Malaria in Calcutta

Following the epoch making discoveries made by Sir Ronald Ross in India and Grassi and his coworkers in Greece on the role of mosquitoes in the transmission of malaria, control of the vectors anopheline mosquitoes, for combating malaria became a matter of strategic compulsion in many parts of the world, where the incidence of malaria was quite high. In India the very first effort in this direction, however, did not succeed due to one reason or other. Detailed and more systematic methodical studies had been initiated much later by a commission appointed by the Royal Society of London to make a probe into the transmission of malaria in India and to suggest measures of controlling it (Rao, 1984).

Based on the information gathered over a span of eight years from the different parts of India, the Government of India, in collaboration with all the State Governments and US Technical Corporation Mission in 1953 embarked upon a National Malaria Control Programme (NMCP) covering the entire malarious area of the country with an aim at reducing the incidence of malaria to much lower levels. This 5-year programme which achieved considerable success (Rao 1955; Jaswant Singh et al. 1957) encouraged to take, as the next logical step, appropriate measures for eradicating completely the
disease from the country. Incidentally, rumblings on the development of insecticide resistance in certain vectors in some parts of the world, including An. stephensi in India forced the malariologists and administrators dealing with the control of malaria to take appropriate corrective measures with a sense of urgency for total eradication of malaria before the resistance phenomenon became a real threat to the programme.

Again the Government of India, in collaboration with the various agencies like the USAID, WHO, UNICEF and all State Governments of the country launched in 1958 another project namely, "National Malaria Eradication Programme (NMEP)" originally for a period of 5 years. Worth noting in this context was that the NMEP differed from the NMCP in having to include even low endemic and potentially malarious areas in the programme and setting up a surveillance unit during the consolidation phase to cover the entire population of the country. Cities and towns with a population over 40,000 were excluded from the purview of the programme, for the antilarval measures were deemed to be economically feasible in there. However, spraying of insecticides over the peripheral areas was continued.
Ultimately there were 39235 NMEP units in the country and a phased programme of withdrawal of spraying was envisaged. In West Bengal the NMEP started working from 1959-60 with a total number of 26 units distributed over 15 districts and Calcutta (Mukherjea, 1964).

In Calcutta the NMEP met spectacular success and malaria was nearly eradicated, though some insignificant records of malaria infestation were available in some clinics (Mukherjea 1964, 1966, 1967 and 1970). As a result of that naturally there was a sharp decline in research on malaria in Calcutta at that time.

The resurgence of malaria in Calcutta in recent years attracted the attention of all concerned and remedy of such a problem had been on the look out with renewed urgency since 1980.

A clear picture of present day malaria is obtained from the data on malaria cases recorded here in Table 3. Malaria cases in the past were at galore. About 400 English soldiers died of malaria in 1690. During 1928 to 1931 over 50 thousand malaria cases occurred in Calcutta in each year. A comparison between the incidence of malaria occurring in Calcutta in the past and that of the recent years (1980-1990) would clearly reveal that though the figure was lower than the past, still it was alarming, thus indicating that
proper control measure must be taken immediately and on war footing to avoid the resurgence of malaria causing havoc as in the past. A glance into the monthwise cases, recorded in Calcutta in ten successive years (1980-1990), would indicate that the highest number of cases occurred in August in all the years. The number of cases in other months namely July, September, October and November were also fairly high. According to Hati (1979) the period between July and November is the malaria season in Central India and also in the Indogangetic plain. Information gathered from the past records on malaria epidemiology also corroborated the results of the present investigation on malaria in Calcutta. The percentages given in the table (3, vide introduction) were not based on total number of positive cases recorded. The table 3 thus did not provide a real picture as to the prevalence of malaria though, still it should not be ignored as it showed the occurrence of fairly large number of cases in different areas, in malaria seasons.

Resting habit

Every malaria problem, especially the vector problem, should first be subjected to field studies to determine its specific nature and define its limits (Samuel, 1926). The literature (Liston, 1901 to Siddons,
1946) available on control of malaria in Calcutta itself revealed involvement of much time and money for undertaking general measures exclusively, even without making any preliminary study of the nature of the local problem.

The recognised potential and incriminated (Siddons 1946, Mukhopadhyay 1980, Hati et al. 1987) vector of malaria in the city of Calcutta is *An. stephensi*. Density of the vector is one of the major factors affecting the epidemiology of malaria. It determines the degree of contact between man and the vector, and for that matter the intensity of malaria transmission. But the information regarding density factor of *An. stephensi* in this metropolis is far from adequate, practically due to non-availability of the species from the conventional resting places. Hence the density factor of *An. stephensi* the age-old problem in this city, remains yet to be settled.

One of the objectives of the present study was to ascertain such a less understood density factor as well as day time resting places of *An. stephensi* in Calcutta. The responsibility to find out day time indoor resting places of *An. stephensi* was assigned, in this study, to a team of skilled insect collectors under
the overall supervision of the present author. At first the team searched the conventional resting places (mainly houses reported by the earlier investigators) and adopted hand collection method in the morning hours. Many areas of Calcutta such as Dhakuria, Beliaghata, Ultadanga (now Bidhannagar) and Behala were surveyed in the rainy season (June to September). The rainy season was chosen as An. stephensi was reported to be the rain water mosquitoes (Basu 1930). The hand collection method in the morning hours soon proved to be expensive, time consuming, tedious and often frustrating as sample size was often meagre, insignificant or nil to project the exact density of the vector.

Due to such uncertainty, the project was about to be terminated. But suddenly the team was able to gather a fairly large number of adult An. stephensi (98 females) from temporary hutments (Jhupries) of Dharmatala area in the month of July. The temporary hutments (Fig. VI) was made by the workers of the Metro rail construction tunnel. Such a success boosted the morale of the collectors, geared up the project work and those hutments (24) were selected for study for one year. After getting high prevalence of An. stephensi similar type of hutments and mix dwellings (human and cattle) were also searched for one year in Sealdah, Bowbazar and Central Avenue areas of Central Calcutta (Fig. VI).
To determine biotopewise prevalence of *An. stephensi*, two biotopes namely human habitations (temporary hutments and brick built rooms) and cattle-sheds (mix dwellings) were searched. In Dharmatala area, *An. stephensi* comprised 2.1% (357) of the total mosquitoes. In Sealdah area, the species consisted of 2.69% (336), a little higher than Dharmatala. Maximum collection was made in the month of July in both biotopes under study. The percentage catches of cattle-sheds in Bowbazar and Central Avenue were 21.27% (130) and 14.1% (91) respectively showing prevalence of *An. stephensi* in the cattle-sheds which were almost similar to the temporary hutments in structure (Table 23). Very insignificant catches were accomplished from the brick built rooms of Central Avenue (3, 0.03%) and Bowbazar (7, 0.17%).

According to the observations made by the earlier investigators (De 1923, Senior White 1934, Knowles and Basu 1934, Ganguly 1935, Roy et al. 1938, Senior White 1940, Siddons 1943, Mukhopadhyay 1980) *An. stephensi* was of secretive habit and its presence was not easily detected. But from the present study it would be clear that *An. stephensi* lost its secretive habits being found in handsome number from cattle-sheds or mixed dwellings and temporary hutments of Calcutta. Such a percentage of *An. stephensi* was sufficient to transmit malaria in Calcutta.
Percentage finding of mosquitoes is an old method (Rao 1984). The determination of 'per man hour' figure has been used most extensively, not only to study fluctuations in the abundance but also to assess the success or failure of control measures (Service 1976). The maximum per man hour collection of *An. stephensi* as observed in this study was in the month of July in all habitations. Very poor per man hour collection was recorded in the brick built rooms of Central Avenue (0.007) and Bowbazar (0.006) whereas the maximum density was reported from the cattlesheds of Bowbazar (0.45) and Central Avenue (0.15). The collection made from the hutm ents of Sealdah, where per man hour collection (0.84) was found to be higher than all the habitations searched, should be regarded as an exception.

It would be clear from the present observation that *An. stephensi* was maximum in cattlesheds and temporary hutm ents than that of the brick built rooms in Calcutta. But the previous investigators (Strickland *et al*. 1936, Roy *et al*. 1938, Senior White 1940 and Mukhopadhyay 1980) recorded that *An. stephensi* were available, though in small number, inside the brick built houses of Calcutta. The present study clearly indicated that the day time resting habitats of *An. stephensi* changed abruptly.
Now An. stephensi are rarely obtained from the brick-built houses and these also inhabit cattlesheds or mixed dwellings and temporary hutments in this city. Such changes may be due to exitorepellatory action of DDT or other residual insecticides sprayed indoors of the brickbuilt rooms in the past. Interestingly it becomes the first hand information in Calcutta.

Mukhopadhyay (1980) got 23 adult An. stephensi outdoors in Calcutta. He collected those species from various situation like roof gutter, roof hole, garden rubbish and under heaps of loose bricks etc. adjacent to the breeding places of An. stephensi. No outdoor collection was made in the present study. Further study is therefore, essential to reinforce the idea whether An. stephensi preferred to rest undisturbed indoors or outdoors in Calcutta.

In Mysore (Karnataka), it showed a decided preference in human habitations for day time resting places (Nursing et al. 1938). In Kutch of Guzrat it preferred to rest in poor habitations and cattlesheds indoors (Afridi et al. 1938). In Poona (Barber and Rice 1938) and Vizagapatnam (Senior White and Rao 1941) An. stephensi were available in human dwellings. In Madras, maximum preference was found in mixed (human and cattlesheds) dwellings (Bhaskar Rao et al. 1946)
In Salem, Tamilnadu (Batra et al. 1979) it was obtained from "pucca"houses, cattlesheds and godown indoors and walls of the wells outdoors. According to the present study, An. stephensi preferred to take rest in day time in cattlesheds (mixed dwellings) and temporary hutments in Calcutta. Such an observation escaped the attention of the earlier workers concerned with Calcutta.

Very high density of indoor-resting of An. stephensi was reported from different parts of the country and abroad (Subbarao and Apparao 1945, Bhaskar Rao et al. 1946, Subbarao et al. 1984, Singh et al. 1985, Sharma et al. 1985). In Iran, maximum density of An. stephensi was found to be 200 per room (Zahar 1974) though such a high density of An. stephensi was not recorded during the present study. Maximum density per room per day was noted to be 16 in the present day Calcutta in the temporary hutments of Dharmatala.

Resting sites

Determination of indoor resting sites of any arthropod vector is of paramount importance for estimating the population indices before and after control operations for an appropriate evaluation; to adopt proper chemical control measure; to collect specimens for blood meal analysis as well as for determination of infection rates, age grading and other population
In the opinion of Liston (1901) adult
An. stephensi preferred to rest upon coloured funnels, cotton clothes, wood works, furs etc. and were easily found in wardrobes which were left open during night and filled with clothes, beneath tables or a desk left in a corner of a room or behind the dark curtain. Contrastingly in this study An. stephensi did not like to take rest on the articles listed by Liston (1901) and these were captured (only 7 An. stephensi) from inside an empty drum (1), cemented wall (1), blade of a ceiling fan (1), hanging clothes (1), wooden furniture (1), nylon string (1) and mosquito net (1) in brickbuilt rooms (Table 26). List of resting sites of An. stephensi as described by Roy et al. (1938) also did not corroborate the findings of the present study.

In the present study, maximum collection of An. stephensi was found from ceiling (54) and minimum from outer side of an earthen pitcher (4) in the temporary hutments (Table 27). In the cattlesheds, maximum collection was obtained from the ceiling (29) and minimum from thatched wall (4). An. stephensi mosquitoes were collected from folds of blank umbrella, nylon string, inside and outside of earthen pitcher, cobweb, bamboo pole, iron-piller, mosquito net, hanging clothes, ceiling
around wooden cot and thatched wall in temporary huts. In cattlesheds, in addition to that objects, it was also found from the body of the cattle, cemented wall and gunny. Such precise information regarding resting sites of *An. stephensi* is for the first time in Calcutta.

In Punjab (Pakistan), Rafi (1955) witnessed resting of *An. stephensi* throughout the height of the wall inside rooms. The observation made in this study does not fully corroborate this view as in the present cases *An. stephensi* was found in lower number in brick-built rooms in Calcutta. But in respect of temporary hutments and cattlesheds the present finding tallies with that of Rafi (1955). In brick built room, *An. stephensi* mosquitoes were obtained from the ground level (0m) or even on a blade of ceiling fan situated on the fourth floor of a room at a height of 14.4 m. in Bowbazar area and the observation confirms the earlier findings of Monouchchri (1976) in Iran.

Dwellers of the temporary hutments or mixed dwellings used to cook food inside the shed. As a result, all the house-hold materials that remained inside rooms became black due to smoke. *An. stephensi* preferred to take rest on those black objects in such a situation - an interesting observation reported for the first time here.
From the earlier records as regards blood meal analysis in Calcutta it was evident that *An. stephensi* of Calcutta was attracted by cattles rather than man (Roy et al. 1938). But in recent years they are more attracted by man as they are found in abundance in the temporary hutments and mixed dwellings in Calcutta indicating thus this mosquito's gradually coming closer to man. Possibly it acquired such habit from *Aedes aegypti* of Calcutta—the wariness and stealthily habit associated with less humming noise of which is well known. All the factors mentioned above are very important from the epidemiological point of view.

**Seasonal variation**

Seasonal variation of *An. stephensi* globalurise is not same. In Delhi (Bhatia et al. 1958), maximum prevalence was found in the summer (1577) followed by rainy (264) and winter (113) seasons. In Madras (Bhaskar Rao et al. 1958), maximum abundance was obtained in the winter season (976) followed by rainy (678) and summer (363), whereas in Mandora, Haryana, highest number of collection was reported in the month of February (Subbarao et al. 1984). In Bandar Abbas, this species was active throughout the year with two peaks, one in April-May and other in August-September
(Monouchchri et al. 1976). Such type of bimodal peaks was not observed in Calcutta. In Pakistan (Reisen et al. 1982), maximum prevalence was noted in the months of November and December. Temperature, humidity, rainfall and insecticides or larvicides spraying of a particular locality played an effective role for such seasonal variation. Seasonal variation of *An. stephensi* in Calcutta could be assessed from the monthwise prevalence presented by Senior White (1940). The then seasonal variation was as follows: in seasons of summer (82), rainy (53) and winter (34) respectively. The result of the present study depicted that seasonal variation at present was at variance to some extent. The prevalence was higher in the rainy, followed by winter and summer in both the biotopes searched (Figs. I, II & III).

**Larval survey**

Different workers reported different breeding spots of *An. stephensi* from the various parts of the world. *An. stephensi* used to breed in garden tubs (Iyenger 1920, Covell 1944), cisterns (Iyenger 1920, Covell 1928 and 1944, Mukhopadhyay 1980), shallow pits (Iyenger 1920), filtered and unfiltered water (Basu 1930), open earthen drains (Roy 1931), ponds (Roy 1931), earthen handis (Knowles and Basu 1934), earthen tubs (Knowles and Basu 1934), jars (Knowles and Basu 1934), Kerosene tins (Knowles and

In this study, 100 brick-built houses of Bowbazar and tunnels under construction for Metro rail in Calcutta were searched for detection of An. stephensi larvae both indoors and outdoors for one year.
In brick built houses of Bowbazar, altogether 4918 containers and reservoirs (2087 indoors and 2831 outdoors) were thoroughly investigated. These are cemented tanks (1811), iron buckets (1035), plastic buckets (439), tin drums (384), tin cans (187), flower vases (247), earthen vats (100), earthen handis (98), earthen pitchers (93), wooden barrels (90), cisterns (72), glass phials (69), earthen barrels (68), kerosene tins (64), iron frying pans (45), battery boxes (38), porcelain cups (34), earthen flower vases (33), and cemented floors (11). These containers may be regarded as the man made breeding source of *An. stephensi* inside houses. Besides those containers, larvae of *An. stephensi* were also found in and outside the Metro rail construction tunnel, inside various articles viz. coconut shells, wooden boxes, tin mugs, motor mudguards, tea pots, tyres, tins, plastic bottles, glass phials etc. In the rainy season, when the rain water accumulated in such containers or thrown away pots, making merrily breeding sources of *An. stephensi* in Calcutta.

Two types of water supply system exist in Calcutta by Calcutta Municipal Corporation - the filtered water for cooking, drinking and bathing purposes which usually stored in masonary vats under the ground level. Affluent dwellers lifted the stored water to the overhead tanks (on roof or at an elevated place) by an electric
pump and from there water used to be distributed through pipeline in the house. The silt laden water of the river Hooghly generally used for flushing privies and for gardening served as another source of water supply. In both types of water, larvae of An. stephensi were detected. The poor rented dwellers of the study area mainly depended on the filtered drinking water supplied by Calcutta Corporation at some specific periods of the day. Such dwellers generally stored water in the above mentioned domestic receptacles for house works. In such type of containers, if water was kept stored for a long time, in all probability adult An. stephensi could then become veritable sources of breeding of larvae of An. stephensi. If such breeding places are destroyed (source reduction), the population of An. stephensi is bound to fall considerably and there by the incidence of malaria in Calcutta can be checked to a large extent.

Association of An. stephensi larvae with other mosquito larvae is an important phenomenon in regard to control operations. In the present study, out of 3003 positive containers, 953 (31.7%), 904 (30.1%), 590 (19.6%), 334 (11.1%) and 222 (7.3%) were found to contain the larvae of An. stephensi alone; association of An. stephensi and Ae. aegypti; An. stephensi and An. subpictus; An. stephensi, Ae. aegypti and C. quinquefasciatus; and An. stephensi and An. vagus respectively.
House, container and breteau indices of *An. stephensi* larvae in Calcutta.

House index, container index and breteau index were initially employed on *Ae. aegypti* larvae by Curtin and Johns (1961). Due to non-availability of *An. stephensi* from the conventional resting places, Mukhopadhyay (1980) first adopted those indices on *An. stephensi* larvae in Calcutta. It was noted that house index was highest in August (28.8%) and lowest in February (4%). The index declined steadily after August 1976 and reached the lowest level in February 1977 and then spurted up again. Container and breteau indices were also highest in August and after August the indices declined steadily (1.40%, 6). These figures again started going up from June.

In this study, the number of containers searched indoors, were more or less same in each month during the study period, but the number of water holding containers outdoors increased in the rainy season i.e. from June to September. In October also the population was possibly due to spill over of the previous months. The increase of population was due to rain water accumulated in the indiscriminately scattered containers outdoors. For this reason, the house, container and breteau indices were higher in outdoor
situation in the rainy season as compared to other seasons. In June, the house, container and breteau indices were 16, 9.8 and 47 respectively. The indices reached their peaks (house index 24, container index 17.0, breteau index 86) in August. On the other hand, as expected in the summer months those indices were lower. In April, the corresponding figures were as low as 7, 6.9 and 28 respectively (Table 30).

The observation of Mukhopadhyay (1980) and that of the present study on the relationship between malaria incidence in Calcutta and above three indices were analysed statistically. According to Mukhopadhyay (1980) 'r' values (product moment of correlation coefficient) in respect of house, container and breteau indices were 0.9149, 0.8765 and 0.8561. The corresponding 'r' values in the present study were 0.9944, 0.9653 and 0.9998 respectively. So from the present study, it was clear that breteau index would be the best sampling technique since degree of relationship (r) was the highest amongst those three indices.
A new approach for determining critical density of *An. stephensi* in Calcutta in relation to malaria transmission.

After being convinced that Breteau index was the best sampling tool, the monthwise data of this tool was put against monthwise malaria incidence of Calcutta and monthwise per man hour density of adult *An. stephensi* from hutments (Table 46), cattlesheds (Table 47) and buildings (Table 48). It was felt worthwhile also to get an idea, for the sake of comparison at least, such index and per man per night collection of *An. stephensi* off man landing experiment. The data of these tables revealed that out of five sampling methods (per man hour densities of hutments, cattlesheds, buildings, per man per night density of manlanding experiment and Breteau index) Breteau index proved statistically (Table 50) to be the best sampling technique. In the opinion of the present author this device can, therefore, effectively and easily be employed for determining the density of the vector, specially where the density of *An. stephensi* remains obscure.
Vertical distribution of An. stephensi larvae

According to Mukhopadhyay (1980), out of 204 containers distributed in different floors of building, 158, 18, 11, 2 and 15 were collected at ground, first, second, third and fourth floors respectively in a normal condition. On the contrary, the present finding revealed that An. stephensi preferred to breed at upper floors (Figure V and Table 31) showing rather a geophobic pattern of breeding behaviour when equal opportunities were available to each floor. So it might be inferred from the above two observations that normally An. stephensi used to breed significantly in greater numbers at ground level but when chances were available, it became upper breeder (18 m high). Covell (1928) reported An. stephensi generally breeding below ground level or on the roof of a building 24-30 m high in Bombay city. Findings of Covell and the present author that high rising buildings were the dangerous sources of An. stephensi in big cities were interesting indeed. Previously on one occasion only "The insect borne diseases Department" of Government of West Bengal, noticed breeding places of An. stephensi on the 8th floor of a multistoried building (personal communication).

A comparative study with C. quinquefasciatus and Ae. aegypti was also carried out during the present study. It was found that Ae. aegypti and
C. quinquefasciatus behaved as geophilic when equal opportunities were available in each floor, more particularly, C. quinquefasciatus as no larvae of it were detected above 10.8 m (third floor). Larval association of Ae. aegypti and An. stephensi was also noticed in such experiment, but such an association of An. stephensi and C. quinquefasciatus was never detected.

The present study indicated that the sky scrapers in Calcutta contributed a great deal as the breeding source of An. stephensi though, yet they preferred temporary hutments (Jhupries) and cattlesheds as their day-time resting places. It was difficult to assess whether those mosquitoes after having their blood meal from persons living in multistoried buildings got into the Jhupries/cattlesheds for taking rest undisturbed or they had their blood meal from persons living in Jhupries or they used to imbibe human blood whenever such a host was available in and around the cattlesheds. It would be worth noting in this connection that the average flight range of An. stephensi was reported to be 0.8 to 2.5 km (Galvao 1948).

The vertical distribution experiment carried out in this study suggested that it could effectively serve as a biological weapon for control of malaria, if ovitraps could be widely distributed in all houses,
in all localities of Calcutta and residents of those houses took pains in inspecting regularly and with meticulous care the traps for the purpose of destroying larvae. Such an experiment also indicated that where density of the adult mosquitoes was not known, this ovitrap collection method can be formulated to determine the density of vector in any locality. Interestingly, such type of experiment was conducted for the first time in Calcutta as well as in India.

Collection of different mosquito larvae from Metro rail construction tunnel

The Metro rail is deemed to be a symbol of extreme urbanisation. From Table 32, it would appear that the water holding habitats in and outside the metro rail tunnel provided opportunity for breeding of different species of mosquitoes throughout the study period. Out of 786 (20.6%) occupied habitat, 13.7% (108), 20.5% (161), 14.6% (115) and 51.1% (402) were found to contain larvae of *An. stephensi*, *Ae. aegypti*, *An. subpictus*, and *C. quinquefasciatus* respectively implicating thus that the larval breeding propensity of filarial vector was greatly enhanced due to Metro rail construction than those of the other mosquitoes,
still the channels of Metro rail as the breeding sources of *An. stephensi* and *Ae. aegypti* should not be overruled.

Seasonwise prevalence of waterholding containers with larval infestation showed quite higher larval breeding during rainy season as it was pointed out by Paiva way back in 1912 in Calcutta. When seasonwise larval breeding of individual mosquito species was considered, *An. stephensi* and *Ae. aegypti* larval breeding spots were found to be significantly higher during the rainy season than those of other seasons which might be due to influence of a number of climatological factors, especially the higher rainfall and suitable relative humidity, which prevailed during the rainy season (Wattal 1964, Rao 1967, Basu 1930). As these two mosquito species were absolutely fresh water breeders (Basu 1930, Paiva 1912), their breeding propensitv was found to be significantly lower during the winter and summer seasons, only due to paucity of fresh water. The breeding of *C. quinquefasciatus* larvae was however, found to be in the range of 12.0% and 12.21% in the water holding habitats during the winter and summer seasons respectively, the figures being quite higher than that in the rainy season (8.39%). From this observation one might infer that *C. quinquefasciatus* preferred to breed in foul water in winter and the post winter months, a finding which confirmed the earlier report of Paiva (1912) in this regard.
Bloodmeal analysis

By means of screening epideomiological situation in a malarious area, the identification of the source of blood, taken by female vector mosquitoes has been given prior emphasis in the modern field of malariology (WHO 1966, 1972). The city of Calcutta is malarious since its inception but scanty information (Roy et al. 1938) exists regarding the blood feeding spectra of An. stephensi in this metropolis possibly due to paucity in the collection of adults from nature (Basu 1930; Senior White 1934, 1940; Strickland et al. 1936) as the day time resting habitat was not clearly known. In recent situations (temporary hutments and cattlesheds) owing to the discovery of its favourite day time resting habitat (Hatiet al. 1988), an opportunity was offered for large scale analysis of fresh bloodmeals of wild caught An. stephensi, the findings of which were reported here.

In Calcutta, Roy et al. (1938) first carried out blood meal analysis of 172 An. stephensi by micro precipitin test, which showed that only 3.4% (4) had taken human blood while the rest 95.5% (111) had taken bovine blood. These figures changed significantly at the present moment. An. stephensi were now found to take 76% human blood, indicating that this species came closer to human contact, thereby increasing the intensity of malaria transmission in Calcutta.
Some investigators (Hati et al. 1979, Mukhopadhyay 1980) threw light regarding human blood index (HBI) of *An. stephensi* in this city. But their sample size were too small to give exact idea regarding the actual blood feeding spectra of the species. The present finding agreed with the findings of Senior White 1940 and Siddons 1943, who found infected *An. stephensi* in dwelling houses and observed that dwellers one after another was attacked with malaria as *An. stephensi* had taken more human blood and lived in houses.

Human blood indices of Poona (Barber and Rice 1938), Delhi (Afridi et al. 1946), Madras (Bhaskar Rao et al. 1946), Bellary, Karnataka (Rao 1984), Vizagapatnam (Senior White 1947) and Hyderabad State (WHO 1966) were 0, 1.19-46, 0.8, 11.0, 15.0 and 41.0-47.0 respectively (Table 8), showing lower HBI as compared to Calcutta (76%) in the present situation under consideration of this study.

Outside India in Iran (WHO 1966), Iraq (WHO 1966), Saudi Arabia (WHO 1966) and Pakistan (WHO 1966, Reisen and Boreham 1982) HBI was lower as compared to India.
So the present finding as to HBI gives a picture different from that presented by the previous researchers indicating that man was decidedly the preferred host of *An. stephensi* in Calcutta in his dwelling houses (HBP 94.6%). But opportunities being available percentage positivity (HBP 40.9%) of bovid blood might rise considerably compared to that in human habitations (0.8%). However, the calculated HBI is as high as 76, showing the preponderance of arthropophilic race of *An. stephensi* in the city of Calcutta (Table 33).

It is an established fact that the vectorial capacity and reproduction rate of malaria vectors vary as the square of the proportion of the blood meals obtained from the human host (WHO 1966), which is also evident from the present finding concerning Calcutta, showing thus a drastic change in the epidemiological picture often with 50% of annual cases occurring in the State of West Bengal, India.

Mukhopadhyay (1980) searched 21 cattlesheds for 48 hours in the morning after sunrise and in the evening after sunset and did not find a single *An. stephensi*. However, his observation lacked any information about the structure of cattlesheds as well as the period of collection. Eventually, he remarked that *An. stephensi* of Calcutta were probably losing
their zoophilic character. As An. \textit{stephensi} mosquitoes were found in fairly large number in cattlesheds, it was not possible to mention from this study that An. \textit{stephensi} lost their zoophagic habits in Calcutta (Table 33). According to Sweet and Rao (1937) the type form of An. \textit{stephensi} was more arthropophilic and var \textit{mysorensis} was zoophagic. So it might be inferred that the both forms of An. \textit{stephensi} were inhabiting in Calcutta in the present situations under study.

**Manlanding experiment**

The direct collection of mosquitoes coming to bite man provides important epideomiological information and it can be measured with a reasonable degree of accuracy, if properly planned and carried out (Pant and Pull 1980), who recommended fortnightly observation indoors and outdoors by a team of collectors.

Amongst the nocturnal man-biting mosquitoes in Calcutta where malaria is endemic, the size of population (both diurnal and nocturnal) of An. \textit{stephensi} was determined by 24 hours collection for the first time. The results revealed that no An. \textit{stephensi} came to bite man at day time. This finding corroborated the earlier observations of Choudhuri (1936) in Calcutta. But at night, a total of 54 An. \textit{stephensi} mosquitoes landed on human bait in the whole year. Such a huge number of
An. stephensi never landed on human bait experiment in Calcutta on previous occasions (Senior White 1940, Das et al. 1971 and Mukhopadhyay 1980). Therefore, the present observation that man-vector contact is gradually increasing in such micro-environmental situations in Calcutta.

It was already known that An. stephensi in nature did not bite at day time. But in the laboratory, it was found to bite human volunteers greedily in day time, provided it was starved for 24 hours previously. This change of habit was possibly induced by necessity which knows no laws as day biting was an abnormal behaviour.

Manlanding experiment conducted here offers some noteworthy information. According to Samimi in Southern Iran, An. stephensi landed on man bait both indoor and outdoor, but the proportion of landing outdoor was greater than landing indoor. This study showed the reverse results, in that landing indoor (42) was greater than outdoor (12) which was significant at 5% level.

In Iran Zahar (1974) and Monouchchri (1976) found high percentage of An. stephensi on animal baits. In Calcutta Senior White (1940) caught An. stephensi employing cow as a bait. Mukhopadhyay (1980) placed an
animal bait outdoor in Calcutta. But no An. stephensi was attracted to such bait suggesting thereby that An. stephensi was gradually tending to be more anthropophilic than it was in the past. This finding incidentally complimented that of blood meal analysis in the present study.

In the study period spanning over a year altogether 6 species of mosquitoes namely C.quinquefasciatus (PMC 12.37), Ar. subalbatus (PMC 0.31), An. stephensi (PMC 0.09), Ae. aegypti (PMC 0.08), An.subpictus (PMC 0.03) and An. vagus (PMC 0.01) landed on human bait both indoors and outdoors. An. stephensi occupied the third position among the different species of mosquitoes collected. Mean per man per night contact of An. stephensi was found to be 1.12, which was perhaps sufficient to transmit malaria. The data also showed that An. stephensi was becoming an important night biting species of mosquitoes in Calcutta. Per man hour collection at night was 0.023 in 1980 (Mukhopadhyay 1980) this figure as reflected in this study increasing to 0.09 in recent years.

The seasonal prevalence of man biting An. stephensi had also been studied. The results showed that they were attracted to human beings more in the rainy season (June-September 43, 79.62%) than the
other two seasons namely, the winter (October to January 8, 14.81%) and the summer (February-May, 3, 5.55%). It might be noted in this context that the prevalence of malaria was high in the rainy season, which was the natural transmission season of malaria in Calcutta (Table 6).

There are reasons to believe that the size of population of An. stephensii has considerably increased in recent years in Calcutta. A comparison may be made between the results of the earlier study conducted by Senior White (1940), Das et al. (1971) and Mukhopadhyay (1980). In their study, the nocturnal man-biting An. stephensii consisted of 3, 1 and 27 respectively, whereas in the present observation it was 54 (0.72%)—thus the increase was really alarming.

The biting activities of An. stephensii on man bait was studied in detail, both indoors and outdoors at different hours, different months, different quadrants and different halves of the night. The preferential sites of bite were also noted. They came to bite at 18-19 hrs, 22-23 hrs, 23-24 hrs, 24-01 hrs, 01-02 hrs, 02-03 hrs and 04-05 hrs both indoors and outdoors. Taken as a whole, the peak activity was observed at midnight, around 1 A.M. This observation differed from that of
Nursing et al. (1934), according to whom *An. stephensi* were more active between 21.00 and 24.00 hrs and between 4.00 and 6.00 hrs. In Baghdad, the biting time of *An. stephensi* was over by midnight (Krishnan 1961). De Burca and Jacob (1947) found that the feeding of *An. stephensi* occurred in the open at 09.00 hrs. According to Reisen and Aslamkhan (1978) in January and February no biting took place at 19.00 hrs in Pakistan. Mukhopadhyay (1980) in Calcutta stated that *An. stephensi* mosquitoes came to bite man between 23.00 and 04.00 hrs, with a peak between midnight and 02.00 A.M. So the biting activity of *An. stephensi* was at variance in accordance with the geographical region as well as microenvironmental situation of the study area and also with the location of the baits placed indoors or outdoors.

When the night was divided into four quadrants (Table 40) it was observed that the density of manlanding *An. stephensi* mosquitoes increased in the III quadrant (77.7%, 42) of night. It was also observed that the second half of the night, the total collection of this species was greater (46, 85.1%) than the first half (8, 14.1%) of the night. Human behaviour was likely to affect the mosquito's biting activity. Garrett Jones (1964) and Service (1977) while emphasising on these factors suggested to take into account them during epidemiological investigation.
Monthwise variation was also noticed in respect of prevalence of *An. stephensi* on man bait. The collection reached the peak in the months of July (25.92%, 14) and August (27.77%, 15) and no *An. stephensi* was obtained in the months of December, January, March, April and June. As *An. stephensi* (loc. cit) is a fresh water breeder, it breeds at rainy season thereby increasing the number of manlanding mosquitoes in that season in Calcutta. This reflects an important epidemiological clue in respect of nocturnal man biting mosquitoes. As regards the indoor and outdoor activities of the species, no monthwise variation was, however, noticed. *An. stephensi* was collected in greater number indoors than outdoors in all the months, showing thus *An. stephensi*’s retention of endophilic and endophagic behaviour in Calcutta.

It was further ascertained if there existed any preferential site of biting of *An. stephensi* mosquitoes on the body of man. Out of the total of 54 mosquitoes, 0%, 9.25%, 9.25%, 5.55%, 3.70%, 22.22% and 50.00% were caught off head, neck, chest, belly, back, hand and leg respectively showing that *An. stephensi* was attracted more towards the hand and leg regions of man. When the body was divided as upper and lower extremities, same *An. stephensi* was found in both (27+27) the regions, which did not agree with the findings of Mukhopadhyay (1980) in Calcutta. He caught all 27 mosquitoes from
below the waist line and remarked that this selective habit of *An. stephensi* of biting man below the waist line had some bearing on the epidemiology of malaria, and possibly explained why males in India suffered malaria more than females (Park and Park 1976). From the present study, it would be seen that the protection of hand and legs by the human beings at night would largely preclude the chance of malaria infection in this metropolis.

**Infection and infectivity**

In Calcutta *An. stephensi* was proved to be a good reservoir of both *P. vivax* and *P. faciparum* infection in the laboratory conditions (Iyenger 1933, Strickland et al. 1933, Knowles and Basu 1944). Siddons (1946) first incriminated *An. stephensi* as a vector of malaria in Calcutta. In the year 1946, the natural infection rate was 0.85 (oocyst 0.58 and sporozoite 0.56) which rose to 1.56 in 1980 (Mukhopadhyay 1980). The natural infection rate in the present study was found to be 1.78 (2 sporozoite infections out of 112 specimens dissected), a still higher value in comparison to earlier records (Siddons 1946, Mukhopadhyay 1980) in Calcutta. Besides, the infection rate of Calcutta was higher (0.51) than West Bengal (Neogy and Sen 1962). This might lead to an inference that *An. stephensi* was the potent vector of malaria in Calcutta and the gradual increasing infection rate had a positive bearing on the
severity of the disease in this metropolis in recent years.

An. stephensi proved to be the important vector of malaria both in Bombay and Lucknow. The sporozoite and oocyte infection rates were 2.4 and 7.4 respectively in Bombay, showing thereby that in Bombay the species was more active so far as transmission of malaria was concerned (Bentley 1911). In Lucknow (Banerjea 1930) the recorded infection rate of An. stephensi was 9.3, which was significantly higher than Calcutta. The infection rates were lower in Andhra Pradesh (King and Iyer 1929), Delhi (Hodgson 1914), Madras (Ramsay and Macdonald 1936), Ahmedabad (Singh and Jacob 1939) than Calcutta. Outside India (Afridi and Majid 1938, Mononchchri 1976) the infection rates were found to be lower than Calcutta. Such a variation in infection rates in different parts of the world was perhaps due to variation in temperature, humidity, microenvironment and other climatic factors.

Age determination

(a) Gonotrophic cycle

The study of age distribution in anopheline populations is of paramount importance for understanding the population biology of the mosquitoes and the
epidemiology of the diseases transmitted by them particularly malaria (Beklemishev et al. 1959).

Gonotrophic cycle of An. stephensi was first studied in the laboratory by Mukhopadhyay (1980). In March to December it was 2 to 3 days and in colder days of January and February it extended to 4 days. The present study also revealed that duration of gonotrophic cycle was not same throughout the year. The maximum duration of gonotrophic cycle was found in the winter months (96 hours, 4 days). This finding tallied with that of Mukhopadhyay (1980). Two and half days (60 hours) duration of gonotrophic cycle was obtained in both rainy and summer seasons in the present case study.

Quraishi et al. (1966) stated that the first gonotrophic cycle in Iran was completed in 3 to 4 days and the second in 6 to 8 days after eclosion. Such a change was due to the widespread use of residual insecticides which had greatly increased the importance of determination of the age of malaria vectors. According to the present author mortality appeared to be about 30% per day in studying flight range of An. stephensi mysorensis. It was probably for complete coverage with insecticides of all the resting places of anophelines which resulted in such high mortality during each gonotrophic cycle. As a result the proportion of the females reaching the age at which sporozoites ought
to appear in their salivary glands was practically insignificant so far as the transmission of infection was concerned. The main objective of insecticide treatment was to effect the mean longivity of the vocal vector population.

(b) Proportion parous, daily survival rate and daily mortality rate

Estimation of age composition of mosquitoes related to malaria transmission is an important aspect as mosquitoes of greater physiological age are of greater epidemiological importance (Polovodova 1949).

Daily survival rate can be calculated from the vertical age grading data, if the time intervals between successive age-classes can be determined. For female mosquitoes, the number of previous ovipositions, or reproductive age can be ascertained by counting the number of dialatations on the ovariole tubules (Detinova 1962). Constant daily survival rate can be calculated from the parity rate (Detinova 1954, Collens 1958).

In the present study, 371 An. stephensi were dissected out for determining the parity status, of which, 187 (50.40%), 127 (34.23%), 46 (12.39%), 10 (2.69%) and 1 (0.26%) were found as nulliparous,
primiparous, biparous, triparous and tetraparous respectively (Table 43). In Broach town (Gujrat), Nair and Samnotra (1964) showed that out of 61 An. stephensi females considered only 9 were nulliparous, 22 primipara, 19 bipara and 11 tripara, showing that at least 19% were living up to the epidemiologically dangerous age. This percentage (as reflected in this study) was little bit higher in Calcutta than Guzrat.

The proportion parous of An. stephensi was found to be higher in the monsoon and postmonsoon seasons (Table 45) indicating that during these periods An. stephensi lived longer which was very significant from epidemiological point of view. The only tetraparous female was obtained in the month of August (Fig. XI). The proportion parous in the present study on an average was 0.50 and it was lower than Guzrat (0.85) but more or less same in Salem, Tamilnadu (0.53).

Maximum daily survival rate of An. stephensi was recorded in the months of January, November and December. The maximum mortality rate was noted in the months of March (99), April (98), May (94) and June (96) in the situations under study in Calcutta.
Thus from the foregoing discussion the following points became clear: (1) In various aspects the results obtained here disagreed more or less, while in many others they tallied with those of earlier workers, depending on the situation and its climatological factors, more than anything else; (2) Even if the epidemiological aspects of malaria caused by An. stephensi were studied here in greater details, this attempt was not at all exhaustive, and there remains further scope of research in this area so far as transmission of Malaria vis-a-vis its eradication.