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ABSTRACTS OF PAPERS PRESENTED AT THE SCIENTIFIC SYMPOSIA

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SUNDAY 11 JAN. 59

14-30 hrs SYMPOSIUM ON PLAGUE
to
16-30 hrs

MONDAY 12 JAN. 59

09-00 hrs SYMPOSIUM ON CHOLERA
to
12-30 hrs

TUESDAY 13 JAN. 59

09-00 hrs SYMPOSIUM ON NEUROTROPIC VIRUSES
to
11-00 hrs
11-15 hrs }
to }
12-30 hrs }
and } SYMPOSIUM ON INFLUENZA
14-30 hrs }
to }
15-15 hrs }

WEDNESDAY 14 JAN. 59

09-00 hrs SYMPOSIUM ON INSECTICIDES
to
11-15 hrs

11-30 hrs SYMPOSIUM ON SNAKES AND VENOMS
to
12-30 hrs

I. SYMPOSIUM ON PLAGUE

1. The Pathogenesis of Tonsillar Bubonic Plague in Primates after Exposure to Primates with Pneumonic Plague

K. F. MEYER,

George Williams Hooper Foundation
University of California
San Francisco.

Exposure experiments were carried out in order to learn whether healthy primates in direct (18 animals) or indirect (8 animals) contact with other primates sick with pneumonic plague and known to be exhaling *Pasteurella pestis* would contract the infection. Sixteen of the exposed animals succumbed to septicemic plague, and only 3 to pneumonic plague, after only 24 hours of exposure to the dying cagemates.

At autopsy of the 16 animals, the superficial and deep cervical lymph nodes were haemorrhagic and imbedded in edema, and although there was congestion and edema in the lungs, there was no evidence of pneumonia. The lymphatic tissue forming the ring of Waldeyer was the apparent portal of entry. Enormous masses of plague bacilli were embedded in the severely altered lymphoid tissue on one side, rarely on both sides, of the tonsillar sinus. The so-called tonsillar lymph nodes adjacent to the diseased lymphoid tissue invariably had the characteristics of typical primary buboes. The gastro-intestinal lesions in 2 animals seem to have been caused by ingestion of clumps of bacilli from the epithelial layer of the pharynx. The bacteremia in the group was intense and began shortly after the appearance of symptoms.

Additional studies suggested that the size of the inhaled particles or clumps of bacilli determined whether the course would be cervical bubonic and septicemic or pneumonic. If the particle size were less than 5 microns they were implanted directly into the lungs; if more than 5 microns they were impinged on the mucus overlying the ciliated epithelium and were wafted to the oropharynx where they found a favourable environment in the lymph tissues.

2. Experimental Respiratory Infection with *Pasteurella Pestis* and Observations on Immunity to Plague

D. W. HENDERSON,

Director, Microbiological Research Establishment
Porton, England.

Two forms of plague, both originating in the respiratory tract of the guinea-pig, can develop according to the size of the particle containing *Pasteurella pestis* presented to the host. Small particles initiate a bronchopneumonia which leads to septicaemia and death. Large particles establish a septicaemia and death results more quickly without the development of a pneumonia.

Cross infection to normal animals occurs irregularly when they are exposed to others developing plague by the respiratory route. Such an incident is rare when the initially infected animals are exposed to large particles. Cross infected animals suffer from the disease characteristic of exposure to large particles. Attempts to establish an epizootic by cross respiratory infection have so far failed, due in measure, it is believed, to the type of disease developing in first cross infections.

A pre-existing respiratory infection can interfere with the development of a superimposed infection with *Pasteurella pestis* but this only occurs where attempts to infect are made via the upper respiratory tract.

Preliminary experiments on immunity to respiratory infection with *Pasteurella pestis* will be described and observations will be made concerning the relative importance of various antigenic factors so far found in *Pasteurella pestis*.

3. Virulence determinants in *Pasteurella pestis* and *Past. pseudotuberculosis*

T. W. BURROWS,

Microbiological Research Establishment
Porton, England.

Strains of *Past. pestis* which show maximum virulence for both mice and guinea-pigs are genotypically Fl + VW + P +. These symbols refer respectively to potentialities for the production of fraction 1 antigen as an envelope, the production of V and W antigens and the development of pigmented colonies on certain defined media. In addition to these potentialities the organism must be capable of synthesising purines (Pu+). The determinants are autonomous so that any one or more may be spontaneously lost independently of the others. As a result cultures may, under laboratory manipulations, become different from original isolates with respect to virulence and immunogenicity. Simple tests *in vitro* permit the recognition of population changes involving loss of virulence determinants and assist the recovery of desired genotypes. These tests will be described.

Certain freshly isolated strains of *Past. pseudotuberculosis* (*P. pstb.*) carry the determinants VW + P + Pu + (6 of 11 strains so far examined). Unlike comparable strains of *Past. pestis* these strains of *P. pstb.* do not show maximum virulence for mice, suggesting the existence of another, as yet undemonstrated, virulence determinant in the former species. In contrast, strains of *P. pstb.* rendered VW—or P—still retain high virulence for guinea-pigs whereas similar loss in strains of *Past. pestis* results in avirulence for these animals. Other similarities and contrasts between strains of *Past. pestis* and *P. pstb.* with respect to virulence in mice and guinea-pigs will be described.

4. Casein Hydrolysate Plague Vaccine

M. K. HABBU,

Haffkine Institute, Bombay.

Haffkine Plague Vaccine which was in use in India for over half a century suffered from some serious drawbacks. Therefore, the problem before the Institute was either to improve the vaccine grown in the liquid medium or change over to agar vaccine. Mueller and Johnson's medium modified by Sokhey *et al* (1950) was found to be very suitable for this purpose.

For the production of a potent plague vaccine, studies on the following important problems very clearly connected with vaccine production were found necessary:

- (a) Selection of suitable strains
- (b) Maintenance of strains
- (c) Existence of strains of different antigenic make-up
- (d) Temperature and period of incubation
- (e) Method of killing the cultures.

(a) *Selection of Suitable Strains:* Previous workers used virulent strains for the preparation of vaccine. But Schutze showed that avirulent strains were no worse. But our studies showed the antigenic superiority of virulent strains and hence we use fully virulent strains.

(b) *Maintenance of Strains:* The customary method of maintaining the strains is through animal passage. But there are some drawbacks in this method. Fortunately the newer method of maintaining the strains by freeze-drying has solved this problem. We have, therefore, adopted the freeze-drying method for maintaining the strains.

(c) *Strains Specificity:* Our studies have shown that *P. pestis* does not exhibit any strain specificity. Hence a single strain is now used for vaccine production, in place of 4 or 5 strains used before.

(d) *Temperature and Period of Incubation:* There are serious differences of opinion regarding the temperature of incubation. Some advocate 37°C and others 28°C. But our experiments have indicated that 32°C is the optimum temperature and two week's incubation, the optimum period of incubation.

(e) *Method of Killing Cultures*: Of the several killing agents, we have preferred formalin (0.07%) as it not only sterilises the culture satisfactorily, but also reduces the toxicity of the vaccine, a property not possessed by other killing agents.

A comparison of the immunising and toxicity values of (1) Casein Hydrolysate Vaccine (2) Haffkine Vaccine and (3) Agar Vaccine has shown that the Casein Hydrolysate vaccine is superior to the other two vaccines. The vaccine has also been found to be fairly stable at room temperature for long periods.

The casein hydrolysate vaccine was tested by a laboratory abroad, along with their vaccine by the mouse protection test. They found that our vaccine was nearly 57 times more potent.

The casein hydrolysate vaccine prepared by the above method is a potent and a fairly non-toxic vaccine and has been well received by the public.

5. Conquest of Plague with Particular Reference to Plague in India

S. C. SEAL,

All India Institute of Hygiene & Public Health,
Calcutta.

The chronological order of event that led to the conquest of plague in India is a fascinating story. Plague was reintroduced in India in 1895-6. This involved practically the whole country and finally led to a state of endemicity with at least three foci in northern, central and southern India. Assam and Orissa have been free from plague and also Bengal except the city of Calcutta and recently Gauhati and Shillong in Assam. During the past six decades there was a continuous fall in the incidence of plague deaths reaching very negligible proportion in the last decade, the specific mortality per 100,000 population being 183.3, 133.8, 51.9, 11.7, 6.8 and 1.8 respectively.

The principal plague affected states in India between 1939 and 1957 were Bihar, Bombay, Hyderabad, Madhya Pradesh, Madras, Mysore, Uttar Pradesh and Punjab. The gradual decline was however, interrupted between 1943 and 1947 before the final decline. The last two states where human plague death were recorded till 1957 are Uttar Pradesh, and Mysore. In 1958, no plague cases were reported except from Mysore.

The chronological development of knowledge which led to the conquest of plague in India are:

- (1) Identification of the causative organism by Yersin in 1894.
- (2) Preparation of antiplague vaccine by Haffkine in 1897.
- (3) Establishment of the role of rats and fleas in the transmission of plague by Simond (1898) and subsequently by the Indian Plague Commission.
- (4) Introduction of treatment of plague by antiplague serum produced in buffaloes and horses by Naidu et al (1930), improved further by Sokhey (1935) at the Haffkine Institute, Bombay.
- (5) Introduction of accurate biological assay of the antiplague serum by Sokhey (1936) now replaceable by quantitative precipitin or agglutination and even flocculation test introduced by Seal and Silverman.
- (6) Increasing knowledge on nutritional requirements and improvement of cultural method of plague organism by various workers including Rao, Sokhey, Seal, Wats and others.
- (7) Increasing knowledge on enzymic activities by Herbert, Fauconnier; Shrivastava, and others
- (8) Classification of plague organisms and their differentiation with *P. Pseudotuberculosis* by Gore, Bhatnagar, Seal & others
- (9) Race differentiation of organisms by Bezsonova, Devignat and others.
- (10) Discovery of effective treatment of plague with various sulphadruugs since 1938 and with antibiotics since 1944.
- (11) Preparation of live avirulent plague vaccine by Girard, Robic. Otten and others (1934)
- (12) Improvement of killed antiplague vaccine by using casein hydrolysate both by Seal (1941-42) and Sokhey (1950)
- (13) Improvement of serological tests—agglutination, precipitation, complement fixation and flocculation both qualitative and quantitative by Seal and Silverman.

- (14) Knowledge of the antigenic structure of plague bacillus and allied organisms by Shrivastava, Seal, Baker, et al. Amies and Bhagavan, et al.
- (15) Fuller study of vector efficiency and ecological studies of fleas and rodents by King, Pandit, Webster, George, Chitre, Sharif, Seal and others.
- (16) Utilisation of DDT, BHC dieldrin and other insecticides for the killing of fleas and cyanogas, HCN, SO₂, chloropicrin and other fumigants for killing of rodents.
- (17) Increasing use of rodenticides like sodium Arsenite, BaCO₃, zinc phosphatide. Antu, Castrix, Fanyline, Warfarin, etc.
- (18) Country-wide anti-malaria campaign with DDT, with collateral effect on fleas
- (19) Organised campaigns against rodents and fleas in the various states.
- (20) Gradual development of resistance in the domestic rodent population (particularly *R. rattus*) in many endemic areas.
- (21) Investigation and researches under the auspices of I.C.M.R. leading to most of the above discoveries during the last 3 or 4 decades.

II. SYMPOSIUM ON CHOLERA

1. The Pathogenicity of El Tor and NAG Vibrios

Lt. Col. OSCAR FELSENFELD,

Walter Reed Army Institute of Research,

Washington, 12., D. C.

Speaking here about El Tor and NAG vibrios, it is my pleasure to state that the experiments performed by my co-workers and I are based, to a large extent, on theorems resulting from the observations of my great teachers, Professor Panja of Calcutta and Professor Ferran of Barcelona, as well as on discussions and publications by Indian, Indonesian and Japanese authorities on this subject. I can mention here only a few, like Drs. Pandit, Basu Mallik, Mondal, Seal, Takita and Tanamal. The experiments presented today are a short summary of some tests involving El Tor and NAG vibrios and some of their relationships to so-called "true" cholera vibrios in test tube and in animal experiments.

To quote Professor Seal, cholera workers are divided into two groups. One of them believes that cholera vibrios represent one single species, called the "true" cholera vibrio which alone can be made responsible for the symptom—complex called cholera, while others accept a variability of the causative organism of this disease. The variability may be due to dissociation, mutation and other changes. For practical purposes, one has to differentiate "true cholera vibrios" from strains which belong to the El Tor group and non-agglutinable (NAG) organisms, some of which are members of the Heiberg I class, while others belong to different groups. El Tor vibrios are generally considered non-pathogenic, except in the Celebes where several outbreaks, caused by such strains, have been noted since 1938. In one instance, Mukerji faced, in Lucknow, an outbreak of cholera which was caused by El Tor vibrios. The literature available to me does not show any other instances in which El Tor types were involved in epidemics in India or in the Near East.

Non-agglutinable vibrios were found in cholera outbreaks by Pandit, Seal, Yajnik, Prasad, and others, in the absence of "true" cholera vibrios. The first question was; why does the El Tor vibrio cause no, or only mild, disease in Egypt and India, while it produces serious illness in the Celebes? Tanamal has developed several methods to identify El Tor strains, in addition to the hemolytic and V. P. positive properties of this microbe which are generally accepted as differentiating means. Neither Tanamal or other authorities on El Tor vibrios, were able, to my knowledge, to differentiate El Tor strains of different provenance. Recently, Watanabe and I began to use hemolytic and lethal dose determinations in an attempt to establish further differences among El Tor vibrios. Slide I shows an experiment carried out by absorbing sera prepared against different El Tor strains supernates. There was a considerable reduction of the antihemolytic and antilethal activities of the sera after such absorptions, except that the antilethal activity of the anti-El Tor Celebes serum was neither in the experiments

nerve block. The effect of organophosphorus compounds on cholinesterase activity and on acetyl choline levels in susceptible and resistant insects seems to be different. The toxicity of the organophosphorus compound to a species is affected by the activity of (a) enzymes capable of converting the compound to a powerful inhibitor of cholinesterase and (b) enzymes capable of splitting the compound to inactive products. From the available information regarding the relationship between the structure of the compounds to their toxicity, a number of resistance mechanisms are postulated.

Mode of action of DDT and BHC is not clearly understood. Unlike organophosphorus compounds, no evidence of the inhibition of any enzyme by DDT or BHC has been found. DDT seems to act on the neuromuscular system of the insect and the poisonous effect may be due to the liberation of certain blood toxins in the insect. The cause of DDT resistance in insects has been attributed to the enzymatic metabolism of DDT to the non toxic compounds; storage of DDT away from the site of action and changes in the sensitivity of the insect nerve to DDT, etc. Gamma BHC seems to act as a metabolite antagonist because gamma BHC and inositol have identical stereochemical structures. In resistant insects enhanced rate of detoxification of BHC has been observed.

3. Outlook for Future.

P. J. DEORAS

Haffkine Institute,
Bombay.

Some authors suggest the use of alternating insecticides. Busvine (1957) thinks that probably there will be no reversion if deposits of the previous chemical persist for some time. The significant fact that experimental studies have shown that resistance in house-fly develops much less rapidly with pyrethrins should not be lost sight of. Ascher (1958) has shown that cetyl bromide was less toxic i.e., produced less K. D. for the normal susceptible strains. It was almost equally more toxic to all the strains showing some type of resistance developed under some type of selection pressure. Crow (1952) mentioned that DDT resistant and multi-resistant strains knocked down by alkali bromides when orally ingested, and much more quickly than normal strain. Mitlin, Babers and Barthel (1956) reported a crude preparation of the diisopropyl tetra-chloroethyl phosphate and that the active preparation when applied as a selective agent killed more of DDT resistant houseflies as compared to S. Strains. The third compound mentioned by Meltzer 1956 and quoted by Brown (1958) is phenylthiourea or phenylthio-carbamide discovered in experiments with DDT resistant strains of *Drosophila*.

In the Phenomenon of resistance one encounters the hand of the process of evolution. Insects have been on this planet long before mankind was born and they have withstood many an onslaught. In the zeal to eliminate some of the harmful ones, one may encounter more disaster than otherwise, unless properly planned. Busvine (1957) has expressed that control measures should aim only at the level of mortality necessary to prevent transmission and that more than this is, wasteful and potentially dangerous. What is needed to be aimed at, is the keeping in check the vector population level at a stage where it will not be potentially harmful.

Hess (1952) mentions that unless new approaches are developed in future this phenomenon will bring an end to the DDT-era. The real solution to the insecticide resistance problem lies in return to fundamental research. We need to know more about the insect physiology and more still about its ecology, biology and their habits. This type of knowledge has been sorely neglected as many seemed to believe that in order to control insects, one needed to know only the procedure of DDT and other residual insecticidal sprays.

In medical entomology, the behaviour modification of vector species has now posed a problem for vector control, and intensive studies are the need of the day on the bionomics and behaviour of hematophagous arthropods.

4. Investigations on Problems of Insect Resistance to Insecticides Carried out at I.A.R.I.

S. PRADHAN

Pusa, New Delhi.

Investigations have been in progress on various factors responsible for both inter-specific and intra-specific variations in insects' resistance to insecticides but only those aspects are listed

below on which results have already been published. The word 'resistance' is used here in its unrestricted scientific sense and includes all types of cases described as vigour tolerance, behavioural resistance, resistance developing as a result of continued control operations, etc.

(a) *Bioassay of the relative toxicity of insecticides to different species taken one by one:—*

Record is being accumulated of the values of LC.50 of various insecticides to each of the various species of insects so that when these tests are repeated after a few years the development of resistance if any as a result of our control can be accurately judged.

- (b) *Analysis of the external and internal components of insect resistance*
- (c) Factors affecting stomach poison effect.
- (d) Epicuticular structure & insect resistance.
- (e) Effect of particle size on toxicity of insecticidal formulation.
- (f) Lipoid solubility and insect resistance.
- (g) Factors associated with increased resistance in higher instars of locust.
- (h) Effect of temperature on insect resistance.
- (i) Effect of humidity on insect resistance.

5. Development of Resistance an Index of the Intensiveness of Control Operations.

S. PRADHAN

Pusa, New Delhi.

It is intended to invite attention to the idea that perhaps the development of resistance may be taken as an index of the intensiveness of the control operations carried out. In other words, instead of starting from the point that control operations have been carried out in such and such area and then to check up as to where resistance has developed and where not, one can probably argue the other way also. Assuming biological variation to be an universal factor it may not be wrong to interpret that, except in the case of total eradication which is rare, there alone really effective and extensive control operations have been carried out where resistance has developed. In other words, depending on the frequency of the resistance gene in the population a certain minimum degree (which should be actually very high) of insecticidal pressures should be necessary for the development of resistance as a result of control operations.

Suppose for a moment that before any control operation has been carried out, the population has been containing 5% resistant individuals (R) and 95% of the susceptible ones (S). That means the ratio of S and R will be:

S : R :: 95 : 5 (The general level being that of S with 5% R).

Now if the first control operation has killed say 50% of the population then obviously those killed will contain mostly the susceptible individuals i.e. the ratio will change to:—

S : R :: 45 : 5 i.e. the population will contain 10% R.

Similarly, after 2nd cycle of control operation

S : R :: 20 : 5 i.e. the population will contain 20% R.

After 3rd cycle of control operation

S : R :: 7.5 : 5 i.e. population with 40% R.

After 4th cycle of control operation

S : R :: 1.25 : 5 i.e. population with 80% R.

On the other hand if the control operation is so effective that as a result of every operation about 80% of the population is killed, then after the 1st cycle of control operation,

S : R :: 15 : 5 i.e. a population with 25% R.

And after the 2nd cycle of control operation

S : R :: 0 : 5 i.e. a population with 100% R.

Again if the control operation is still more effective and 95% of the population is killed in every control operation, then after the very first cycle of operation the position will be:

S : R :: 0 : 5 i.e. a population with 100% R.

The proof for this broad generalisation is indicated in equally broad abbreviations. These Are:—

- (a) The development of resistance has become more of a challenge in public health control operations and it needs hardly any discussion that by the very nature of circumstances

the control operation in the field of public health have to be much more intensive and extensive than in the field of plant health.

- (b) Even in the field of public health the resistance problems have obviously cropped up more frequently either in economically more advanced countries or in countries wherein these economically advanced countries have been so much interested as to get really both intensive and extensive control operations carried out.
- (c) Although the agricultural pests showed the development of resistance as early as 1914, this problem is still much less serious in the field of agriculture wherein the very vastness of the problem does not allow such a high degree of insecticidal pressure as can easily lead to the development of resistance in agricultural pests.
- (d) In agricultural pests the earliest to create a resistance problem was a scale insect infesting garden plants which constituted the best set of conditions for the development of resistance. Not only in the scale insect the power of locomotion is much limited but also more intensive control operation is possible in small environments of the gardens forming, so to say small islands here and there.

Three rational expectations of really effective control operations are:

- (a) Development of resistance which is in the very nature of protoplasm.
- (b) Palliative effect which is soon nullified by the building up of the population by the progeny of the survivors which however form a small proportion of the total untreated population in which they get diluted.
- (c) Erradication which is only an ideal to strive for, specially in large continental areas.

This is to be tried by knock out intensity of operation.

Out of these three possibilities (a) is happening in the field of public health, (b) in agriculture and (c) is practicable only under specially suitable conditions.

Thus in the context of the present seminar it may be pointed out that if the foregoing line of thought is correct, then resistance must develop sooner or later if really intensive and extensive control operations are carried out.

VI SYMPOSIUM ON SNAKES AND VENOMS

1. The Newer Knowledge of Snake Venoms and the Anti-Venomous Therapy.

AFRANIO DO AMARAL

Instituto Butantan, Sao Paulo, Brazil.

Until recently our knowledge of the real nature and composition of snake venoms was rather rusty as it was based on the study of materials either crude or improperly prepared, the results of most investigations not being reproducible. The realization of this fact has lead to the search of proper methods of extraction, preparation and purification of venins aiming at their standardization so as to facilitate the further tedious analysis of the chemical, pharmacodynamic and immunological aspects involved.

Fundamentally, a great difficulty should be overcome: the real composition of a venom from the same morphologic species is apt to vary according to certain local conditions (including particular food) under which a serpent lives, such variations sometimes corresponding so closely to ecologic niches as to suggest the existence of biochemical races within a species. Therefore, any venom to be used in any serious research must fulfill some basic requirements: a)—the *snakes*, proceeding from definitely identified localities, must be kept, under proper conditions, in separate cages bearing number and label; b)—the *poison*, extracted through electric stimulation of bite and received into sterile and neutral laboratory glass (with top covered by thin rubberized cretonne and edge protected by rubber ring so as to prevent injury of snake's fangs and teeth), must be immediately centrifuged (for discarding any insoluble elements) and dried either in vacuum desiccator over CaCl_2 or, if possible, in a suitable lyophilizer, the dehydrated venom finally being stored in a neutral-glass bottle filled with an inert gas (N) and kept in the dark at -10° .

Venoms must be carefully tested by the probit method on standard homozygotic mice, every precaution being taken to warrant statistical computation of results and all causes of error being avoided.

Presently, venoms are considered a complex combination of toxic and antigenic principles, most of which are enzymic in nature. Out of the numerous, important principles recognized therein (particularly crotoxin and its cleavage fractions named crotamin, toxin III, crotactin and lecithinase A; neurotoxins, especially pneumotoxic and optotropic factors; ophio-hemolysins; phospho-lipidases such as phospholipase or lecithinase A and cholinesterase; hyaluronidase and respiratory inhibitor; coagulases, namely, fibrinoplastic and thrombokinetic factors; anti-coagulate or fibrigenolytic factor; proteases and resp. inhibitors; nucleotidase; riboflavo-protein as related to ophiaminoacid oxidase, and others) a few have already been prepared in crystalline form, to wit: crotoxin, from Cascavel (*Crotalus terrificus terrificus*) venom; hemolysin, as distinct from neurotoxin, cardiotoxin and Ach-esterase, from Cobra (*Naja naja*) and Krait (*Bengarus fasciatus*) venoms; A-lecithinase, from Cobra venom.

Crotoxin is both neurotoxic and hemolytic. Proteases, found virtually in all venoms, start and nurture protein-splitting chain-reactions occurring in ophio-toxicoses.

Specificity reflects a definite combination of certain principles in any venom. Its derangement will follow any handling or treatment that may destroy, totally or partly, such delicate principles by enhancing splitting or cleavage of their basic constituents and by altering their balance and normal proportions.

Most of those principles are antigenic, although a few seem to be non-anti-toxinogenic, a fact that might explain why, in certain cases, not all active principles in a venom are neutralized even by an otherwise potent mono-specific antivenin. Incomplete neutralizing power of antivenins appears more frequently to be due to improper, not to say careless, handling or treating the corresponding venins.

Therapeutically, antivenins, which in warm regions should be lyophilized at the time of preparation, may be used either as whole serum or as enzyme-digested and concentrated plasma. Although the latter has the advantage of bearing a relatively higher potency per ml in titration, the former seems to hold specific antitoxic fractions for a wider range of noxious principles as contained in the corresponding poison. Anyhow, titration must be based on bio-assays, preferably employing standard and homozygotic mice and 10 LD 50 doses, with a series of antivenin concentrations vs. one fixed dose of the venom.

Treatment in general and application of antivenin in particular observe a series of biochemical, pharmacological and physiopathological notions as derived from the special effects of the corresponding venom on the tissues.

It is well known that, in the light of clinical pictures, ophiotoxicoses are generally divided into 2 main types, with several sub-types. As a rule, the bite (or stab) inflicted by solenodont serpents (Viperids and Crotalids), with the striking exception of the Neurotropic Cascavel, is followed by a succession of local symptoms, a sort of chain-reaction, in which the leading role is played by proteases, anti-hyaluronidase (hyaluronic acid, the 'limiting factor', being left free to act), coagulases, phospho-lipidases and esterase, assisted perhaps by other enzymes or even toxins. Split products from such reactions, being mostly cholinergic, are responsible for hypotension, depression and other symptoms indicative of the extension of the process through the system.

In the Cascavel case, the main neurotoxic and hemolytic manifestations are provoked by crotoxin (including its cleavage products), fibrinogenolysin and hyaluronidase, although the 'hypo-nephron syndrome' as associated with tubular lesions and impaired renal circulation, seems rather to be caused by the successive action of proteases and lecithinase A.

Intoxication ensuing the bite of proteroglyphodont serpents (Colubrids and Elapids), deriving mainly from such substances as neurotoxin, hemolysin (eventually from phospholipase, cholinesterase and like principles), is characterized by serious systemic symptoms, the virtual absence even of local swelling, apparently being due to predominance of hyaluronidase, the 'spreading factor', acting directly on the affected tissues.

Based on these notions, the newer anti-ophiotoxic therapy is threefold, since it should include specific, general and local measures.

Antivenins, both uni-specific and multi-specific, are the best agents for neutralizing at least the majority of the active venom principles. Dosage must be proportional to amount of poison supposedly ejected by serpent and to its pharmacodynamic potency. Concentration of venom per kg of body weight, being greater in young or light persons and small animals, calls for larger

All virus studies during the period 1949 to 1954 were carried out only in rhesus monkeys. With the successful establishment of tissue culture techniques early in 1955, all subsequent studies were carried out using only rhesus monkey kidney cell cultures.

The results of the studies carried out can be summarized as follows:—

A. Studies on clinical cases:—266 strains of polioviruses were isolated from 1263 faecal samples collected from clinical cases. The distribution of the virus types among them were 183 type I, 39 type II and 44 type III.

B. Studies on apparently healthy children for presence of poliovirus:—778 faecal samples from apparently healthy children under 10 years of age were examined for virus content with a view to determine the distribution of poliovirus in the community and the possibility of picking up avirulent strains of poliovirus. 13 strains of polioviruses were recovered, their type distribution being 9 type I, 1 type II and 3 type III. This study has revealed that about 2.0 per cent of the apparently healthy child population carries polioviruses in their intestines and excrete them in their stools. This can throw much light on the immunity status of the population at large.

C. Serological studies for virus neutralizing antibodies:—562 sera collected from among the population from different age groups were tested in tissue culture for the presence of virus neutralizing antibodies and more than 80 per cent of the sera from persons above the age of 5 years show the presence of antibodies against all the three types of poliovirus.

With this epidemiological background it is worthwhile considering the measures to be adopted to reduce the incidence of the disease in our country.

4. The Problem of Immunisation against Poliomyelitis in India

P. V. GHARPURE

Officer-in-charge, Polio Research Unit of the Indian Council of Medical Research,
Bombay—8.

It is needless to say that the question of active immunisation arises only when an infectious disease occurs in a community and is responsible for appreciable morbidity and mortality.

Though poliomyelitis is not a new disease in India it has appeared in an epidemic form since 1949. As a background to the discussion on prophylaxis, it will be useful to survey the present position of the disease in India. The data available though incomplete are adequate as a useful guide, and information regarding Bombay and Suburbs is substantial. It may also be mentioned that the disease occurs all over India.

The aforesaid facts convey one thing: that in recent years poliomyelitis has been occurring steadily. A rough estimate is that the incidence of paralytic poliomyelitis is 1:10,000 and the mortality is roughly 10% of the clinical cases. In the last 10 years this position has remained more or less unaltered.

The disease has affected the child population with a peak at 2-3 years. The number of cases in persons above 14 years of age have been few, e. g., in Bombay 34/1657, 1949-57. Out of these 34 2 were foreigner.

One more feature requires to be mentioned. The disease has shown a characteristic seasonal incidence, though occasional cases occur throughout the year the bulk is between May and September, with a peak in August.

The three facts :-(i) Low incidence and low mortality, (ii) Age incidence and (iii) Seasonal incidence have a direct bearing on the types and size of prophylactic measures.

Corroborative evidence can be sought in serological studies for the detection of neutralising antibodies against polioviruses in the population.

Sera from adults, age group 18-25 years have shown more or less a 100% immunity status. The distribution of the sera State-wise in the country further adds to the value of such a study.

Serological studies of individuals of younger age groups including very young children give results corroborating information provided by the study of the incidence of clinical disease.

It will not be incorrect to conclude from the results that in the population studied, immunisation against type 1, has commenced very early, before 1 year and the process of immunisation against the other two types has progressively added to the immune child population bringing the age groups above 5 years more or less insusceptible.

Along with the facts mentioned, the incidence and distribution of the poliovirus in the population requires to be taken into account. In the studies carried out so far 267 polioviruses have been isolated with 183 type 1, 40 type 2, and 44 type 3. Of this group 252 are from clinical paralytic cases, with 175 type 1, 36 type 2 and 41 type 3.

In addition to the polioviruses, collected from paralytic cases, the finding of polioviruses in apparently healthy children (age group 0-10) has added importance. In one survey it was found that 10% of the stools give positive isolations of poliovirus. If still younger children are included, this percentage can be expected to be higher.

Speaking of polioviruses in human intestine a fact which has recently come to the notice is the occurrence of non-paralytogenic strains, one type 1 and 2 type 3.

Existence of pathogenic and non-pathogenic polioviruses in the child population can flood-light the mechanism of immunisation as it is progressing in nature.

The question of selecting the agent for active immunisation needs careful consideration. The agents available mainly fall into two categories :—(i) inactivated Tri-valent (Salk) Vaccine (ii) Avirulent live Vaccine. The former is fit for parenteral use and the latter for oral use.

The problem of immunisation of any population must depend upon the immunity status of the population. If the two facts, viz., the low incidence and the high immunity level are taken into consideration, one is able to find an answer to the question—is active immunisation indicated? If the answer is in the affirmative the age group to be chosen is to be decided. A rough estimate of the child population up to 10 years is that it forms 10% of the total population. It can safely be stated that a mass vaccination programme is not indicated. Prophylactic Vaccine may be introduced on a voluntary basis like the triple antigen against pertussis, tetanus and diphtheria choosing the very young age group: 6 months will be the ideal age and the interepidemic period the best time to give it.

Further, the question of the total cost cannot be ignored. Use of Salk Vaccine entails administration by injection of three doses, 1 cc. each to each child. The present price per child is about Rs. 9/- to Rs. 12/-. This is the approximate cost of the imported vaccine. If the vaccine is made in this country it is likely to be cheaper but not appreciably so, as the procedures required to be adopted in its manufacture are complex, time consuming and expensive.

The parenteral administration of Trivalent inactivated vaccine would be an ideal method of active immunisation against poliomyelitis bearing in mind the fact that the paralytic poliomyelitis observed in this country is due to all the three types, as has been mentioned above. In fact there appears a rise in type 2 and type 3 cases in recent years.

The next question which deserves discussion, is the active immunisation with a live virus vaccine. It is felt by some workers that this would be the method of choice.

It would be a correct ambition to make polio extinct; but the problem of choosing the measures of achieving this requires very careful consideration.

The object of this note is to place before the listeners facts as they are. It is a fact that in recent months Salk Vaccine has arrived in the country, and has been administered to a few thousand children. The question of extending this programme and also the question of adopting immunisation by oral administration of avirulent polio virus are matters for next consideration.

I propose to leave the subject here.

IV SYMPOSIUM ON INFLUENZA

1. Antibody Pattern to Influenza B Viruses in the Student Population.

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This study is a successor to a similar study against A viruses. The same random sample was tested. Normal inhibitors were removed by periodate technique. Normal inhibitors and antibodies to eight strains, B/32/52 Hazra; B/33/52 Salunke, B/36/56 Sholapur, Crawley, B/Coonoor/Chellamma, B/Coonoor/Pearl, Lee and Warner were looked for. Out of the 83 sera

studied only 12 had normal inhibitors to these strains, six sera had no antibodies at all, 7 sera showed antibodies B/32/52 and 23 sera to Lee. The antibody patterns to other strains varied.

2. Re-infection of Mice with Influenza Virus.

Lt. Col. S. L. KALRA
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Poona.

During the last pandemic, virus was not isolated from the lungs of the majority of the fatal cases. One case of 3 reinfections at monthly intervals was noticed. This led to the following experiments.

Mice were infected intranasally and re-infected 3 and 6 months later (challenge group) and normal controls were inoculated intranasally at the same time. The control and the challenge groups were sacrificed daily in twos, a diagrammatic sketch of the lesions in the lungs was made; the lungs were ground up in broth and were examined for the presence of the virus and the antigen by HI and C. F. test: the serum was examined for HI titre.

In the challenge group the virus was present for 3 to 4 days, lesions in the lungs persisted for 9 to 12 days and the lung tissue acted as an antigen so long as the lesions persisted. From the control group the virus was recovered for one week; the tissue acted as an antigen so long as the virus was present; the serum titre rose gradually. In the challenge mice the lesions were worse after 3 months than after 6 months or in the controls, although the serum titres were high in the challenge group from the first day.

The Lesions in the 3-month-challenge group were probably partly allergic because there was heavy infection after 24 hours when the controls were normal. There is also the possibility of the formation of incomplete virus in the challenge group because the lung tissue acted as an antigen when no virus was isolated.

3. A Study of Asian Influenza Strains.

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Bombay 12.

Vaccines were prepared against A/Singapore and A/47-Strain at this Institute and the study of antibody response in the human beings against a monovalent vaccine of each and against a bivalent vaccine is recorded. Antisera raised in the animals against several Asian strains have been tested against several strains, and the results are tabulated. It appears that both vaccines prepared from local as well as a strain from Singapore give identical antibody response. There still appear to be some biological differences in the property in the various viral strains isolated. But these need not necessarily be antigenic in nature.

4. The 1957 Influenza Pandemic : Its Lessons & Its Problems.

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Pasteur Institute,
Coonoor.

The Influenza pandemic of 1957 is the first one to occur after the isolation of the virus types A.B.C. and the subtypes and strains, since 1933. It has been studied widely from various angles, particularly those relating to the biological characteristics of the pandemic strains. In some respects the pandemic has behaved according to expectations while in others, it has raised new problems. The lessons learnt from the pandemic as well as the problems posed by it have to be studied against the background of our previous knowledge of the subject to decide our programme for future research.

V SYMPOSIUM ON INSECTICIDES

1. Insecticide Resistance & Public Health; The Present Situation & Future Prospects.

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A sound assessment of the present incidence of resistance depends on the International adoption of satisfactory test methods and the adequate collation of information. So far as pests of public health importance are concerned, the World Health Organization has been very helpful, in both respects. Progress has been made in the standardisation of test methods, especially for mosquitoes, despite many difficulties some of which are inherent in the variable nature of the insects themselves. Much information on resistant strains is accumulating and several good summaries have been published as well as frequent circulation of mimeographed documents.

The present situation has good and bad aspects. The optimistic view is that resistant strains are scattered and many of them only exist over limited areas; so that at present they are scarcely impeding control of insect-borne diseases as a whole. The more serious view is that the trouble is continuously growing and that in no case has resistance been overcome (except by abandoning a class of insecticides for a new type).

Basic researches on resistance have progressed considerably, despite technical difficulties. They have not yet provided any practical solution; but they do provide the best data from which to judge future prospects of resistance, which seems to depend largely on two factors: (i) the frequency of occurrence and the nature of resistant genes in natural populations of insects, and (ii) the intensity of selection. In regard to point (i): since resistant strains have occurred in almost every major insect genus of public health importance, except *Simulium*, *Phlebotomus* & *Glossina*, it is evident that resistant genes are widely distributed. Toxicological studies on "Resistance Spectra" suggest that several different mechanisms exist to protect insects from DDT-type insecticides or organophosphorus poisons; but the combined defence against BHC and the dieldrin series seems to be rather similar in several entirely different pests. Concerning point (ii): it is likely that the persistent residual action and possibilities of extensive use of the new chlorinated insecticides tend to enhance their selecting effect on insect populations. This may explain the frequent cases of resistance to these compounds. Prediction of resistance possibilities for any particular insect must involve detailed knowledge on its ecology to assess the proportions of the populations which may encounter insecticide.

Since these prospects are not too hopeful, the problem urgently requiring attention is how to use modern insecticides to eliminate disease, with the least chance of developing resistance before this aim is accomplished.

2. Mode of Action of Organophosphorus Insecticide on Insects in Comparison to DDT and BHC.

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Since the discovery of organic synthetic insecticides a considerable amount of work on the mode of action of these insecticides has been carried out on a variety of insects. The development of resistance of insects to insecticides has given a further impetus to these studies. Much, however, remains to be done in this direction as these researches may lead to the clearer understanding of toxic mechanisms involved in the susceptible and resistant insects. In this paper an attempt has been made to review the mode of action of some of the synthetic insecticides such as organophosphorus compounds, DDT, and BHC.

It may be generalized that the insecticides kill the insects by interfering with some of their sensitive mechanism(s) essential to life. The sensitive mechanism(s) may or may not involve enzymes and may be a simple or a complex one.

Organic Phosphorus compounds exert their insecticidal action by the inhibition of cholinesterase. The lethal agent seems to be acetylcholine which accumulates in the tissues and causes

demonstrated here, nor in 6 other tests, reduced totally by El Tor Egypt supernates. Thus we had to conclude that the El Tor Celebes strains contain a somewhat different lethal toxin. Quantitative studies of the lethal toxin produced by different El Tor vibrios showed that those from the Celebes manufactured lethal toxin at a faster rate and to a higher titer than El Tor vibrios of other provenance. We had no opportunity to test the Lucknow strains. Some Celebes strains produced as much as 1,000 MLD per ml culture supernate. It is of interest to add that the lethal toxin of El Tor vibrios, contained in the supernate, is heat-labile, antigenic, dependent on the Fe concentration in the medium, and gives the Ramon flocculation test with antisera. These properties are not present in the "true" cholera vibrio supernates to the same degree. Thus one has to conclude that El Tor vibrios are definitely different from "true cholera vibrios", also, in respect to their toxin production, and that there is a certain difference between the choleric Celebes and the little or non pathogenic Egyptian and Indian strains of El Tor. It has to be emphasized that these observations are based on experiments in vitro and in mice and that conditions in man may be different.

Of the NAG vibrios, our experiments were limited to those so kindly supplied by the Haffkine Institute in Bombay and by Professor Panja and Basu Mallik in Calcutta. One of these organisms, NAG, II belonging to Heiberg group I, was used as a parallel test organism in the evaluation of cholera vaccines. Slide II shows that while this NAG vibrio differed in agglutination tests from the true cholera and from the El Tor strains, it showed cross-protection with El Tor strains. The El Tor vibrios used in these experiments were of the Ogawa serologic subtype. The observation of this cross-protection led to the inclusion of this NAG vibrio in a series of examinations of human sera. The results are shown in Slide III. While no agglutination titers were observed in this group of persons against this NAG vibrio, mouse protecting anti-bodies were discovered. This organism was pathogenic not only for mice but produced typical disease in young rabbits when tested according to the technic of Dutta and Habbu. It produced a lethal toxin of 60 MLD per ml culture medium. Thus this observation may be considered further proof of the pathogenicity of NAG vibrios.

It is not the object of this report to discuss if the El Tor and NAG vibrios used in these experiments could be derived from "true" cholera vibrios. Mutations in this direction have been observed by most of us who are working with vibrios. Our group is accepting the fact that the cholera syndrome may be produced by organisms different biochemically and serologically from the classic cholera vibrios. Our greatest concern is the prevention of such disease. If you recall Slide II, routine cholera vaccines did not protect mice sufficiently against the El Tor Celebes vibrios. Thus, the problem is not only that of the laboratory but is also of importance in establishing preventive measures.

2. Genetic exchange in *Vibrio cholerae*

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With the evidence now available of the occurrence of genetic exchange in certain bacterial species such as conjugation (sexuality) in *B. coli*, phage-mediated transduction in *Salmonella* and DNA induced transformation in pneumococcus and *H. influenzae*, it seemed pertinent to investigate whether any such mechanism could be demonstrated in *Vibrio cholerae*. The essential requirement for such genetic studies was the availability of strains with differential characters which could be used as markers in genetic analysis. In *V. cholerae* it was seen that strains differing in nutritional requirements, antigenic structure and drug resistance patterns could be utilised for such a purpose.

When certain pairs of such genotypically different strains were grown in broth, a significant number of colonies could be isolated by selective procedures which deviated in some character or other from the parental types. The fertility of such crosses depended on lysogenic state (phage 129) of either one or the other parent employed, which was suggestive of phage-mediated transduction. However, certain peculiarities of this phage rendered this improbable. Though plaques appeared in cross-matching tests of 129 lysogenic cultures with sensitive strains, it was not possible to obtain high titre preparations of this phage. Filtrates and lysates showed poor plaque count and none at all when passed through filters of sintered glass which would retain vibrio cells. There appeared to be a close correlation between the viable vibrio count of the preparations and the plaque count on sensitive strains indicating that phage 129 may have no extra-cellular existence. However, the dependence on the lysogenic state for effective recombination suggests the role of a fertility factor played by this phage, similar to the F factor in

F + strains of *B. coli*, though the latter does not show any detectable effect on sensitive F— strains.

Crosses between F + and F — strains of *V. cholerae* (129 lysogenic and sensitive) were regularly fertile, but the recombination rate was reduced in F + x F + matings. These studies were carried in detail with strain 162 and its mutants (hypoxanthine-, hypoxanthine-, histidine-, and hypoxanthine-, glutamic acid- respectively) and strain 387 and its mutants (methionine-, aspartic acid-). It was seen that the genotype of the recombinants, as tested by unselected markers, were generally of one parental type. Of particular interest was the isolation of some recombinants which were Smooth derivatives of Rough Strains of *V. cholerae* and others which were unstable prototrophs continually segregating into parental auxotrophic and recombinant types.

The evidence presented supports the possibility of 'conjugation' between *V. cholerae* strains.

3. Immuno-chemistry of the Polysaccharides of *Vibrio Cholerae*.

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(Central Drug Research Institute, Lucknow).

In view of the importance of polysaccharides in the antigenicity of micro-organisms, work was started in India in 1932 on the isolation, purification and characterization of polysaccharides from cholera and non-cholera vibrios. On the basis of the discovery of three types of polysaccharides and two of proteins, vibrios were classified into six groups. The relationship of the above fractions to the H and O antigens of the vibrios was, however, not clear. Further, the samples of polysaccharides then prepared did not have any immunological activity. Later, by employing improved methods, serologically active samples were obtained, some of which appeared to protect mice against cholera infection. No difference was, however, detected in the preparations from the two sub-types, which may be due to the degradation of the polysaccharides during preparation or to some difference in the protein fractions.

Recent work on the enzymes in *Vibrio cholerae* has shown that it is endowed with the enzymic mechanisms operating the glycolytic as well as the direct oxidative pathways. Since the biosynthesis of polysaccharides is effectively linked with these two major pathways of carbohydrate metabolism, specific differences among the antigens of the sub-types of *V. cholerae* and other vibrios may be reflected in the mechanism of its biosynthesis. Investigations are, therefore, planned to be taken up with the use of labelled glucose and amino acids in the medium for the growth of *V. cholerae* and recovery of the specific activity in the metabolic products. The success of such an investigation would depend upon the isolation of enzymes, nucleic acids and polysaccharides in as near the natural form as possible. The results of isolation of polysaccharides by different methods would be discussed in the paper. Further, attempts are being made to localize the antigens in the cell and characterize them by gel diffusion technique. The cells have been ruptured mechanically or sonically and fractionated by differential centrifugations. Certain interesting results have been obtained and these would be described and their importance discussed.

4. Role of Cholera Toxin in Production of Signs and Symptoms of the Disease.

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It is generally accepted that the intestinal manifestations of cholera are due to the action of endotoxin of *V. cholerae*: the question to what extent this toxin is responsible for the production of general symptoms and signs of the disease has remained a subject of much doubt.

Dutta and Habbu (1955) have developed a method of producing cholera in infant rabbits which resembles human cholera. Using the same method, we tried to study the effect of toxin on infant rabbits, 8-10 days old.

Toxin was administered by intraperitoneal, intramuscular and pergastric routes.

With intraperitoneal and intramuscular routes the animals showed postmortem picture similar to that described by Dutta & Habbu (1955) though they did not develop diarrhoea. With pergastric method the animals showed clinical, biochemical and histopathological picture similar to those seen in experimental cholera and also in human cholera cases.

The toxin used in these investigations was prepared after Gallut's (1944) method.

5. Some activities of bacteria-free products of *Vibrio cholerae*.

S. N. DE

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Bacteria-free filtrate of untreated cultures of *V. cholerae* shows haemolytic and mucinase activities and swells the ligated loop of small intestine of rabbits. It also causes fall of blood pressure but the fall is no greater than that with the control medium.

Sterile filtrate of washed suspension of *V. cholerae* exposed to ultrasonic vibrations, causes marked fall of blood pressure, while control material has no such effect. The filtrate has no haemolytic, mucinase or enterotoxic activity.

6. Studies on Typing of *Vibrio Cholerae* by Bacteriophages.

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The typing of bacteria by specific bacteriophages and the precise identification of the carriers and the line of spread of an infectious disease have proved to be one of the most important advances in the study of epidemiology in recent years. In the present studies, results obtained in phage typing of cholera vibrios isolated in epidemics in Calcutta are recorded.

Large number of strains of cholera bacteriophages isolated from the stools of cholera patients, lysogenic strains of *V. cholerae* and water sources could be classified into four distinct groups by their patterns of lysis of *V. Cholerae*, neutralization tests by antiphage sera, plaque morphology, thermal death points, generation times and cross reactions on the phage-resistant secondary growths of vibrios.

879 strains of cholera vibrios were isolated from cholera patients in Calcutta. On being tested with the four groups of cholera phages these vibrios were found to belong to seven types and sub-types. The universally lysable type I vibrios were the predominating phage type of vibrios in Calcutta during the period under observation. Phage types of the vibrios were found to be stable on maintenance in the laboratory for several years. Strains of *V. cholerae* isolated in localized epidemics originating from one source belonged to the same phage type.

III SYMPOSIUM ON NEUROTROPIC VIRUSES

1. The Inter-Epidemic Survival of Mosquito Borne Viruses

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Epidemics due to viruses having mosquito vectors may either recur to a lesser or greater extent each year or may only occur at prolonged and irregular intervals.

In the case of the mosquito borne encephalitides this phenomenon has been studied particularly in the United States but also to some extent in Australia.

At one time it was thought that such viruses might survive in those blood-sucking mites which commonly abound in bird nests, but the very low percentage of mites found infected and the failure to find infected mites except when there was a high incidence of the disease in the area, has made this theory seem highly improbable. In the case of the North American equine encephalitides three main theories are not supported, firstly that migrating birds bring the virus back from tropical endemic areas in the spring. The second theory for which there is some good evidence is that the virus survives for considerable periods in inactive, hibernating mosquitoes. Prolonged survival in mosquitoes which has been demonstrated for Western Equine Encephalomyelitis virus has not yet been demonstrated by any member of the Japanese B group. The third is latent survival in birds. Increasing evidence is being obtained that, at least in tissue cultures, viruses of the Japanese B group can become latent in mammalian cells. The possibility of a reactivation of virus in spring under the physiological stress of nesting undoubtedly exists.

In the case of Murray Valley Encephalitis Virus in Australia it has seemed unnecessary to postulate any complicated means of survival since there is good evidence of the existence of small highly endemic areas where there is adequate mosquito activity throughout the year to explain virus survival on a short term bird-mosquito cycle carried on throughout the year.

The reasons for the irregular occurrence of epidemics in wet tropical areas remain obscure.

2. Advances in Rabies Treatment.

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During recent years, important claims have been made regarding rabies treatment, involving *Inter alia*, (i) the use of hyperimmune serum with and without vaccine therapy, and (ii) the use of chicken-embryo-adapted rabies Vaccine. All these claims were investigated at the Pasteur Institute, Coonoor, with a view to assess the various claims themselves, their relative merits and whether they hold good when Indian strains of street virus are used for challenge. The results are presented of treatment with antirabies serum alone, with 5% Semple vaccine, with a combination of serum and vaccination, and with high-egg-passage Flury vaccine with and without serum.

Antirabies serum alone, given before or after the infection, while definitely prolonging the incubation period, had no saving effect. Serum seemed to confer some protection when it was obtained from animals of the same species which had survived an infection with the particular challenge virus.

By pooling batches of vaccine from several infected sheep brains it was possible to produce a vaccine of fairly uniform potency, the antigenicity of which was always superior to that of the standard reference vaccine as well as of most of the individual batches. With doses comparable to those administered to human beings it was possible to confer solid protection to animals against virulent strains of street virus when the treatment was started 7 days before challenge. The pooled vaccine also conferred significant protection in the postinfection treatment of animals given moderately severe challenges of virulent strains of street virus.

Combined therapy with serum and vaccine given after infection was of great value under certain conditions. There appeared to be an optimum relationship between the quantity of serum given and the antigenicity and dosage of vaccine administered.

High-egg-passage Flury vaccine given before infection gave very good protection. But serum and HEP Flury vaccine administered after infection was not of value against an Indian strain of street virus.

3. Studies on Poliomyelitis Virus

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With the kind permission of the Director, Indian Council of Medical Research and the Officer-in-charge of the Polio Research Unit at the Grant Medical College with which the author was associated as staff member till last year, I present the following data which have a bearing on the problems of immunization against poliomyelitis.

Except for brief description of an epidemic of the disease in our country in 1823 and in 1938, the disease was endemic in character, occurring only sporadically and affecting children below the age of 4 years. Since 1949 the disease has assumed an epidemic form.

Detailed epidemiological studies carried out so far in Bombay and its suburbs have revealed that (a) the disease has been occurring regularly in recent years, (b) the disease has a low incidence (the incidence of paralytic poliomyelitis being 1 in 10,000) and low mortality (about 10.0 per cent of the clinical disease) in the population, (c) there is a definite seasonal incidence of the disease, viz., between May to September, (d) the occurrence of the paralytic disease has been restricted predominantly to the younger age group, viz., 0 to 3 years, (e) there have been very few cases in the adults, and (f) more or less 80 per cent immunity against the disease exists in the population in persons above the age of 5 years.

Isolation and identification of virus strains from clinical cases of paralytic poliomyelitis and their immediate contacts were undertaken with a view to find out the distribution of the virus types.

doses of the specific. Also, for obvious reasons, the lighter the patient the greater the urgency of serumtherapy, intravenous or intraperitoneal inoculation being advisable in all severe cases. In those types of envenomation marked by local hard edema likely to evolve to necrosis, part of antivenin dose should be mixed with hyaluronidase (up to 5 mg for injection) and administered topically so as to permeate the gel barrier hyaluronic-acid effect) contributing to the gangrenous outcome. However, in order to prevent rapid, uncontrolled, dissemination, throughout the system, of noxious split products from venom-tissue interactions, an extra dose of antivenin mixed with ephedrine should be given intravenously, heart and kidney functions being carefully watched.

General medication, both vasopressor and adrenergic, is highly recommended should hypotension and depression with tendency to collapse appear. Strong hot drinks (tea or coffee) are also advisable, followed by blood transfusions and intravenous administration of saline-glucose solution or Ringer's sodium lactate solution in order to counteract dehydration, further blood loss and liver injury. In severe cases of certain types of toxicosis, neuroplegic medication (e.g., substances of the phenothiazine group) may be needed to retard absorption of venom principles by tissues. Analgesics such as novalgin (never opium or morphine, apt to aggravate cholinergism) are useful in those cases with acute pain, since pain, by causing vaso-dilation, aggravates the cholinergic syndrome in most types of envenomation.

Local application of warm wet dressing or immersion of affected limb in warm bath of brine or saturated magnesium sulfate solution is indicated to hasten resorption of edema and healing of sore areas. Finally, constriction and judicious incisions (associated with strong suction so as to possibly remove lysolecithin or any other split product from hemo-proteolytic reactions) around the affected region might be attempted, when extremity is involved, against grave local symptoms caused by venom of certain species (Gaboan viper, Texas rattler and others), should specific medication and other rational means be unavailable.

2. A Preliminary Electrophoretic Study of Unimmunised and Immunised Horse Sera using Haemotoxic and Neurotoxic Antigens.

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and

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In the production of Antivenene sera, when one used haemotoxic venoms (Russell's Viper or Ankiostrodon Rhodostroma) the titre of the serum is higher than when one used neurotoxic venoms (Cobra or King Cobra). It also requires less antigen and shorter time in the former than in the latter.

Electrophoretic study of serum was performed in two groups of animals: unimmunised group both haemotoxic and neurotoxic antigens were used. Four races of horses were concerned, although one race was represented by only one animal, and another by two. Results are shown in Tables 1 and 2. Owing to the limited number of samples, this part of the work must be regarded as preliminary.

The unimmunised group comprised 13 animals, viz. 6 Thai ponies, 6 Australian cobs and 1 Australian griffin. Taking Thai ponies as a group it was seen that their gamma globulin had a lower percentage compared to the whole group (22.7% as against 24.0%) as well as to the group of cobs (22.7% as against 24.3%). This point might be significant in connection with the observation noted earlier that Thai ponies are poor if ever successful producers of antineurotoxic serum.

A comparison between the immunised and unimmunised groups gives the impression that with immunisation there was a rise in the beta and the gamma fractions of globulin, with corresponding fall in the albumin; while the alpha globulin was practically unaffected. The most remarkable point was perhaps the sharp rise in the beta fraction. Taking the mean of the unimmunised group as a reference, the beta globulin was higher in the immunised group by 30.9%, while the gamma was so only by 25.8%. The rise of the beta fraction becomes even more distinct if we leave out the ponies immunised against cobra venom, which theoretically may have

points of attack different from the other two haemotoxic antigens. The remaining three animals gave 35% as mean value of beta globulin, which was 38.8% higher than the control; the figure for gamma globulin of this group was only 10.1% higher than that of the unimmunised group.

In contrast to what has been mentioned, the two mules inoculated with cobra venom showed definitely high gamma globulin, which is 54.1% higher than the control, while the beta was only 13.0% higher. Unfortunately we do not have figures for normal mule serum and have to use the general mean for reference. Nevertheless, the difference is big enough to suggest that formation of antibodies to cobra venom is probably associated with increase in production of gamma globulin. This is further supported by the observation made above that Thai ponies are unsuitable for immunisation against cobra venom, since they have low gamma globulin by nature.

Conversely, as suggested by our findings, immunisation against the venom of the pit viper and Russel's viper is probably associated with increase in production of beta globulin.

In our series of electrophoretic patterns we have not been able to find separation of the immunised sera into an extra band named T by Van der Scheer (J. Immunology 39:65, 1940) between the alpha and beta fractions.

3. Enzymes in Relation to the Neurotoxic Action of Cobra Venom.

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It is well known as a result of several physiological investigations, that Cobra venom brings about pronounced disturbances in the nervous system. The factors concerned are termed "Neurotoxins" and fractionation studies have indicated that they are proteins in nature. Although cobra venom "neurotoxins" have been obtained in a high degree of purity, the biochemical mechanisms involved in their action are not well understood.

Investigations which will be described were carried out in order to determine the primary biochemical changes taking place in the normal cell, which may be responsible for the toxic symptoms produced by cobra venom. The results of these studies demonstrate that the toxic factor in cobra venom inhibits *in vitro* and *in vivo* some of the enzymes concerned with respiratory activity of the central nervous system. The enzymic reactions by which energy is produced in a transportable form e.g. ATP in tissues, are particularly sensitive to the action of cobra venom toxin.

Evidence will be presented to indicate that the phospholipase present in the cobra venom, is responsible for the inhibition of the respiratory and energy metabolism in the central nervous system. These inactivations are produced by the enzymic destruction of the mitochondrial structures upon whose integrity the enzymes concerned especially with energy production in tissues, depend for their activities. The possible role of these biochemical alterations in cell metabolism, will be discussed in relation to the toxic action of cobra venom.

4. Proteolytic Activity of Venoms of Indian Snakes.

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Proteolytic activity of venoms of cobra, krait, Russells viper and saw scaled viper was measured over the pH range 1.5 to 10.5 using heat denatured hemoglobin substrate. The venoms showed the presence of more than one pH optima in each, indicating the multiplicity of the proteolytic enzymes. Gelatinase activity of these venoms was also measured using viscosimetric method and the results obtained with haemoglobin substrate were confirmed.

The proteolytic activity of Russell's viper venom (RVV) was studied in greater detail. It had two proteases, one active at pH 3.6 and the other at pH 9.0. A large number of inorganic and organic substances known to affect enzyme activity were tested for their activating or inactivating effect on the proteolytic action of R. V. Venom at pH 3.6 and 9.0. Heparin which inhibited the activity of protease completely at pH 3.6 and partially at pH 9.0 was tested for its protective action *in vivo*. It protected mice against 1.5 CLD of RVV when mixed and injected intravenously.

It is well known that proteolytic enzymes affect blood coagulation. The acceleration of coagulation of recalcified human plasma was measured with the four venoms. Venoms of Russell's viper, saw scaled viper and Krait accelerated coagulation of blood while cobra venom inhibited

coagulation at a high dilution. Calcium ions were not necessary for acceleration of coagulation with saw scaled viper venom but was necessary with the other two. Heparin inhibited the thromboplastic action of RVV but not of saw scaled viper venom (SSVV). However, EDTA concentration necessary to inhibit the action of both RVV and SSVV were about the same.

VII OTHER ABSTRACTS

1. Difference in Primary and Secondary Immunizability of Inbred Mice Strains.

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Primary immunizability is the amount of antigen necessary to confer a certain degree of immunity in a single dose. Experiments with different inbred mice strains showed that the primary immunizability to tetanus toxoid differed up to ten times. The endpoint immunity is determined by challenging the mice with high doses of toxin, and the immunity measured on a score scale that includes death times and survivor symptoms.

Secondary immunizability is measured by challenging the mice after two doses of toxoid. These doses were varied in amount and in time interval between doses. The second dose had the same dose response curve in all mice strains, but the immunity level after two doses was found to depend on a combination of the size of the first dose and the time interval between doses.

Differences in secondary immunizability did not occur if high first doses were combined with long time intervals, but strains that had low primary immunizability showed lower immune response than other strains, if the first dose was low and the second dose was given within a short period after the first dose.

2. Urinary Elimination of Polyols and Particularly of Cyclohexitols.

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The mesoinositol is normally excreted in small quantities by the urinary route and is very frequently (in 67% of the cases) accompanied by its isomer scyllitol. For studying normal and pathological elimination, the amount of mesoinositol only is estimated by measuring the effect of deionised urinary extract on the growth of "inositolless" *Neurospora crassa*. The normal level is about 30 mg, litre (12 to 56 mg) independent of the effect of food intake. This level is raised considerably in persons with glycosuria, the inosuria being related to glycosuria and not to hyper-glycemia. This increase does not occur in diabestes insipidus. During chronic nephritis a great increase in the elimination was noted (220 mg litre) as well as a rise of free inositol in the blood plasma (97 mg litre as against a normal level of 7 mg). It thus appears that glucose hinders the tubular reabsorption of mesoinositol while in chronic nephritis it is probable that the "diabetes" of inositol is related to the elevated level of this substance in blood.