of 500 sera were collected from both human and animal origin. The samples were from selected sources such as sewer workers, stray dogs, clinically suspected leptospiral cases of human and dogs, buffaloes and also aborted cases of cattle, sheep and goat. Leptospiral antibodies were detected by both microscopic agglutination test and sensitised erythrocyte lysis test. The percentage of infection in stray dogs was found to be quite high (31 per cent). Results of S.E.I., test and microscopic agglutination test were found to be agreeing well with each other. The work is in progress.

Toxigeny and phage typing of *C. diphtheriae*

About 200 strains of *C. diphtheriae* were isolated from suspected patients. They were tested biochemically for their differentiation into gravis, mitis and Intermedius. Mitis were 60 percent, gravis 35 percent and intermedius were 4 per cent while the remaining were diphtheriods. The strains were then subjected to toxigenicity test both by Elek's plate method and rabbit intracutaneous test.—Eighty-five per cent of the strains were found to be toxigenic by both the methods.

A set of twenty-four phages had been obtained from Rumania—W.H.O. International Reference Centre. The strains were subjected for phage typing. About 80 per cent strains belonged to type No. 19 only and 6 per cent showed a phage pattern while the remaining 14 per cent strains were untypable.

A few (twenty) strains were received from Calcutta, Aurangabad and Poona and of these fourteen strains belonged to type No. 19; four strains were untypable and two strains from Calcutta showed phage pattern. Thus No. 19 phage was found to be prevalent.

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

These studies were undertaken with the object of determining the level of immunity against diphtheria in contact of diphtheria cases. Throat swab examinations of 500 school children and contacts of patients was done to find out carriers amongst them. Schick test was performed on 142 non-immunized

school children under 10 years of age, out of whom only 8 were Schick positive and 12 were carriers. When throat swab examination was performed, of the 375 immunized school children, 28 amongst them were found to be carriers.

Studies on Mycophages

This study was undertaken with a view to understand the Phenomenon of transduction in mycobacteriophages. So far about 10 samples were enriched with various strains of atypical and typical mycobacteria to isolate the mycophages. One phage was isolated. This phage will be studied along with the four standard phages obtained from U.S.A. All these phages were propagated on respective host strains. One strain 607B, which is a host strain for one of the standard phages, exhibited lysogeny. Change in the colony characteristics of the lysogenic strain was observed. This will be studied further. The effect of dimethylsulfoxide (DMSO) and acriflavine on the susceptibility and resistance of mycobacteria to drugs like Streptomycin, PAS and INH was studied in Youman's medium by dilution method. The effect, of DMSO was studied in the concentration ranging from 1 per cent to 50 per cent and acriflavine in 0.5 percent. It was observed that the strains are susceptible to the higher concentrations of DMSO whereas 0.5 per cent acriflavine and 1 per cent DMSO did not show any adverse effect on the growth of mycobacteria. No definite change in the permeability of the drugs into the cell when these chemicals were incorporated, was observed. Some of the mycobacterial strains were phage typed. This work is in progress.

DIRECTOR'S LABORATORY

A comparative study of drugs against amoebic infection

Since the introduction of emetine as an anti-amoebic agent in the therapy of amoebiasis, many synthetic compounds have come into the market. In certain cases emetine remains the drug of choice. There is still enough scope for finding out an ideal anti-amoebic agent. *Berberine hydrochloride*, an alkaloid isolated from *Berberis aristata* Linn. has been known to have anti protozoal action and its effect on amoeba was studied along with the currently used drugs for the treatment of amoebic infection. Different chemotherapeutic agents have been studied *in vitro* as well as *in vivo* against *E. histolytica*, isolated from an acute case of dysentery.

Amoebiasis in rats and hamstars.—White rats weighing 20-30 g were infected under ether anaesthesia, intracaecally with about 5000 trophozoites. Each group consisted of a varying number of animals, one for the test drug and one for the control (untreated). The animals were autopsied at the end of 6 days. On assessment, the degree of infection for each rat varied from 0 to 5 which was fixed arbitrarily.

Hamsters used were from this Institute's stock. They weighed 40 to 55 g. The animals were infected intrahepatically under ether anaesthesia, the dose being 5,000 amoebae for each animal. They were autopsied at the end of 96 hours. Livers were examined and the nature of the abscess was noted. Smears were prepared from the periphery of the abscess and examined for the presence of amoeba. Arbitrary scores were given from 0 to 5.

Administration of drugs.—Each treated rat was given 6 doses of the drug, under test, orally (except emetine hydrochloride which was given subcutaneously). The first dose was given at the time of infection (afternoon), thereafter, once daily for 5 days.

In the case of golden hamster, all the drugs were administered orally except emetine hydrochloride which was given intramuscularly. The first dose was administered 3 hours before the challenge inoculation (afternoon) and the second dose in the evening, subsequently each dose twice a day till 8 doses were completed.

In vitro tests showed that of all the compounds emetine, chloroquine, camoquine, vioform, dehydroemetine, metranidazole and berberine were basically antiamoebic, the drug metronidazole being the most potent. The amoebicidal concentration was 5 μ ml. Chloroquine required 200 μ ml. under similar circumstances. Berberine hydrochloride was as effective as emetine hydrochloride, the amoebicidal concentration being 10 μ ml.

In the intestinal amoebiasis of rat, berberine could reduce the infection to the level of 17.2 per cent which was almost equal to that of emetine hydrochloride. Combination of berberine with BqB (broxy-quinoline brobenzoxaldine combination) was of no advantage.

In the hepatic infection of hamster berberine was as potent as emetine in reducing the amoebic liver infection. The dose required to do so was 150 mg/kg/day.

Metronidazole at a dose of 100 mg. kg/day reduced the infection in hamster to the level of 50 per cent and thus it was less effective than emetine. In hepatic infection in hamsters the combination of berberine and BqB proved to be of no special benefit.

Growth behaviour of *E. histolytica* in different media

The growth of the culture of *Entamoeba histolytica* was studied in different media. Of all the media studied, Dobell's medium (Horse Serum) Powlova's medium and LMS medium were found to be superior to other media used. Powlova's medium and LMS medium can be conveniently used for testing drugs *in vitro* against *E. histolytica* as these are monophasic (liquid) media giving an abundant growth of amoeba.

Various media have been found to support the growth of *E. histolytica*. Due to variation in their ability to support the growth of amoeba, a comparative study would be useful in selecting a proper medium for a particular strain of *E. histolytica*. The media used contained different complex materials such as blood serum, egg and liver extract as constituents.

The media used were (i) Dobell's medium with egg albumin. (ii) Dobell's medium with horse serum. (iii) Liver extract Marmite-Serum medium (LMS.) (iv) Powlova's medium. (v) Whole blood medium No. 1. (vi) Whole blood medium No. 2. (vii) Whole blood medium No. 3. (viii) Locke-egg serum medium (ix) Ringer-Egg serum medium.

The above media were inoculated with a 48 hour old culture of *E. histolytica* and were incubated at 37°C. Counts were made every 24 hours using a haemocytometer counting chamber. Growth curves were drawn from the readings. The amoebae survived upto about 13 days in Dobell's medium (horse serum) Pavlova's medium and LMS. medium. The survival period of amoebae in other media was found to be about 7 to 8 days.

Pharmacological observations on the fractions isolated from seeds of BANAKADALI (Ensete Superbum, Cheesm, N.O. Musaceae)

Seeds of *Banakadali* are used in the treatment of small pox and measles by the physicians practicing in the indigenous system of therapy. Three fractions have been isolated and tested for anti-variola and anti-vaccinia effect and have shown encouraging results. Before putting up clinical trial, pharmacological properties of the fractions should be known. Therefore, pharmacological investigation has been undertaken. The present report concerns the pharmacological actions of one of the potent constituent of the seed of *Banakadali* named as VIDR-2 GD. Actions of other two fractions are being investigated.

A dose of 0.5 mg. to 7.5 mg. did not produce any marked effect on the isolated perfused heart of frog. When adrenaline and the drug were given concurrently in the anaesthetised cat, the action of the drug VIDR-2 GD predominated, and later on adrenaline action was observed but not to the extent produced by adrenaline alone. VIDR-2 GD caused hypotension. Topical (5 mg/ml) and intravenous administration of the drug (250 mg/kg) produced congestion of the mesenteric blood vessels of the rat and mice. The stasis of circulation due to topical application was restored to normal but after washing with normal saline. Perfusion of the frog's vessels with the drug (2.5 mg.) showed dilatatory effect with increase in outflow. The drug VIDR-2 GD did not have any effect on the bronchial musculature in a dose of 250 mg/kg. It neither enhanced nor antagonised the effect of histamine induced broncho constriction. It did not produce any appreciable action of tracheal chain of guinea-pig in concentration of 20 to N 100 ml. In a dose of 30 mg./ml it caused immediate contraction of guinea-pig ileum but after atropine the drug effect was antagonized showing chloinoergic like effect. The amplitude of peristaltic movement of guinea-pig was increased with 5 ml. of the drug and adrenaline in large dose removed the tonicity. The tonicity of uterus of guinea-pig and rat was increased in concentration of 20-40 ml. and adrenaline checked the tonicity. It has shown slight diuretic effect in rats with a dose of 2.5 mg/kg. The drug potentiated the pentobarbitone induced sleep in mice and rats in a dose of 180 mg/kg. but without any sedative effect when given alone. The drug could not check metrazol induced convulsions in rats and mice in a dose of 180 mg/kg given intraperitoneally half an hour and one hour before metrazol. But the animals were protected for further half an hour before death than the metrazol treated control animals. In the dog and rabbit the drug reduced the whole blood clotting time, recalcified plasma clotting time and bleeding time.

The drug caused hypoglycemia by 15 per cent at the end of 5th hour with dose of 100 mg/kg in rabbit. After 24 hours the blood sugar remained low by 8.5 per cent. It did not have any inhibitory effect on the growth of *E. histolytica* upto a concentration of 50 mg/ml. Acute toxicity studies showed that a dose upto 5 mg./kg. orally, 2,500 mg/kg. intraperitoneally and 1,000 mg/kg. intravenously did not produce any deleterious effect during 6 days observation. Persistent hypotensive action for about 20 minutes was observed in the case of dose between 50 to 100 mg/kg in the spinal cat.

In the spinal cat after clamping both carotid arteries, 50 mg/kg of VIDR-2 GD caused lowering of blood pressure significantly but the effect was transient and the original level was restored. After occlusion of right or left carotid artery individually, the induced hypotension was brought down with the above dose when the drug was given intravenously. But the hypertensive state returned and the blood pressure came down to original level after releasing the occlusion. After atropine sulphate (1 mg/kg intravenously) in the spinal cat, VIDR-2 GD caused transient lowering of blood pressure but the effect was reduced than in the non-atropensied cat with the same dose. After cutting both vagii in the spinal cat, 50mg/kg VIDR-2 GD caused the same hypotensive effect.

Preliminary pharmacological observation on the alkaloid, glycoside and gluco-alkaloid fractions isolated from *Cynodon*, Linn, Pers, N. O. gramineae (Durva Hariali)

The shrub is used as astringent, diuretic, aromatic, stomactic, anti-diarrhoric and in epilepsy, insanity etc. in indigenous system of therapy, for which a detailed pharmacological study has been undertaken with the crude extract as well as with 3 fractions isolated from it.

The alkaloid fraction did not show any marked effect on the blood pressure and respiration of cat in a dose of 15 mg/kg. The mesentric capillaries of rats and mice showed slowing effect of the blood flow. The flow was interrupted and thin, instead of a continuous stream with a dose of 50 mg/kg intravenously. The isolated ileum of guinea-pig has shown increase in tonicity with a concentration of 0.1 mg/ml. and there was sustained increase in tonicity for a long time with a concentration of 0.2 mg/ml. Normal tone and rhythmicity returned after washing. The guinea-pig uterus could not respond to the drug in a concentration of 0.2 mg./kg. ml. Five mg/kg given intravenously lowered the blood sugar in the rabbit to the extent of 15 per cent (maximum) at the end of 1st hour. It reduced the clotting and bleeding times in the adult rabbit with a dose of 2.5 mrg/kg intravenously. It did not have any inhibitory effect against the growth of *E. histolytica* *in vitro*

The glycosidal fraction in a dose of 8 mg-18 mg/kg. caused hypotension in the cat but the action with a dose of 10mg/kg was antagonized by atropine. The drug did not produce any effect on guinea-pig isolated ileum. It did not show any significant result on the bronchial musculature of guinea-pig when 25 mg/kg were given intravenously. A dose of 10 mg/kg, given intravenously, to adult fasting rabbits lowered the blood sugar level to the extent of 7 per cent (maximum) at the end of the 1st hour. It did not have inhibitory effect on the growth of *E. histolytica* *in vitro*.

The gluco-alkaloid in a dose of 3-8 mg/kg, caused slight hypotension in the cat which was not significant but after atropine the same effect was observed. It did not show any significant effect on bronchial musculature of guinea-pig with a dose of 2.5 mg/kg intravenously. Ten mg/kg given intravenously in the fasting adult rabbit, caused lowering of blood sugar by 3.1 per cent (maximum) at the end of first hour.

DEPARTMENT OF ENTOMOLOGY

Plague epidemiological studies were continued in the Western Maharashtra region and are being followed up in the Thana District. A rat control campaign was organised in 171 villages situated in Panvel block.

Plague epidemiological studies in Maharashtra State

The role played by plague epidemics in the past is well-known. Although since 1951 the disease has shown a considerable decline whether this decline is of a permanent type or of a short duration remains to be seen. The history of plague infection shows its periodical disappearance and sudden reappearance even after a long lapse of time. The factors influencing reappearance of plague infection are many. One of them is the possibility of persistence of infection among wild rodents. Plague epidemiological studies are being carried out with a view to know the existence of persisting infection among rodents, the susceptibility status of hosts to plague infection and to study the susceptibility status of vector species to different insecticides.

Western Maharashtra : Plague epidemiological studies were carried out at Satara, Radhanagri, Kudal, Sawantwadi and nearby villages. Rats were collected from houses, outskirts of the town as well as from the forest locations by trapping. In all 229 rats were trapped, out of which 221 were *R. rattus*, 5 were *Leggada budooga*, 2 were *G. ellioti* and one *Mus musculus*. The rat density was 6.0 at Satara, 15.76 at Radhanagri and 8.8 at Sawantwadi. *R. rattus* predominated, constituting 95 per cent of the total rat catch.

Seven hundred thirty-five fleas were recovered from these rats. *X. cheopis* was observed to be the most predominant flea species. In the course of our plague epidemiological studies for the first time, *X. brasiliensis* fleas were recovered in good number at Radhanagri.

Rats from Radhanagri area of Kolhapur district showed a high degree of resistance to plague infection (70 per cent) whereas rats from Sawantwadi area showed only 44 per cent resistance.

Panvel rat elimination project : Rat control campaign was taken up in 171 villages of Panvel block on 9th June 1968 in collaboration with the Kolaba Zilla Parishad, Public Health and Agriculture Departments of Government of Maharashtra, and was recently concluded.

The work primarily constituted the extension of the work done last year in four villages of Panvel taluka. It involved trapping of rodents with the help of wonder traps, followed up by baiting with zinc phosphide. Two bordering villages outside Panvel block were taken as control to evaluate the results. The initial rat densities in the control villages outside Panvel block viz. Ris and Jasai were 20.0 and 69.0 respectively on 16th June 1969. Two villages were similarly taken from experimental group and studied on a comparative basis for rat densities. The rat densities in the experimental villages viz. Dapoli and

Pargaon were 44·0 and 130·8 respectively on 10th June 1968. The densities at the conclusion of campaign in the control villages were observed as 56·0 and 59·0 whereas in the experimental villages selected for comparison viz. Dapoli, Pargaon these were observed to be 5·0 and 18·0 respectively, showing a reduction in experimental villages.

As a result of trapping and baiting in 171 villages 20,476 rats were destroyed. Out of these 18,705 belonged to the species. *R. rattus*, 320 *B. bengalensis*, 348 *B. gigantea*, 1937 *Suncus caeruleus*, 8 *Leggada budooga*, and 58 *Golunda ellioti* and *M. musculus*. Rats collected from Morbe area from Panvel block were challenged with a standard dose of *P. pestis*. It was significant that susceptibility varied from place to place even among the villages situated within a range of two miles.

Plague studies in Thana district : Plague epidemiological studies were initiated in Thana district on 28th February 1969. The studies carried out in 6 places till 31st March 1969 indicate the predominance of *R. rattus* and *X. cheopis*. These studies are in progress.

The results of susceptibility studies carried out with *R. rattus* to plague indicate that the rats from Poorna show considerable susceptibility (46 per cent) as against *R. rattus* from Bhiwandi (30 per cent).

Rat sera examined by HA test were found to be negative to plague.

DEPARTMENT OF IMMUNOLOGY

Research

The insolubilisation of pepsin was finally achieved after repeated failures. The product retained its enzyme activity and was able to digest antitoxic sera when tried on a laboratory scale. The fractionation of Russell's Viper venom in fairly large amounts was achieved using starch block electrophoresis method.

Stability of purified antitoxic sera

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

The effects of heat, various antiseptics, amino acids, salts etc. were tested. Preliminary results showed that these factors had no effect in accelerating the loss of potency during storage at 2-5°C and 37°C as compared with controls. An interesting result was that pepsin treated antisera could stand heating at 60°C for 10 hrs. without showing any decline in potency as assayed by neutralization tests using animals. The effect of oxidizing and reducing agents and of enzymes are under test.

Insolubilisation of pepsin

The use of insolubilised pepsin for the digestion of horse antisera has a number of advantages. It does not contaminate the system as it could be removed by centrifugation or filtration. The reaction can be stopped at any stage by simply separating it. It could be used over again if still active. There will be no antigens contributed by pepsin which may cause sensitization. It is also likely that any deterioration in antibody activity of stored antisera, if due to residual pepsin would be eliminated. In preliminary experiments a number of methods and reagents were tested for polymerisation of pepsin with a view to insolubilise it. The results were all unsuccessful. Ethylchloroformate which has been used by Avrameas and Terynck to insolubilise many antigens did not show a precipitate with pepsin. Finally, however, this reagent did show a precipitate when pepsin was copolymerised with other proteins such as gelatin or papain or amino acids such as lysine or glutamic acid. Even here, the results were variable. The product obtained in some experiments showed activity while in others it did not. It was found that factors such as initial pH, amount of reagents added, time and temperature of reaction etc., had their effect on the enzyme activity of the product. The general procedure so far worked out which gave an active product is as follows:—

To 100 ml. of aqueous solution of 1 per cent pepsin and lysine in the proportion of 1 to 0.5, one ml. of ethylchloroformate was added and kept stirred at room temperature (24-25 °C). After 10 minutes of stirring, 2N Na OH was added slowly drop by drop to maintain the pH between 3 to 3.5. In about 15

to 20 mts, a precipitate began to form. The reaction mixture was stirred for one hour and left undisturbed for 1 hr. It was then centrifuged and the precipitate was washed with normal saline 3 or 4 times. The precipitate could be preserved in the active state in wet condition. It lost its activity when dried by washing with acetone and ether.

A sample of insolubilised pepsin was used in wet condition to see whether it acted on horse antitoxic plasma. The results are encouraging. Further work is in progress.

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

The purpose of the work was to fractionate Russell's viper venom in quantity using mild physical methods and to isolate and purify the biologically important factors such as coagulase, L-amino acid oxidase, skin necrotising factor, protease etc. The purified factors could then be taken up for study of their chemical nature and mode of action. It is likely that some of them will have value as laboratory reagents or in therapy.

The first step used in fractionation of the Russell's viper venom was the use of starch block preparative electrophoresis method. In this way about 10 grams of venom was separated into 5 fractions. The proteolytic activity was distributed in fractions II, III and IV and it seemed that the venom had two or more proteolytic enzymes. L-amino acid oxidase and coagulase were present mainly in fraction III. Phospholipase A was present in fraction IV.

Fraction IV was then taken up for further purification by using gel filtration technique on Sephadex G-75. By this method it was possible to separate the phospholipase A from the proteolytic enzyme. The separation of other fractions is now in progress.

Studies in tetanus toxin Production

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

In preliminary experiments it was found that Harvard strain of *Clostridium tetani* could grow in casamino acids medium supplemented with yeast dialysate, cystine, tryptophan and salts. The yeast dialysate could be replaced with thiamine, PABA, biotin, Vit. B 12, pyridoxine, riboflavine, nicotinic acid and pantothenic acid. The optimum amount of each of these vitamins for maximum toxin production was then estimated. The toxin produced in this medium showed a mouse MLD of 20,00,000 per ml and L_{+100} of 700. These results were consistent.

Using the above medium based on casamino acids, the suitability of different sources of sulphur for toxin production was investigated. It was found that sodium sulphide, sodium sulphite and sodium thioglycollate could replace cystine as sulphur source. However, sodium sulphate and sodium thiosulphate could not substitute for cystine. Calcium was not necessary for toxin production and in fact it showed inhibitory action when added to the deferrated medium.

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

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

The adjuvants and immunosuppressors were tested in groups of about 10 rabbits, each of which received three injections each of 50 Lf of diphtheria toxoid at intervals of 2 weeks. The drugs were administered either along with the diphtheria toxoid or separately. The control group received diphtheria toxoid alone by intramuscular injection. The highest antibody level in the sera of these rabbits was about 9.7 units/ml which was reached 7 days after the 3rd injection (i.e. 37 days after the first injection). It was found that there was no significant difference when the toxoid was given intraperitoneally.

Oil emulsion adjuvant by intramuscular injection with paraffin oil, lanolin and aqueous toxoid in the ratio of 2:1:3 showed an average antibody titre at its highest of 19.6 IU/ml.

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

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

The use of rabbits and of diphtheria toxoid for screening adjuvants and immunosuppressors has disadvantages because of the large size of the animal and the cumbersome procedure for estimating the titre of diphtheria antitoxin in blood. The use of SRBC in mice has none of these disadvantages. The procedure is as follows :

Groups of mice were given a single dose of 0.2 ml. of SRBC intraperitoneally. The mice were bled periodically and the haemagglutination titre was determined in perspex plates using 1 per cent SRBC suspension in 2 fold dilution. The maximum titre of 1:2048 was reached on the 15th day after the injection and then the titre gradually came down reaching 1:32 on 41st day. The immunosuppressive effect of 'Endoxan' and 'Imuran' are under test,

DEPARTMENT OF NUTRITION

Studies in *E. coli* metabolism

The *E. coli* organism and its various mutants are capable of growing in a simple medium. The auxotrophic mutants are specially useful for the investigation of many biochemical reactions.

Earlier reports included the work on (a) the proximate principles and amino-acid composition, (b) nucleic acids, (c) pantothenic acid and vitamin B₁₂, (d) vitamin B₁₂ requirements in the presence of cystine and inorganic sulphate. The present report contains the work on the effect of precursors of nucleic acid on the growth of the organism, fatty acid composition, the reversal of growth inhibition due to the presence of cystine, and the synthesis of sulphhydryl compounds from exogenous cystine.

Studies on growth

The growth responses of the strains W and 113/3 of *E. coli* were studied under shaken and stationary conditions using the standard medium of Roberts *et al.* Maximum growth was obtained between 16-18 hrs. The lag phase and the logarithmic growth phase were of 6 and 10 hours' duration, respectively. Effect of the precursors of nucleic acids on the reduction of the lag phase of growth was investigated. Adenine, uracil, adenosine and guanylic acid had no effect on the growth of the organism. Cytidine, cytidylic acid, guanosine and uridine shortened the lag phase by 2 hours and also increased the final growth of the microorganisms.

Fatty acid composition of the lipids of *E. coli*

The lipids in bacteria are essential part of the structure of the cell membrane and are not merely a storage product of the cell. Previously it was shown that variations in temperature of incubation and in the composition of the medium altered the proportions of individual fatty acid in the lipids of *E. coli*.

In the present studies, gas liquid chromatography was employed for the identification and quantitative estimation of the component fatty acids in *E. coli*, (ATCC 9639) and its mutant *E. coli* 113/3 grown in media having different composition. Three different media described by Davis *et al.*, Burkholder and Roberts *et al.* were used for the growth of the organisms. The fatty acids present were mainly C₁₂, C₁₄, C₁₆, C₁₈, and C₁₈:1. Polyunsaturated fatty acids were absent. The variations in the composition of the medium showed some changes in the proportions of individual fatty acids in the lipids of the two strains of *E. coli*. But cystine when added as a sole source of sulphur instead of inorganic sulphur in the medium made a significant alteration in the composition of fatty acids in *E. coli* 113/3.

Studies in cystine metabolism in *E. coli* 113/3

Growth responses of *E. coli* 113/3 as a function of the concentrations of cystine as a sole source of sulphur was studied under both aerobic and semiaerobic conditions. Concentrations of 6 µg and 8 µg/ml. of the medium

gave maximum growth under both the conditions, respectively. The final growth under aerobic condition was less than that under semiaerobic condition (stationary condition). On the other hand, the standard medium containing inorganic sulphur gave maximum growth under aerobic conditions. Apart from this, the growth obtained with cystine as the sole source of sulphur was suboptimal under both aerobic and stationary conditions.

The growth response of *E. coli* 113/3 to graded doses of cystine added to the standard medium containing inorganic sulphur was studied. Cystine partially inhibited the growth of the microorganism. The degree of inhibition was more in the aerobic condition (shaking). The reversal of growth inhibition by the addition of certain metabolites such as glutamic acid, hydroxylamine hydrochloride, vitamin C, pantothenic acid, uridine and guanosine was studied. It was found that glutamic acid, hydroxylamine hydrochloride and vitamin C are much effective and brought about the reversal of growth inhibition to a great extent.

Synthesis of methionine, glutathione and coenzyme A by suspension of *E. coli* 113/3 in relation to vitamin B₁₂ :

The object of this study was to obtain a simpler system for the synthesis of methionine and sulphhydryl compounds by using exponentially growing cell suspensions with clearly defined reactions (precursors). In this system, the reaction mixture contained glucose, ATP, MgSO₄, phosphate buffer, hydroxylamine hydrochloride, cystine hydrochloride, glutamic acid, pantothenic acid and vitamin B₁₂. Vitamin B₁₂ decreased the rate of synthesis of glutathione and enhanced the synthesis of methionine and coenzyme A.

Effect of precursors of nucleic acids on growth :

The effect of supplementing the minimal medium with purine, pyrimidine, nucleosides and nucleotides on the growth of *E. coli* W and *E. coli* 113/3 was studied. The addition of uracil, uridine, cytidine, guanosine, uridylic acid and cytidylic acid enhanced the growth rate of *E. coli* 113/3. The addition of adenine, adenosine, guanine and guanylic acid in the medium lowered the growth rate. In the case of the wild strain, all the ten precursors viz., adenine, guanine, uracil, adenosine, guanosine, uridine, cytidine, guanylic acid, cytidylic acid and uridylic acid accelerated the growth rate under both the shaking and stationary conditions.

Effect of change of composition of the medium on the fatty acid composition of lipids in *E. coli* :

The effect of different nutrients on the composition of fatty acids in lipids of the two strains of *E. coli* viz., wild and mutant was studied. The cells of *E. coli* 113/3 were grown on glucose salt medium of Roberts *et al.*, Davis-Mingioli's and Burkholder's media. All these media were supplemented either with vitamin B₁₂ or methionine. Similarly, the cells were grown on glucose salt medium containing cystine as a sole source of sulphur in place of sodium sulphate. The strain W was grown on the glucose salt medium of Roberts *et al.*

The methyl esters of CHCl_3 : CH_3OH extractable lipids were analysed by Shandon FB-4 gas-liquid chromatogram (Isothermal) with flame ionisation detector. The stationary phase was diethyleneglycoladipate (15 per cent W/W) on gas chrome P. The results of the experiments are summarised below :

The commonly occurring fatty acids in the two strains were lauric (C_{12}), myristic (C_{14}), palmitic (C_{16}), stearic (C_{18}) and oleic ($\text{C}_{18:1}$). Palmitic, oleic and myristic acids were the major components of the extractable lipids of the two strains. The presence of cyclopropane acids was not detected in the strains grown in the different media. However, the presence of an unidentified component peak which had retention time longer than C_{16} and $\text{C}_{16:1}$ but less than C_{18} was detected in the cells grown in the medium containing vitamin B_{12} . This component is presumed to be C_{17} cyclopropane acid. The fatty acid composition of the cells of *E. coli* 113/3 varied to some extent according to the composition of the medium in which they are grown. The variation was only in the distribution of palmitic (C_{16}) and stearic acids. The changes in fatty acid composition produced by the incorporation of cystine as the sole source of sulphur replacing sodium sulphate in the glucose salt medium of Roberts *et al.* were very marked. The cells grown in this medium showed complete absence of oleic acid with subsequent increase in palmitic and stearic acids.

Effect of cystine on the growth of *E. coli* 113/3

The growth response of *E. coli* 113/3 to graded doses of cystine added to the standard medium containing inorganic sulphur was studied. The culture was grown for 24 hours at 37°C under shaking condition. Cystine inhibited the growth of *E. coli* 113/3. One microgram of cystine per ml. brought down the growth from (optical density) 0.68 to 0.24. At a concentration of $10\text{ }\mu\text{g/ml}$ of the medium, the growth was lowest and was equivalent to optical density 0.175.

Reversal of growth inhibition was tested by incorporation of certain metabolites such as glutamic acid, ascorbic acid, pantothenic acid and hydroxylamine hydrochloride in the medium to elucidate the metabolism of cystine. Glutamic acid, aspartic acid, and hydroxylamine HCl were more effective than pantothenic acid in reversing the growth inhibition of cystine in *E. coli* 113/3. However, these compounds were unable to counteract completely the effect of cystine.

Studies in protein metabolism of *T. pyriformis* W

T. Pyriformis W is a proteolytic protozoon. It can, therefore, grow on intact proteins by liberating and utilizing the necessary amino acids. Earlier studies indicated that the growth of the organism on different proteins varied. It was, therefore, felt that the ability of the organism to grow on a given protein may depend on its capacity to liberate amino acids, besides other unknown factors.

Work has now been undertaken to study the growth responses of this organism to intact protein, acid hydrolysate of protein and enzymic (pepsin and trypsin) digest of casein, whole egg protein and wheat gluten at different levels of nitrogen content. The work is in progress.

Microbiological evaluation of protein quality.

Common vegetable proteins are generally deficient in tryptophane and lysine. Since synthetic methionine and lysine are available at cheaper rates, their supplementation to vegetable proteins on a large scale for human consumption would be promising.

The effect of supplementing with synthetic amino acids such as methionine, lysine, tryptophane and threonine on the relative nutritive values of certain cereals and legumes was, therefore, investigated with *T. pyriformis* W. The food proteins investigated were rice, wheat, 'jowar', 'Shravan ghevda' and the processed seeds of 'gawar'. In addition, a few protein blends such as wheat flour plus groundnut flour (9:1 and 8:2), wheat flour plus copra oil meal (1:1), rice flour plus copra oil meal (1:1) and wheat flour plus leaf protein (9:1 and 8:2) were studied for their relative nutritive values.

The relative nutritive values of both rice and wheat increased significantly on lysine supplementation. Rice did not show improvement with lysine and threonine in combination, as with lysine alone. The nutritive value of 'jowar' was not improved by a single supplementation of either lysine, tryptophane and methionine. But combined supplementation with all the three amino acids improved the nutritive value of 'jowar' considerably. Shravan ghevda and 'gawar' seeds when supplemented with both tryptophane and methionine gave significantly higher relative nutritive value. However, lysine supplementation had no effect on the improvement of the quality of its protein.

The protein blends studied indicated that leaf protein is a better supplement to wheat flour than groundnut oil meal.

Toxicity of isolated proteins from 'gawar' meal

"Gawar" meal contains 48 per cent protein which is a by-product of the gum producing industry. The nutritive value of this waste was taken up for study.

It was reported last year that raw gawar (*Cyamopsis tetragonoloba*) meal when incorporated in the diet at 20 per cent protein level produced growth inhibition and mortality in young rats. The toxic factors present were destroyed by autoclaving for 30 mins/15 lbs pressure. It was, therefore, thought interesting to study further the nature of these toxic factors. In the first place, proteins isolated from 'gawar' meal were tested for their toxicity by feeding them to young weanling albino rats. Rats fed on a diet containing 25 per cent isolated protein as a sole source of nitrogen died within ten days.

Further feeding trials on adolescent rats given a basal diet complete with 20 per cent casein and 5 per cent isolated protein from 'Gawar' meal, were also carried out along with a control group on the basal diet without the test protein. The results showed that the isolated protein at 5 per cent level in a complete basal diet inhibited the growth of the animals. The toxic factors in 'Gawar' seem to be associated with the protein moiety.

Studies in lipid metabolism

The object of the scheme was to study the overall effect of dietary fats on different aspects of lipid metabolism. The dietary facts studied include groundnut oil (refined), hydrogenated oil, coconut oil (highly saturated) and safflower seed oil (highly unsaturated).

Previous report contained the work on (a) effect on the tissue lipid serum, liver heart and adipose tissues, (b) the esterase activity of intestines and pancreas were studied *in vitro* using stearic, oleic, linoleic and arachidonic acids.

Effect of dietary fats on oxidative phosphorylation and the possible physiological correlation of the same with the subcellular fatty acid spectra

Very little data is available on the effect of dietary fats on oxidative phosphorylation which is one of the important biochemical phenomenon in the cells. This study was made as follows :—

Two dietary fats namely safflower seed and coconut oils were given at 20 per cent levels in the diet of weanling male albino rats for a period of 8 weeks. Cholesterol (total and free), phospholipids and fatty acid patterns were estimated in serum, liver and heart. The cholesterol esters and phospholipid fractions were separated on silicic acid column and their fatty acid pattern checked by gas liquid chromatography.

P/O ratios were determined using glutamate, malate and succinate as substrates for liver mitochondria. Since the phospholipid is an integral part of the system, the fatty acid pattern of the mitochondria, and microsomes were also determined. The phospholipids were fractionated on thin layer chromatography.

The liver total fatty acid pattern, especially that of the subcellular particles seems to be related to the dietary fat. The fatty acid composition of the mitochondrial (liver) phospholipids also varied with the dietary fat. The oxidative phosphorylation (P/O) capacity is more in the unsaturated fat fed group as compared to the saturated fat groups for substrates like glutamate and malate. However, in the case of succinate no such change is noticed. These results probably suggest that changes in the phosphorylation capacity are due to changes in the mitochondrial lipids particularly the phospholipids.

Effect of dietary fats on liver enzymes.

Some of the enzymes which are important in lipid metabolism such as α -glycerol-P-dehydrogenases, glycerol kinase, citrate cleavage enzyme, malic enzyme, malate dehydrogenase and isocitrate dehydrogenase from the livers of rats fed various fats (coconut oil, safflower seed oil, hydrogenated vegetable fat, fat-free diet and fat-free plus cholesterol) for a period of 8 weeks were determined. These were estimated spectrophotometrically. The enzyme levels were all higher in the fat-free groups and low in the unsaturated fat fed groups, the saturated fat group being intermediate.

Relationship of carbohydrate and dietary fat on tissue lipids.

There is no data available on the role of dietary carbohydrates on tissue lipids.

Work has been undertaken to study the effect of sucrose and starch in varying proportions on tissue lipids.

Sucrose and corn strach were used as sources of carbohydrate with coconut oil (highly saturated) and safflower oil (highly unsaturated) as the dietary fat.

Groups of weanling male rats were fed diets containing the two dietary fats and varying amounts of sucrose and starch along with casein, vitamin and salt mixtures. The feeding experiment was conducted for a period of 8 weeks after which the animals were sacrificed. The analyses of serum, liver and heart for cholesterol (total and free), phospholipids and gross fatty acids pattern are underway.

DEPARTMENT OF BIOCHEMISTRY

BIOCHEMICAL STUDIES ON *V. CHOLERA***Studies on in vitro protein synthesis**

The incorporation of leucine-1-C-14 into protein was studied using sub-cellular systems of cholera organisms. It was seen that the supernatant enzymes of *El-Tor cholera* were more active than those of *V. cholera*, since in addition to the higher synthesis using its own system, they also enhanced the synthesis of protein in *V. cholerae*. However, these enzymes are less active than those of Salmonella organisms, viz *S. typhi*, *S. paratyphi A* and *S. paratyphi B*. This is confirmed by the fact that neither *V. cholerae* nor *El-Tor cholerae* supernatant could stimulate the protein synthesis in the three Salmonella organisms, whereas the supernatant enzymes of Salmonella organisms, especially *S. paratyphi A* stimulated the protein synthesis in the cholera organisms.

Synthetic ability of the three Salmonella strains

The Salmonella organisms namely, *S. typhi*, *S. paratyphi A* and *S. paratyphi B* are of special interest to this Institute with reference to the production of T.A.B. vaccine. A study of the chemistry, metabolism and the variation in the three strains of Salmonella organisms may perhaps be of some use in this direction.

Metabolic studies using the antibiotic chloromycetin

Studies on the growth kinetics of organisms in the presence of chloromycetin showed that para B is inhibited twice as much as the other two. 2.5 µg./ml. of this antibiotic produced about 80% inhibition in the case of *S. paratyphi B*.

Effect of chloromycetin on the incorporation of phenylalanine

Incorporation of phenylalanine into the proteins was studied at log phase in the presence of chloromycetin. Incorporation of this amino acid was inhibited more by *S. paratyphi B* as indicated by the total and specific activities.

Effect of chloromycetin on the incorporation of adenine and guanine

Incorporation of adenine and guanine into the cells was carried out at intervals of 0, 3, 6, 9 and 12 hours of the growth of the organisms using 2.5 µg./ml. of chloromycetin under stationary and shaking conditions. The treated cells showed higher incorporation of these nucleic acid precursors during the early stages of the growth.

Effect of chloromycetin on the RNA. subfractions

Studies on guanine-8-C-14 incorporation into soluble RNA and ribosomal (16S and 23S) RNA revealed that the chloromycetin treated cells have higher specific activity in all the sub-fractions, thus indicating that the RNA synthesis is enhanced by the drug. Further, it was observed that this enhanced activity is much more in the ribosomal RNA fractions. In all these studies, *S. paratyphi B* showed maximum activity.

Effect of chloromycetin on the metabolism of glycine and aspartic acid

Good inhibition was observed in the case of glycine incorporation into nucleic acid and proteins. The same amount of chloromycetin caused comparatively less inhibition of aspartic acid utilization. The specific activities of the various fractions—acid soluble RNA, DNA and proteins were compared in normal and treated cells. Only the RNA fraction of treated cells showed higher specific activity, while it was lower in all the other fractions. However, the protein synthesis was inhibited much more than the other macromolecules. This effect was more prominent in the case of *S. paratyphi B*.

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

In vitro protein synthesis in the Salmonella

Studies on the synthetic ability of *S. typhi*, *S. paratyphi A* and *S. paratyphi B* were carried out using cell-free protein synthesizing systems namely (a) the ribosomes (to provide the platform for the protein synthesis) and (b) the supernatant fraction (1,05,000 g.) containing the messenger RNA, a primer, soluble RNA and peptide synthesizing enzymes from the three organisms. A mixture of 19 natural 'cold' amino acids and one 'hot' residual unit (leucine-1-C-14) along with a source of energy supply (ATP and ATP generating systems), GTP (for peptide bond synthesis) was used. The ribosomes and supernatant enzymes from the three organisms were exchanged in order to study the compatibility of the homologous and heterologous systems for protein synthesis. The studies indicate that there is a definite difference in the concentration of the protein synthesizing enzymes in these three organisms and that the *S. typhi* seems to have the maximum concentration of the enzymes.

Serum amino acids and proteins in children suffering from Kwashiorkar and marasmas

Protein-caloric malnutrition of early childhood is a generic term including Kwashiorkar and marasmas. Serum samples of some malnutrition cases of children from the Sion Hospital were analysed for serum proteins, free- α amino N, free amino acids—essential and non-essential.

There was much variation in total protein values, while albumin values were lowered and globulins elevated. The α -amino N content of serum in these cases was lower as compared to normal (8-10 mg%) The ratio of non-essential to essential aminoacids was found to be about 3 in both Kwashiorkar and marasmic cases. Other workers abroad have reported values ranging from 5-10.

DEPARTMENT OF PHARMACOLOGY

Two more techniques for the evaluation of new drugs were added to the battery of tests performed in the department e.g. testing of diuretics and testing of antileprotic drugs using *in vitro* methods. A modest beginning was made for toxicological evaluation of new and investigational drugs.

Diuretic activity of some synthetic diuretic agents

Synthetic derivatives of S-triazine and thiodiazole were prepared by the Chemotherapy Department of this Institute. They were screened for their diuretic activity.

Thirty-five compounds from these two series were tested in rats. Four compounds, viz R-1966, R-1998, R-2006, R-2117 and R-2121 showed good activity. Further screening of new compounds is in progress. The compounds which have shown promising activity have to be further screened in dogs to explore the full range of action.

Screening of drugs for their antidiabetic activity synthetic drugs

A large number of synthetic compounds (sulphonylureas and other compounds) have been studied for their hypoglycaemic activities, but none have proved an ideal drug. Even well-known compounds have been shown to possess some side reactions. We have undertaken the study of oral synthetic antidiabetic compounds to get a substance with greater activity and least toxicity.

About 40 compounds of triazole and triazine (synthesised by Chemotherapy Department) have been tested for their antidiabetic activity in experimental animals. Out of these, about a dozen substances were found to possess good hypoglycaemic activity. Among the active compounds two were found to be better than the well-known compound tolbutamide in certain respects when tested in normal animals or in diabetic animals. In normal rats, the activities of the two compounds were higher than those in rabbits. Results showed that the hypoglycemic activity lasted much longer than that of tolbutamide. Acute toxicity (oral) in albino mice for the two compounds indicated that these compounds were less toxic than tolbutamide. In alloxanised rats, unlike tolbutamide the two compounds have moderate hypoglycemic activity.

Caffeine and antidiabetic compounds

Majority of people in the world consume caffeine containing beverages daily. Caffeine alone has been shown to be mildly hyperglycemic. It was, therefore, of interest to study the effect of caffeine on well-known antidiabetic drugs, viz. tolbutamide, chlorpropamide and phenformin.

A low dose of caffeine showed some beneficial effect on the hypoglycemic activity of antidiabetic compounds in normal experimental animal. On the

other hand, a high dose of caffeine exerted a definite adverse effect in both the species of animals tested, viz., rabbits and rats.

Chronically caffeine treated rats (normal) showed significant adverse effect on hypoglycemic activity of tolbutamide and chlorpropamide but not on phenformin.

Caffeine did not exhibit any effect on hypoglycemic activity of tolbutamide and phenformin in alloxan diabetic rats.

Effect of antidiabetic compounds, when tested on caffeine diuretics in rats did not show any adverse or beneficial effect.

Toxicological evaluation of new and investigational drug.

Many drugs are synthesised in the Institute and some of them have shown promising activity. Some of the pure fractions from indigenous drugs have also shown good results. Before clinical trials can be undertaken it was essential to evaluate the drug from toxicological aspects.

Chemo-R, 1238 was shown to be effective as anticancer agent. Preliminary pharmacological studies were undertaken and it was shown that the drug did not cause any appreciable changes in blood pressure and respiration on anaesthetised cat. It did not exhibit antiacetylenoline, antihistamine and antirenaline like activity.

Diuretic activity of two indigenous plants—*Musa paradisiaca* Linn.

Though there are potent diuretic agents available for use, there is still demand for better agents with less effects.

Various extracts of *Musa Paradisiaca* Linn. and *Celosia argentea* Linn. were tested for their diuretic activity in rats using excretion of sodium as an index for their activity. The alcohol extract of bark of *Musa Paradisiaca* Linn and chloroform extract of *Celosia argentea* Linn. showed promising results. Further work to isolate the pure fractions and to examine the full range of diuretic activity is under progress.

Screening of drugs for their antileprotic activity

There is still a demand for a more effective drug than DDS. Also there are fears of resistance being developed to DDS.

M. leprae (ICRC bacilli) was grown in liquid medium and the inhibition produced by acidino-diamine compounds prepared by Dr. S. M. Deshpande of Benaras Hindu University was observed and compared with that of the standard drugs. The work is under progress.

Screening of drugs for their antidiabetic property—indigenous drugs

In the literature on Indian medicine a number of plants are mentioned as useful in diabetes mellitus. Although some work has been done in our country, there is still a good scope for investigating plants for antidiabetic activity. Screening of plant extracts for this type of activity has been continued.

Extracts of plants were prepared by using ether, alcohol or water and concentrated under reduced pressure. The residue was suspended in gum acacia and orally administered to experimental animals. The percentage of fall in blood sugar was noted as expression of hypoglycemic activity.

During the year under review, the following plants were tested : IDR 2 (Kelful), IDR-29 (Katuka) IDR-30 (Aragvadhya), IDR-31 (Vridha) and Nagkeshar. Significant hypoglycemic activity was obtained only with preparations for two plants. viz., IDR-2 and IDR-29. Other plant extracts showed practically no activity.

Antibacterial activity of Indigenous Medicinal Plants

Various plant species are used in the indigenous system of medicine. Some selected species from Ayurvedic, Unani systems etc., were used for the preparation of extracts whose antibacterial and antifungal actions were studied.

The alcoholic extract of *Nyctanthes arbortrists* inhibited *V. cholerae* and *Shigella shiga*. The water soluble fractions of the above extract inhibited *S. aureus*, *V. cholerae* and *Sh. shiga*.

Asparagus racemosus : Various extracts were prepared and tested. All were found to be ineffective.

Caesalpinia sappan : Activity against *V. cholerae*, *Sh. shiga* and *S. aureus* was detected in alcohol and water extracts.

Mesua ferrea : Petroleum ether and methanolic extract of flowers inhibited *S. aureus* and *V. cholerae*.

Fraction CD of *Ensete superbum* inhibited *S. aureus* and to a very slight degree *V. cholerae*.

Withania Somnifera : The reinickate salts inhibit *S. aureus*, *V. cholerae* and *Sh. shiga*.

Activity of some of these against *Candida albicans* and *Microsporon canis* was also tested. Alcoholic ext., glycoside of CDR-21 and *Ensete superbum* (GD), were found to be active against *Candida albicans*. Gly. fraction alcohol and petroleum ether extracts of CDR 21, Kurchi HC 1, were active against *Microsporon canis*. (berberine hydrochloride was tested against these two organisms and was found to be active).

DEPARTMENT OF VIROLOGY

Tissue culture studies in rabies

The cultivation of rabies virus in hamster kidney tissue cultures was continued. Methods of demonstration and titration of the virus are being investigated in an effort to circumvent the laborious and time consuming methods of mouse inoculation.

Assessment of rabies virus in HKTC by demonstration of interference with a CPE producing virus

This is a preliminary study to establish techniques for the assessment of rabies virus in tissue cultures, which can subsequently be applied to research work on tissue culture adapted virus. (See previous report). The CVS strain of rabies (derived from mouse brain) has been adapted to HKTC using methods previously described. At the 6th and 7th passage in HKTC, the harvested fluid produced symptoms of rabies in inoculated mice. Titration of this HKTC virus is being undertaken. Three enterovirus strains, Coxsackie A₉, B₂ and B₅ (derived from MKTC) and a vaccinia strain from calf lymph, have been adapted to HATC for possible use as indicator virus. All showed marked CP changes in HKTC. Adaptation of Paris strain fixed virus (from sheep brain) to HKTC, grown in a defined medium, is under way.

Rabies pathogenesis

Two approaches are at present being studied. Histological changes in the brown fat, which is considered a possible target organ for the virus, are being studied from the day of infection to the day of death with parallel estimations of virus content in this tissue. Another approach is the attempted simulation of the human condition by prolonging the "incubation" period of the disease in laboratory animals. This would allow more time to set up experiments for identification and localization of the virus during the latent period. Such methods may, with suitable modifications, be useful in determining (a) whether the virus can be attacked during this phase of its existence, and (b) whether a bitten person or animal is harbouring the infection or not i.e., whether antirabic treatment is required or not.

Role of brown fat in pathogenesis of rabies.

In a preliminary study on the brown fat, in rabies infected mice, it was found that progressive histological changes were seen in this tissue from 48 hours after infection onwards i.e., long before rabies virus can be demonstrated or any symptoms of rabies appear. In order to establish the relationship, if any, between these histological changes and the rabies infection, a repeat study was made using three groups of mice. Group I—infected intramuscularly with approximately 10LD₅₀ fixed rabies virus. Group II—control mice inoculated I.M. with equivalent quantity of Semple's vaccine (to study the response of brown fat to non-infective antigen), and Group III—control uninoculated mice of the same age and weight. Two mice from each group were sacrificed every

24 hours and pools of their tissues studied for histological changes and for presence of virus. Details of the procedure have been given in the previous report.

While detailed study of all the histological sections is yet to be completed, a preliminary sampling shows that progressive histological changes occur in the brown fat of infected mice as follows:—

Reduction of individual cell size with relative increase of the nuclear size; the large vacuoles of the cells of normal brown fat give way to multiple small vacuoles, or to a granular texture of the cytoplasm, and there is increase of congestion and of extravasation of red cells. Similar though less marked changes are seen in the brown fat of the vaccine infected animals upto 3 days, after which the histological changes appear to regress. No such changes were found in control uninoculated mice. A detailed analysis will be presented when the study is completed.

Prolongation of incubation period of rabies in small animals

When antirabies serum was first introduced for prophylactic treatment of rabies it was shown, in guinea pigs, that administration of the serum to infected animals resulted in prolongation of the incubation period, but that the animals finally succumbed to the infection. Though this was a highly artificial model it was felt that prolongation of the incubation period in this manner might enable us to devise experiments for identification and localization of the virus in such infected animals. Mice were used in the first instance for reasons of cost. Groups of mice were infected with rabies fixed virus followed, after an interval, by antirabies serum. Earlier experiments were inconclusive and it was found necessary to standardise and balance the dose of virus and antiserum used. Twelve tests were carried out, 3 tests each using sheep brain, rabbit brain and mouse brain virus respectively.

Groups of 12 mice each were inoculated subcutaneously with 10 fold dilutions (10^{-1} , 10^{-2} and 10^{-3}) of each virus (10^{-1} dilution represents approximately $20LD_{50}$ by this route). Six mice in each group were kept as virus controls. Thirty minutes later, each of the 6 remaining mice in each group was given 10 units antirabies serum intraperitoneally. All the control mice and approximately 50 per cent of the test mice (treated with antiserum) died of rabies. The study is being continued.

A study of interference produced by rabies virus in different host systems

Rabies virus has been reported to produce interference with other virus infections, and to be an efficient producer of interferon. The study which has just commenced, proposes, initially the use of HEP flury virus in chick embryo, or in tissue culture, as an interfering virus to NDV or other myxovirus agents. If successful, it is proposed to prepare purified interferon by the method of Lampson *et. al* for further study.

Interference phenomena in RSV infection

RSV is a complex virus requiring certain prerequisites (helper viruses etc.) to produce infection. An effort is being made to study this phenomenon, using rabies or non arbovirus agents, for the production of interference. A preliminary study in 1-3 day old chicks showed that while tumours were produced in all chicks infected directly with RSV only 50 per cent of birds developed tumours, if they were pre-infected with rabies fixed virus. The mechanism of this blockade phenomenon is under further study.

Influenza

Surveillance studies on cases of upper respiratory infections were continued. As in previous years, the bulk of the cases were those referred from the staff clinic of the Institute. In September and October 1968 the number of cases showed a sharp rise and a number of influenza virus strains, all except one resembling the A₂/Hong Kong/68 virus were isolated. The single exception was an infection with Influenza Type B. Reports received confirmed the existence of an epidemic outbreak, and a vaccine incorporating the A₂/Hong Kong/68 and A₂/India/57 antigens, was prepared as an emergency measure. The epidemic was short lived. Cases declined sharply from November 1968 and no further isolations were made.

Isolation of influenza virus and serological studies of patients

A total of 19 throat washings from suspicious cases were processed for influenza. Of these 17 were received during the epidemic period (September and October 1968). Fourteen isolations of influenza were made. Eleven isolations were found to resemble the A₂/Hong Kong/68 virus and one was a type B virus. Two recent isolates are still to be typed. These 11 patients, as well as clinical cases from whom no isolation was made, showed a rise of haemagglutination inhibiting antibodies to the A₂/Hong Kong/68 virus, and of CF antibodies against the Group A antigen. Many of the cases also showed moderate rise of H. I. antibody against older A₂ virus strains, notably the A₂/Taiwan/64 strain suggesting that the Hong Kong strain is a variant A₂ strain and not a new antigenic type. It was noted that while cases occurring early in the epidemic showed a rise in H. I. antibodies against the A₂/Hong Kong/68 strain to almost the same degree as to their own isolates, the later cases showed a more marked response against their homologous isolates. These findings suggest that the A₂/Hong Kong virus was not a stable variant and may explain the rapid decline of the epidemic in Bombay.

Study of the characters of A₂/Hong Kong/68 type virus strains isolated in Bombay

The characters of the strains isolated were studied for comparison with older strains of influenza virus.

Agglutination.—The Hong Kong isolates studied were found to agglutinate the following cells: fowl, guinea pig, rat, monkey, hamster and human "O" group. No agglutination was seen against mouse, sheep, horse and rabbit erythrocytes.

Ether sensitivity.—The strains were ether resistant.

Heat.—The strains were heat stable after exposure to 56° C for $\frac{1}{2}$ hour.

Antigenic analysis.—Neutralization was found with antisera against A₂/India/47/57 and to a greater extent, antisera against A₂/Taiwan/64. The Hong Kong virus appears to be an A2 variant with antigenic similarities to the 57 and the Taiwan viruses.

Serological survey of the population of Bombay for influenza antibodies

Sera collected from different age groups in Bombay City during 1967 were tested for antibodies to various strains of Influenza A and B. The numbers of sera tested were as follows :—

0-6	months	..	50
1-12	months	..	50
1-3	years	..	50
Maternal and cord blood		..	100 pairs
Adults	..	..	150

The sera were tested for H. I. antibodies, titres of 1 : 8 or higher being considered positive. No significant differences could be noted between the different age groups, as the numbers positive were too small for analysis. The sera tested showed presence of antibodies as given below :—

A ₂ /Taiwan/64 ..	..	..	40.6 per cent.
A ₂ /Tokyo/67 ..	..	..	37.0 per cent.
A ₂ /India/47/57 ..	..	..	16.0 per cent.
A ₂ /Hong Kong/68 ..	..	..	6.6 per cent.
B/Singapore/64 ..	..	..	34.0 per cent.
B/Massachusetts/66 ..	..	..	27.0 per cent.

All sera were devoid of antibodies against A classic and A prime virus strains.

Note.—It was found that the A₂/Hong Kong/68 virus is affected by non-specific inhibitors to a very great extent (see below) and these results will have to be confirmed by more precise techniques.

Thirty per cent of cord blood showed lower titres than the corresponding mother's blood (2 to 4 fold). The remaining pairs showed identical titres.

Elimination of non-specific inhibitors in sera to A₂/Hong Kong type influenza virus strains

Preliminary HI tests against the Hong Kong type isolates tested at allantoic passage 2 or 3 showed unexpectedly high HI titres in both acute and convalescent sera. Similar inhibition was found with sera of various animal species tested. This inhibition was attributed to the marked sensitivity of these virus strains to non-specific inhibitors which were not removed by periodate treatment. Virus strains at the 6th allantoic passage level were then used for the test. More consistent results were obtained, but non-specific inhibition was still present to a marked degree. Various methods of treating the sera were attempted viz. trypsin and periodate (as recommended by the World Influenza Centre), heat,

cholera filtrate and kaolin. No method was found to remove the non-specific inhibitors completely, but treatment with kaolin was found to give the best results, on preliminary tests. The kaolin method was therefore used in subsequent tests and is being studied with a larger number of sera from human and animal sources.

The Enterovirus Unit continued the studies in progress on epidemiology of poliomyelitis and enterovirus infections.

Epidemiology of poliomyelitis in Bombay

Poliomyelitis in Bombay this year attracted the public health authorities because of the unusually high incidence of paralytic polio in Greater Bombay. The need for prevention of paralytic poliomyelitis was acutely felt by all concerned.

During the period under report (April 68—March 69) 1030 cases of paralytic poliomyelitis were recorded by the Enterovirus Laboratories (Table 2).

Epidemiological studies carried out indicated that there has been no shift in the age as well as seasonal pattern of the disease. The paralytic disease was mainly restricted to children below 3 years. As many as 92 per cent of the cases were observed in children below 3 years. The highest number of cases were recorded during the month of July (Table 2). The geographical distribution of the cases indicated that this year larger number of cases were recorded in the suburban community as compared to the metropolitan area.

Result of virological studies carried out on material collected from cases of poliomyelitis are shown in Table 3.

Poliomyelitis in Ceylon

At the request of the W.H.O., facilities for virological and serological studies were extended to Ceylon. During the period under report materials from 287 cases of poliomyelitis were referred to the Enterovirus Laboratories. The results of virological studies carried out so far are shown in Table 3. It is interesting to note the predominance of type 3 poliovirus in material from Ceylon as compared to the results from Bombay wherein type 1 poliovirus constituted an overwhelming majority.

Aseptic meningitis

To study the possible role of enteroviruses in the etiology of aseptic meningitis, work has been continued. Results of virological studies are shown in Table 4.

Study of paralytic illnesses among vaccinees

Introduction of oral polio vaccine for mass immunization has brought to light two problems viz. problem of failure of vaccination and incidence of paralytic illness amongst individuals successfully vaccinated with oral polio vaccine. Both these problems are now known to occur in every part of the world where oral polio vaccine has been used on a large scale. Failure of immunization as demonstrated by seroconversion studies has been particularly noted in tropical and under developed countries.

During the course of laboratory studies dealing with materials received from Ceylon it was noted that a significant number of children had developed paralytic illness in spite of administration of oral polio vaccine. Up to the present time the entervirus unit has received materials for virological and/or serological studies from 509 suspected cases of paralytic poliomyelitis. Of these, as many as 153 cases have had oral polio vaccine (1 dose—70 cases, 2 doses—36 cases and 3 doses—44 cases; in 3 cases details were not known). Of the 153 cases referred to above, materials for virus studies were available from 102 cases. Polio virus was isolated in 33 (type 1—10, type 2—12 and type 3—11) while a non-poliovirus was detected in 34 cases. No virus could be isolated from 35 cases. The serological studies have been carried out on 62 cases, who were vaccinated months or years before the on-set of illness. The sera were collected, in most cases within 10 days of on-set and results are shown in Table 5. It will be seen that in a significant number of cases the vaccinees have failed to respond to the antigens administered to them.

Survey of measles antibodies in the population of Bombay

The study on antibodies against measles in different age groups was undertaken to collect essential information about this disease e.g. age of exposure, antibody levels in adults etc., as described in the previous report. In addition batches of gamma globulin prepared at the Institute from pooled normal adult sera, were tested for antibodies to see if they could be utilized in place of immune sera if need arose. A total of 500 sera was tested for NT, HI and CF, antibodies to measles as follows: 100 from normal adults, 100 pairs from mothers and cord blood (new born) 200 from children of various ages. Antibodies were found in nearly all adults and a significant proportion of children over 3 years showed the presence of antibodies, cases in each age group showing high antibody titres are shown in Table 6.

Twelve gamma globulin samples tested showed high antibody titres (Table 7), which make them suitable for use in situations where passive immunization against measles is required.

TABLE 7
Antibody titres of commercial human globulins

No. of batches of commercial human globulins tested	Antibody titres	
	HAI	CF
Gamma globulin—		
4	1 : 640	1 : 128
1	1 : 640	1 : 256
4	1 : 320	1 : 64
Placental globulin—		
2	1 : 160	1 : 32
1	1 : 320	1 : 64

The Institute undertook serological studies for a trial of measles vaccine to be carried out at three centres in India, viz., Bombay, Indore and Trivandrum. Six hundred vials each of vaccine and placebo, using a coded numbering system, were distributed to each centre for use in vaccination of children between 6 months and 2 years of age. Pre-vaccination and post-vaccination (1 month) blood samples were collected from each child vaccinated, the sera separated, and the paired samples sent to this department for testing along with the patient's case record. Some paired sera have been received and are being tested. The trial is still continuing.

Chemotherapy and chemoprophylaxis of vaccinia and variola

A number of compounds, derived from indigenous drugs supplied by the Department of Pharmacology, were tested for antiviral activity against vaccinia virus in eggs. Twelve day old eggs were inoculated on the chorioallantoic membrane (CAM) with virus (diluted to give 100 PFU/per inoculum) and drug mixtures in different dilutions. Virus controls for infectivity and drug controls for toxicity were run with each batch, 5-6 eggs being used for each substance or mixture. The virus used for all tests was vaccinia virus of second passage in MKTC, which has been found (see previous reports) to show consistent infectivity titres and is very stable on storage at -20°C. Virus derived from CAM suspension of infected eggs was also tested but the results were not consistent, and the tissue culture virus was preferred for subsequent tests. Pock counts were made on a special illuminated glass plate divided into squares for ease of counting. Ten compounds (fractions from *Banakadali*) tested during the year, showed no antiviral activity. A crude extract (VIDR-2T), previously found active was retested to confirm the validity of the technique. This compound showed antiviral activity in the expected dilutions. Some of the extracts prepared for test were insoluble in water and were dissolved in Tween 80. A Tween 80 control was included in these tests, and it was found that Tween 80, alone showed slight, though consistent, antiviral activity.

Studies are also being made on MKTC, HKTC and chick embryo tissue culture to see if a more reliable test can be devised in these systems, thus obviating the use of eggs.

Studies on rats as possible vectors of human virus infections

A large number of rats, trapped in human habitations were available to us in connection with other studies. Very little data is available on virus infections in rats, which by their habits of contaminating food stores etc. may be in a position to transmit infection to human beings. A study was undertaken to discover if these rats harbour any viruses of importance to humans.

Rats were collected from houses in rural and urban areas, and pooled tissues from pairs of rats were collected for processing and for microscopic examination. Each rat was bled before it was killed and the serum stored for antibody studies at a later date. The tissues collected were (1) brain, (2) brown fat, (3) lung, (4) spleen and (5) intestinal contents. Suspensions

of each pooled tissue were passed in adult mice, infant mice and in MKTC for evidence of any virus pathogenic to these host systems. (Details were given in the previous report).

Work done so far is summarized below : Three hundred and twenty five specimens have been collected from rural rats and two hundred from urban rats. Two hundred and ten specimens have been studied so far for agents pathogenic to infant mice, adult mice or MKTC. Eighty-one strains pathogenic to one or more of these systems have been isolated, while 39 specimens are still under process.

Anti sera have been prepared in rabbits against three of isolated strains. Thirty-two strains were studied for heat inactivation test. Sixty-five were resistant to 56°C for $\frac{1}{2}$ hour and 30 were resistant to 37°C for 1 hour. Nine strains were studied for ether sensitivity. All were resistant. Thirty-two strains were studied for bile salt sensitivity. All were resistant.

Some of the strains appear to resemble the EMC virus which is a common virus of wild rodents. A standard strain of EMC virus and a local strain isolated by the Virus Research Centre, Poona, have been obtained for comparative study.

Study of rabies in normal dogs for immune or passive carrier state

A study was commenced on normal dogs obtained from the Municipal Kennels in Bombay which are scheduled for destruction. The dogs were kept for a week and daily collections of saliva were made by swabs held in the cheek pouch. This was eluted in cold 2 per cent horse serum saline, treated with antibiotics, and the eluate was inoculated into 6 infant mice intracerebrally. Simultaneously a bacterial sterility test was put up of the inoculum.

The dogs were kept under observation for a week longer, to rule out the possibility of their being in the pre-rabid state, when salivary virus may sometimes appear. They were then killed and the blood, the brain and salivary gland collected. The blood was stored for later antibody study and the brain and salivary gland were processed by routine methods for rabies virus by inoculation of infant and adult mice. Samples from 10 dogs are under study.

It is proposed to study 100 dogs in this way and then to assess the results for planning further studies.

TABLE I
Antibody Levels in Paired Sera of Influenza Patients

TABLE
Antibody levels in Paired

Patient	HAI Titres					
	A2/India/ 47/57		A2/Taiwan/ 64		A2/Tokyo/ 3/67	
	A	C	A	C	A	C
K.N.P., B/Bombay/21-68	8	8	32	32	16	16
L.R., A2/Bombay/23-68	64	64	32	128	8	16
M.C., A2/Bombay/24-68	8	16	16	32	8	8
S.N., A2/Bombay/25-68	..	8	..	512	..	8
G.N.K., A2/Bombay/27-68	8	8	32	128	8	8
S.I.N., A2/Bombay-28/68	8	16	8	16	8	8
G.S.S., A2/Bombay/29-68	8	8	16	64	8	8
V.S.S., A2/Bombay/30-68	8	8	32	64	8	8
P. V. G., A2/Bombay/32-68	..	16	..	32	..	8
P. K. S., A2/Bombay/33-68	8	16	8	128	8	32
A.N.D.N., A2/Bombay/35-68.	8	8	8	32	8	8
Mrs. R. K. 	..	8	..	256	..	8

No antibody detected for A (classic) PR₈ (1934), A prime—FM₁ (1947), B-Lec (1940).

1
Sera of Influenza Patients

A2/Hongkong/ 1/68		A2/Bombay/ 23/68		Homologous isolates		CF titres			
						A		B	
A	C	A	C	A	C	A	C	A	C
8	8	8	8	8	256	8	8	8	64
8	256	8	256	8	256	8	32	8	8
8	256	8	128	8	256	8	128	..	..
..	128	..	128	..	256	..	64	..	8
8	128	8	128	8	128	8	64	8	8
8	512	8	128	8	256	8	32	8	8
8	256	8	128	8	256	8	128	8	8
8	256	8	256	8	256	8	64	8	8
8	256	..	128	..	256	..	64	..	8
16	256	16	256	16	256	8	32	8	8
8	64	8	128	8	256	8	16	8	8
..	128	..	1,024	..	..	..	128	..	8

TABLE 2

Pollomyelitis, Greater Bombay, April 1968—March 1969

Month	Total No. of cases	Age distribution			
		1 year	1-2 years	2-3 years	3 years
April 1968	55	27	17	8	3
May 1968	81	34	26	14	7
June 1968	161	68	66	17	10
July 1968	267	108	105	32	22
August 1968	216	93	82	29	12
September 1968	100	40	40	13	7
October 1968	58	17	23	9	9
November 1968	30	14	11	2	3
December 1968	21	8	7	3	3
January 1969	16	5	4	5	2
February 1969	10	3	3	3	1
March 1969	15	5	5	2	3
Total	1,030	422 (40.9%)	389 (37.8%)	137 (13.3%)	82 (8.0%)

TABLE 3
'Poliomyelitis, Virus Studies, April 1968—March 1969

Sample		Total No. collected	Total No. tested	Total No. positive	Poliovirus type			Coxsackie	ECHO	Untyped
					1	2	3			
Greater Stool Bombay	..	657	476	266	148	0	12	2 (A ₃ —1) (B ₄ —1)	1 (Echo 7)	103
Rectal Swabs	..	642	591	245	137	2	8	2 (B ₁ —2)	1 (Echo 7)	95
Ceylon Stool	..	287	173	112	35	2	52	3 (B ₃ —2) (B ₅ —1)	0	16

TABLE 4
Aseptic meningitis virus studies, April 1968—March 1969

Sample						Total Collected	Total tested	Total positive	Identification
C.S.F.	..	..	..	..	..	30	30	3	Polio 1 (2) Unidentified (1)
Rectal swabs	..	..	..	..	..	32	32	10	Coxsackie B3 (1), Polio (1+2+3)—1, Unidentified(8)
Throat swab	..	..	..	..	..	29	29	4	Coxsackie B4 (1), Unidentified (3)
Blood	..	..	..	..	..	26	26	4	Polio 1 (1), Polio 3 (1) Unidentified (2).

TABLE 5

Studies of paralytic illness among vaccinees Serological studies (ceylon)

H/o Oral Polio vaccine		1 dose	2 doses	3 doses
Serological Pattern				
Absence of Type 1 only	..	1*	4	1
2 only	..	1	0	1
3 only	..	1	0	2
Types 1+2	..	0	1	2
2+3	..	6	2	0
1+3	..	6	6	4
Types 1+2+3	..	8	1	4
Presence of types 1, 2 and 3	..	6	3	2
Total	..	29	17	16

*Figures refer to number of cases

TABLE 6

Number of persons showing high antibody titers against measles.

Age group	No. with high antibody titer (number tested)	Titers above		
		HI	CF	NT
0 to 1/2 year	.. 6/50	.. 1:40	1:4	1:32
1/2 to 1 year	.. 3/50	.. 1:80	1:8	1:128
1 to 3 years	.. 12/50	.. 1:20	1:4	1:16
3 to 5 years	.. 14/50	.. 1:20	1:4	1:16

