INTRODUCTION

Phylogenetically bats are among the most ancient eutherian mammals. The earliest form yet discovered is from the early Eocene epoch. They are the largest order of mammals in overall abundance and second only to rodents in numbers of living genera and species. They are distributed throughout the world than any other mammals except man. According to modified classification of Koopman and Jones (1970) the order chiroptera comprises 2 suborders, 16 currently recognized families, about 170 recent genera and some 847 recent species. The Megachiroptera consists of single family the Pteropodidae whereas Microchiroptera is divided into 15 distinct families.

The great deal of diversities among bats were described by Jepsen (1970) as apparently no other class of mammal has ever had so many eccentric and extremely specialized characteristics. Difference in body size, locomotor skill, diet and mechanisms of nocturnal orientation are only a few examples of the wide diversities encountered among the thousand of species in this order. They are the only mammals capable of true flight. Except three families all are inhabitants of tropical zone (Koopman, 1970). Their thermoregulatory mechanism is as diverse as the group itself. The

Megachiroptera regulates body temperature as true homeotherm whereas Microchiroptera exhibits patterns of thermoregulation depending on weather they inhabit (Henshaw, 1970). The variation ranges from constant homeothermy to less precise homeothermy or complete abundonment of control in which body temperature drops to ambient temperature when the animal ceases to move (Hock, 1951; Morrison, 1959). Certain species of bat of temperate zones show variations in body temperature over a range which could not be tolerated by any other mammalian species (Allen, 1939; Griffin, 1958).

Out of the several characteristics of bats which seems most significant from immunological and epidemiological points of view, is the importance of these animals as reservoir hosts for bacteria, viruses and other mycotic agents. Not much is known concerning the association of bats with bacteria which cause diseases in man. Bacteria of the Klebsiella, Aerobacter, Serratia group were found most frequently, followed by enterococci and Proteus species. Bacteria of eight other types were less frequently recovered (Constantine, 1970). Four serotypes of Salmonella known to cause salmonellosis have been isolated from bats (Arata et al., 1968; Klite, 1965).

Association of virus with bat is now a well documented fact. The first report of suspecting bat as carrier of rabies virus causing paralytic disease of cattle and horse came from Brazil (Carini, 1911) and the report of death of human due to rabies transmitted by bats was first to come from Trinidad (Pawan, 1936). Subsequently reports regarding isolation of rabies virus in bat came from different parts of the world, from U.S.A. (Irons et al., 1954; Scatterday, 1954; Sullivan et al., 1954; Venters et al., 1954; Bell et al, 1955; Enright, 1956; Sulkin, 1962), Canada (Avery and Failyour, 1960; Beauregard, 1969), Yugloslavia, Hungary (Nikolic and Jelesic, 1956); Turkey (Tuncman, 1958), Germany, Thailand (Smith et al, 1967) and India (Veeraraghavan, 1955).

The extent of the role of bats that may play in the persistance of rabies in wild life populations by transmission to other wild life hosts is difficult to evaluate at present; but it is known that bats come in contact with other species especially those which prey on cave bat colonies. Studies which have demonstrated oral transmission of rabies virus (Soave, 1966; Fischman and Ward, 1968; Correa-Giron et al, 1970) suggest that this means of interspecies transmission of rabies virus could occur in nature. Also, the demonstration of air borne transmission of rabies virus in bat caves (Constantine, 1967)

indicates another means through which bat could perpetuate rabies virus in nature as well as could endanger man. Role of bat in perpetuation of rabies viruses is supported by the findings that virus particles can be isolated from brain and salivery gland of apparently healthy, normal behaving bats captured in nature (Burns et al. 1956; Sulkin and Allen, 1970).

Several workers experimentally infected bat with rabies viruses (QueirozLima 1934; Pawan, 1936b) and reported that some experimentally infected bats developed furing or paralytic forms of rabies and were capable of transmitting viruses; others showed no overt sign of illness and did not succumb, but are equally capable of transmitting the disease to other animals. Experimentally infected vampire bat provided the first model of symptomless carrier of rabies viruses, a condition which subsequently shown to exist in nature (Smith et al. 1967). However, Tuttle and Kern (1981) raised certain doubts about the role of bat as a carrier of rabies viruses.

They are also ~~been~~ implicated as a reservoir host of certain arboviruses. The isolation of Japanese B encephalitis (JBE) and St. Louis encephalitis (SLE) viruses from bats collected during all seasons of the year indicates that these animals are rare hosts, capable of maintaining persistent foci of virus

activity in endemic areas (Sulkin et al. 1963, 1966a, 1966b, 1970; Miura et al. 1970; Allen et al. 1970).

The first experimental arbovirus infection to bats was reported by Ito and Saito (1952) who infected Pipistrella bats with JBE virus by intracerebral inoculation. Virus was detected in brain tissue but not in blood, liver, pancreas or gastric contents and could be serially passed in bats without loss of virulence. Although brain tissue titers ranged as high as 10^{-7}, none of the infected bats showed sign or symptoms of encephalitis nor did they develop m neutralizing antibodies. Experimental infection of two species of insectivorous bats (Eptesicus fuscus and Pipistrellus subflavus) with JBE virus was reported by Corristan, et al (1956). Subcutaneous inoculation induced viremia which persisted for at least 15 days in some bats. The animals survived infection without overt signs of illness. Another report of experimental arbovirus infection to several species via intranasal or intraperitoneal route showed no disease symptoms despite high levels of virus were present in blood for as long as 26 days post-inoculation (Corristan et al. 1958).

The importance of an animal host in the biological life cycle of an arbovirus depends on the establishment of a viremic

state of variable duration which provides infective virus for feeding vectors. The best evidence that bats do not develop a firm immunity following experimental arbovirus infection was the demonstration of spontaneous recurrent viremias and susceptibility of reinfection in a small group of big brown bats maintained in the laboratory for 2 to 3 yrs. Recurrent viremia in a natural host in the absence of reinfection would depend on the presence of latent foci of infection in one or more tissues from which active virus could be shed periodically into the blood stream. It is known that various host tissues other than blood are involved in the infection processes. Following subcutaneous inoculation, evidence of virus replication was demonstrated in brown adipose tissue of infected animals for extended periods of time and to a lesser degree in the brain and kidneys of some animals (Allen et al. 1964; Sulkin et al. 1963). Viral invasion and multiplication in the brown adipose tissue of these animals is particularly significant, since this tissue could sequester virus particles in viable state during the period of hibernation and could then provide seed for recurrent viremia upon arousal of bats in the spring, thus enabling hibernating species to harbour certain arbovirus over the winter in temperate zones (La Motte, 1958). The perpetual existence of viral agent of human disease in this class of animal instigated the scientists to delve in more details

the potential role of bats as reservoir hosts.

The fact that bats can harbour the dreaded virus and bacteria as reservoir hosts automatically stimulates the questions about immune system and immune responses in these animals. What are the organizations of lymphoid cells in this evolutionarily old group of the mammals? Can they mount a systematic immune responses against specific antigen?

Although the bats represent an old group of mammals little informations have been accumulated regarding their immune system and immune responses.

Lymphoid organs - In the vertebrate classes immune system is mainly constituted of the lymphoid organs like spleen, lymph node, thymus and bonemarrow and they are the main sources of the cells responsible for immune responses. The spleen and lymph nodes are the major antibody producing organs in mammals and the thymus is necessary in early development to potentiate the immunologic capacities of the other lymphoid organs (Good and Papermaster, 1964; Miller and Mitchell, 1969). Several reports have been accumulated on the splenic structure, function, ontogeny and role of spleen in the development of other lymphoid organs and in various immune responses (Auerbach, 1978). Shape

of spleen varies in different animal groups. The spleen in fishes is a flattened and elongated structure, globular in amphibians and oval in reptiles and birds (De Lanney and Ebert, 1962; Kanakambika, 1971; Soilendri, 1973; Pitchappan, 1980), while elongated in most other cases.

Phylogenetic considerations of spleen reveal a gradual appearance of white pulp follicles, thymus dependent areas and germinal centers (Cooper, 1973; Manning and Turner, 1976; Cooper, 1976; Cohn, 1977). Precise germinal centers are absent in lower vertebrates. The existence of germinal centre have been studied so far in a limited number of species of fishes, amphibians and reptiles and in most of the cases germinal centres are absent (Evans et al, 1966; Perren, 1967; Marchalonis et al, 1969; Borysenko and Cooper, 1972; Wetheral and Turner, 1972; Turner and Manning, 1973). But their existence in higher orders like birds and mammals indicate the more sophistication in spleenic structure and function. Cooper et al,(1965), reported that germinal centers are bursa dependent. It was also been proposed that the germinal center is the innovation of homoothermy and the high avidity IgG and the typical anamneses are the outcomes of germinal centre function.

Study of lymphoid organs of bats are fragmentary and they are mostly concerned with the gross morphological changes

during hibernation, vascular system of lymphoid organs etc. The weight of the spleen was measured in course of hibernation in certain bats (Kallen, 1960). Schiwatschewa (1967) made histological study of lymph nodes of some species of bats. Forman (1974) studied the structure of Peyer's patches and their associated nodules in relation to food habits of new world bats. Virtually there is no elaborate report on the organization of the lymphoid cells or the histological architecture of these organs in bats.

All vertebrates, higher than cyclostomes possess a discrete thymus at early stage of their life history, although this key organ may be lacking in adult form (Marchalonis, 1977). Study of evolutionary changes in the histological architecture of thymus reveals that though thymus was the first lymphoid organ to appear during ontogeny and phylogeny, it remains almost the same throughout vertebrate evolution (Pitchappan, 1980). Importance of the thymus in various immune functions have been established throughout the vertebrate class (Cooper, 1973; Weissel, 1972; Manning, 1975; Charlema, 1974).

Thymus is a large lymphoid organ. In most mammalian species it is located in the mediastinum anterior of the chest. Epithelial cells of embryonic thymus are the outgrowth of the

third and fourth branchial pouches, around which mesodermal elements aggregate. During embryological development certain cells in the epithelium of the thymus, under the inductive influence of the surrounding mesenchymal cells, have been stated to divide and to form the precursors of the lymphoid cells (Auerbach, 1961). According to Moore et al (1965, 1967a, 1967b, 1970), the stem cells for lymphoid cells migrate out of the yolk sac during early embryonic life and seed in the anlage of thymus. In the postnatal life most of the lymphoid cells of the thymus are continually replaced by new blood borne cells, mostly of bone marrow origin. The thymus becomes lymphoid late in foetal life and the organ rapidly increases in size after birth, reaching a maximum weight between the fourth and sixth weeks of age in case of mice (Metcalf, 1960). After attending maximum a characteristic loss of weight occurs (age dependent involution) which proceeds rapidly for the first few months, then continues at a very much slower rate for the remainder of the life of the animal (Bloom and Fawcett, 1976). Without the detail study of this organ, the understanding of the least known immune system in a particular animal like bat would remain incomplete.

Immune response - Besides the study of lymphoid organs, the investigation about immune responses in the bats is

also necessary for proper understanding of the immunobiology of the bats. There are certain investigations concerning humoral immune response in the bats and these are mostly on the basis of measuring the neutralizing antibody titres to certain virus and bacteria.

Experimental JBE virus infection provided rather interesting informations. There was no clear evidence of the production of complement fixing (CF) antibodies in infected animals although antibodies detected by hemagglutination inhibition test to group B arbovirus in naturally infected bats have been demonstrated (Whitney, 1963; Williams et al, 1964; Stanley and Chee, 1964; Pavri and Singh, 1965). In another series of experiment with JBE viruses Leonard and his associates (1968) showed that immune response in bat is quantitatively less but qualitatively similar to that produced in guineapig and at least two types of bat antibodies having characteristics of 19S and 7S immunoglobulin were demonstrated. Hatten et al (1968, 1970) showed for the first time the difference in the immunoglobulin classes in bat on the basis of sensitivity to mercaptoethanol and concluded that their response is different from other homeothermic animals.

Another interesting point is that simultaneous demonstration of virus and antibodies in bat blood was not uncommon

(Sulkin, et al, 1966a). Bats with circulating neutralizing antibodies have been shown to undergo spontaneous recurrent viremias and were susceptible to reinfection with JBE viruses, suggesting that the immune responses of these animals would not repress completely their effectiveness as reservoir hosts for the agent.

Immune response of bat to E. Coli phage Øx 174 have been reported by some workers. Quantity of antibodies production in bats following immunization with Øx 174 was less than in guineapig and rabbit (Heck, 1965; Hatten et al, 1968).

Fletcher (1961) showed that bats maintained at room temperature and immunized with Salmonella O and H antigens developed agglutinating antibodies in limited amounts. Heck (1965) showed that immunization with human serum albumin (HSA) did not elicit sufficient amount of antibody response as could be detected by quantitative antigen binding capacity technique of Farr (1958) or by passive hemagglutination technique. Whereas antibodies could be detected by Tanned cell hemagglutination test after immunization with keyhole limpet hemocyanin (KLH). Antibodies to KLH were demonstrated by 7th day following the injection of this antigen into animals kept at 37°C. Predominance of 19S antibodies after 7 days of injection have

been noted. The proportion of 7S antibody increased during the following 3 weeks until day 28, when most of the antibodies was 7S with small amount of 19S still detectable. It was also reported by Heck (1965) that synthesis of 19S antibody was more prolonged in bat as compared to that of rabbit.

So far humoral immune response in bats has only been studied on the basis of serum titres. To our knowledge, there is no study of humoral immune response in bats in reference to the antibody synthesizing cells. Antibody secreting or plaque forming cell (PFC) assay can be employed for this purpose. Nowadays this is a standard technique for studying humoral immune responses in different species of animals (Miller and Mitchell, 1968; Mitchell and Miller, 1968; Auerbach and Ruben, 1970; Kanakambika and Muthukkaruppan, 1972; Seto, 1980). Study of humoral response on the basis of PFC technique also helps in understanding the immune response in reference to the different lymphoid organs and in furtherance of our knowledge about cellular mechanism of antibody mediated response.

Cell mediated immunity (CMI) is another arm of immune responses in higher organisms. Thus some knowledge about cell mediated immunity of a least studied animal like bat is necessary for understanding of the different aspects of its immune responses.

Initial works to establish the CMI reaction in the bat was not much fruitful. Some workers tested the CMI in bats against Histoplasma capsulatum (Mycotic agent). They reported striking lack of cellular reactivity of bats to this agent (Emmons et al, 1966; Hasenclever et al, 1969; Hasenclever, 1972) and prolonged survival of viable organisms in the tissue (Taylor et al, 1962).

McMurray and Thomas (1979) reported the findings on development of delayed hypersensitivity to two protein antigens in two species of bats (Desmodus rotundus and Carollia perspicilla) and also showed PHA induced blast transformation of blood lymphocytes in these two species and effect of mitogen concentration, incubation time on their manifestation of cell mediated immunity in terms of blast transformation.

After reviewing these reports concerning physiological specialities of bats, the role of bats as reservoir host of virus, bacteria and other mycotic agents and preliminary works about immune responses in bats, it is apparent that more detail and systematic study of the lymphoid organs and immune responses in bats are worthwhile. Moreover, it has been speculated that these flying mammals represent an offshoot of the primitive order Insectivora, a group which stands near the origin of all other placental mammals (Peterson, 1964). So, study of the

immune system and immune responses of this mammal of primitive origin might contribute informations concerning the phylogenetic development of the immune responses.

The present investigation has been initiated mainly to study the organization of the lymphoid organs and the immune responses of a megachiropteran species, Pteropus giganteus. It mainly concerns with the following aspects. i) The study of the organs, generally responsible for immunological functions has been made on the basis of gross morphology and histological features in normal and immunized conditions, distribution pattern of lymphoid cells in different organs and in peripheral circulation. The possible heterogeneity in the cell population of these lymphoid organs has also been investigated. ii) Antibody mediated immune response of bat to a particulate antigen like Sheep's red blood cells (SRBC) has been measured in term of plaque forming cell assay and hemagglutination titre for primary and secondary response. Degrees of immune response were also studied in reference to varying amount of antigen and for a long duration.

In the study of immune response of a wild species, the use of naturally occuring infecting viruses as antigens could be a problem in view of ignorance about previous antigenic experience of the animals. Thus we decided to use sheep

red blood cells (SRBC) as routine antigen to investigate systematically the mechanism of antibody mediated immune response in this interesting group of mammals.

As a corollary to the humoral immune response, the study of electrophoretic separation of different classes of proteins in the serum of normal and immunized bats were made and the total amount of each class of the protein was also determined.

iii) Cell mediated immune response in bats has been investigated by contact sensitivity test with Dinitro Fluoro Benzene (DNFB) and in another series of experiment, mixed lymphocyte reaction assay was employed.