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PRELIMINARY APPRAISAL OF THE USE OF AGE-GROUPING
METHODS IN ANOPHELINE MOSQUITOS¹

by

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Introduction

In April 1959 a course sponsored by WHO, on advanced entomological techniques applied to malaria eradication, was held at the London School of Hygiene and Tropical Medicine. At this course some new methods of age-grouping of mosquitos were demonstrated according to the technique worked out by Detinova and Polovodova. The lectures translated into English proved to be of such interest and importance that they have been prepared and re-edited as a WHO/Mal document, No. 238.²

The participants of the London course have returned to their various countries, and many of them have had opportunities for trying out and assessing the new techniques. It seems likely that much of the work done on these lines would still be incomplete or inconclusive and that some workers might still not have achieved a complete degree of competence in their mastering of this admittedly difficult technique or in its interpretation. Nevertheless, it is felt that a preliminary assessment of the value of this method applied to different species of anophelines in different countries and in different climates, would be of interest.

¹ Part of the present document was issued as a Working Paper No. 27 for the Scientific Group on Malaria Research which met in Geneva in November 1959. Appendix 1 provides some interesting data from North Viet Nam.

² "Course in advanced entomological techniques applied to malaria eradication" (London, April 1959) WHO/Mal/238, 30 September 1959, 187 pages, 30 figures

Contents: I. Preface; II. Introduction by Professor G. Macdonald;
III. Age-grouping methods in Diptera of medical importance by Dr T. S. Detinova;
IV. The ovary and ovariole of mosquitos by Professor D. S. Bertram; V. Appendix.

About the middle of October 1959 a circular letter was sent by the Division of Malaria Eradication to all the participants of the London course, including both WHO staff and national staff, as well as to several other entomologists who were known to be working on this technique although they did not take part in the London course.

Sufficient material has already been received in response to our inquiry to justify a preliminary summary of collective experience. It is hoped that this summary, together with the combined body of fact and/or opinion provided by all these field workers who have been asked to contribute, will form a valuable basis for present appraisal and for future planning of the use of the newer methods of age-grouping.

Reports have been received from entomologists working in West Africa, East Africa, Madagascar, Baroda and Mysore in India, Nepal, Cambodia, Territory of Papua and New Guinea, Netherlands New Guinea, Iran, Mexico and Brazil.

The general experience so far confirms the impression gained by most of those who attended the London course, that the technique is not easy and that it takes considerable time to master it. The process of perfecting this technique may prove very time-consuming, especially if the entomologist is working unaided. Under such conditions it may not be possible to deal with more than six dissections per hour, or approximately 30 per day. As a result, several contributors very naturally do not feel that they are yet in a position to commit themselves in the way of providing facts or opinions.

Preliminary errors in interpretation have been made by entomologists who did not have the advantage of attending the course. Some observers were misled by the distinct beaded appearance of the funicle between germarium and follicle and between follicle and follicular thickening. These were obviously teething troubles, due to the absence of practical guidance, and not likely to be made again by the same workers. The following points from reports are of special interest:

1. Dr M. Mofidi and E. Shahgudian (Institute of Malaria, Teheran, Iran)

Work has been carried out in the Jiroft area of south-east Iran during the hot weather (maximum temperature 38-43°C, minimum 25-32°C). At this time of the year Anopheles stephensi (mysorensis) are abundant but there is no transmission "under the unfavourable climatic conditions".

Conditions in an untreated area were compared with those which had been sprayed with DDT and dieldrin (twice in 1958, once in 1959) with the following results:

Age-groups (gonotrophic cycles) of A. stephensi

| | | 0 | 1 | 2 | 3 | 4 | Sac | Gravid | Total |
|---------------------------|-----------|-----|-----|----|-----|-----|-----|--------|-------|
| Sprayed villages | Number | 109 | 113 | 15 | 2 | 2 | 6 | 93 | 340 |
| | Per cent. | 32 | 33 | 4 | 0.6 | 0.6 | 1.8 | 27 | - |
| Unsprayed village (Zakht) | Number | 17 | 20 | 6 | 1 | 1 | 0 | 31 | 76 |
| | Per cent. | 22 | 26 | 8 | 1.3 | 1.3 | - | 41 | - |

These findings reveal no obvious differences in age-groups between treated and untreated areas but the results may be influenced by the unfavourable climatic conditions referred to, and possibly by the fact that in this region A. stephensi has developed resistance to both dieldrin and DDT.

It is planned to continue these observations during the cool weather and the malaria transmission season and this should provide further material for this age-grouping assessment.

2. Dr C. Achuthan (Malaria Unit, Mandya, Mysore, South India)

This work was done in some areas which have been under residual insecticide treatment for over eight years. Polovodova's method was applied to 86 specimens representing 11 species of Anopheles. The author points out several technical difficulties, of which the following are selected examples:

(a) In small-sized species like A. fluviatilis (an important vector) the separation of the ovarioles was more difficult than with large-sized species like A. hyrcanus and A. barbirostris (non-vectors).

(b) Specimens which were judged to be very old by the threadbare condition of the wings proved to be very fragile and the ovaries were liable to crumble to pieces even though the utmost care was exercised. On an average it was possible to dissect out six mosquitos per hour.

(c) The presence of yolk granules in the proximal end of the developing follicles was utilized in order to avoid confusion between undifferentiated germarium cells and the follicular thickenings (dilatations).

(d) The beaded appearance of the funicular stalks is liable to be mistaken for dilatation. (This point has also been noted by Dr Shalaby in Baroda.)

The maximum number of dilatations (corresponding to gonotrophic cycles) encountered was four, recorded in A. fluviatilis. Three dilatations were recorded in A. fluviatilis as well as A. subpictus.

3. Dr M. T. Gillies (East African Malaria Institute, Amain, Tanganyika)

Experience gained in the last few months has amply confirmed the opinion expressed by several entomologists at the London course that Polovodova's full technique is a research tool and normally has no place in the routine assessment of control measures in the Tropics.

"I myself have not been successful in applying it to A. gambiae, although it must be confessed that my attempts have been sporadic rather than intensive."¹

The author points out that although results have been disappointing on the whole, modification of the basic method for the simple breakdown into parous and nulliparous groups has been found to be extremely valuable. These modified methods, in which

¹ We understand that Dr M. Giglioli, working at the MRC Unit in Fajara, Gambia, confirmed that the application of age-grading technique according to Polovodova's method is very difficult in A. gambiae. (Editor's remark)

the presence or absence of pigmented relics or of dilatations in the stalks of the ovarioles is noted without attempting the difficult task of counting the dilatations in individual ovarioles, have been applied to A. gambiae and A. funestus. The technique has been used in two different ways according to the level of experience of the examiners: (a) suitable for entomologists or senior technicians; and (b) of possible application by trained assistants under field conditions.

These techniques, which are described in detail, are still being perfected. They are considered to be specially suitable for dealing with samples of gorged females collected in the day-time, and could only be applied to other samples, e.g. night collections, with certain reservations.

Limitations of space do not permit the inclusion of all these and other technical details described but experience so far with this modified technique at Amani has been encouraging.

4. J. Hamon ("Centre Muraz", Bobo-Dioulasso, Republic of the Upper Volta)¹

Hamon and his colleagues found Polvodova's method very time-consuming. However, by setting up a team of himself and two other entomologists, considerable experience has been gained in the value of different methods of age-grouping in A. gambiae, A. funestus, A. coustani and A. nili.

In each female Hamon's team carried out simultaneous examination of (a) oviducts and ampullae; (b) funicle, and (c) tracheoles. One entomologist extracted the ovaries and identified the females as parous or nulliparous. Then one of the ovaries was transferred to distilled water and the tracheoles examined when dry by the second entomologist. The other ovary was then teased out in saline and examined in the fresh state.

¹ The report by Hamon, J., Chauvet, G. & Thélin, L. under the title "Observations sur les méthodes d'évaluation de l'âge physiologique des femelles d'anophèles" was issued in the WHO/Mal series of documents under the number 246 of 16 November 1959. It should be pointed out that the terminology II (j), II (m) and II (a) used by the authors of this paper refers to the early (jeune), middle (moyen) and late (agé) sub-stages of the stage II ovaries. (Editor's remarks)

In distinguishing parous from nilliparous it was found that method (a) was unreliable while methods (b) and (c) gave equally reliable results. In the method of diagnosis by examination of tracheoles 50-60 specimens can be examined in one hour, but the method is not applicable beyond stage 2. Teasing of the ovaries and examination of the funicle can still be done fairly well in stage 3, but only 12-15 specimens can be examined in an hour.

On the basis of this experience the teamwork of the three entomologists was reorganized to deal not only with age-grouping according to tracheole and funicle diagnosis, but also with the examination of salivary glands for sporozoites. Of particular interest is the fact that their mosquito samples were taken from two different sources, (a) females attacking man during the night, and (b) females taken in artificial shelters in daytime collections.

In this way they were able to show that A. funestus females taken from artificial shelters contained a much higher proportion of nillipars than those captured at night. A. funestus caught at night also had a much higher sporozoite rate than those coming from artificial shelters. (This did not occur with A. gambiae.)

The technique was also used to find out at what ovarian stage the females come to take their bloodmeal, and in the case of parous females to see if those that come for a bloodmeal have already oviposited on the same night (presence of residual sac) or on one of the preceding nights (presence of a dilation). These figures show that although there is no general rule, a high proportion of A. gambiae and A. funestus take the bloodmeal on the same night as egg-laying. (This is a most interesting confirmation of similar conclusions made with regard to A. gambiae several years ago using entirely different methods.) With A. nili and A. coustani, on the other hand, it appears that the majority do not take a bloodmeal on the same night as egg-laying.

Further observations lead the author to conclude that in nature a certain number of females only lay 24 or 48 hours after maturation of the eggs. The result of this is that the average period between two successive layings or feeds is certainly longer than the duration of the gonotrophic cycle.

Age-grouping was also carried out according to the period of biting during the night, showing that there was a higher proportion of parous females in the second half of the night than in the first. This finding, taken in conjunction with the previous figures about night catches and day collections, emphasizes the fact that age-grouping may give misleading results if confined to one type or time of sampling the population.

Comparison between samples from control villages and the DDT-treated zone showed nearly always a decrease in the proportion of parous females in the treated zone. But this was also observed to be the case with A. coustani, which normally does not enter houses. All these results emphasize the importance of careful preliminary inquiry into problems of sampling the mosquito population before one can rely entirely on this age-grading technique to indicate the efficiency of insecticide treatment in controlling the anopheline vectors

5. Dr A. M. Shalaby, (WHO ATME No. 2, Baroda, India)

The team applied Polovodova's technique to A. culicifacies in the Panchmahals district of Bombay State. The greatest number of follicular dilatations found in one specimen was four (August 1959). The following table shows the results of dissections carried out in June, July and August:

| Month | Number dissected | Proportions (%) found with dilatations | | | | |
|--------|------------------|--|----|----|---|---|
| | | 0 | 1 | 2 | 3 | 4 |
| June | 84 | 35 | 63 | 2 | 0 | 0 |
| July | 137 | 31 | 52 | 7 | 5 | 0 |
| August | 97 | 36 | 50 | 10 | 3 | 1 |

The June dissections were made before the first DDT-spraying of the year, while those of July and August coincided with the first and second rounds of spraying. The fact that the proportion of nullipars did not rise is considered to be due either to the development of DDT-tolerance (LC50's up to 2.3 per cent. were recorded in some villages) or to irritability or behaviouristic avoidance.

In the period September-November dissections were made to determine the proportions of nulliparous A. culicifacies entering experimental huts at different periods of the night and among the parous mosquitos the proportions in which the presence of sac-like follicular pedicels indicated that oviposition had occurred the same night. The results were as follows:

| | Period of the night | | | |
|--------------------------------------|---------------------|-------------|-------------|-------------|
| | 1st quarter | 2nd quarter | 3rd quarter | 4th quarter |
| Number dissected | 400 | 185 | 83 | 42 |
| Proportion parous (%) | 71 | 78 | 58 | 79 |
| Proportion of parous with "sacs" (%) | 35 | 58 | 45 | 61 |

Notes on technique: At least two to three months of continuous practice are necessary before one is sure of distinguishing the real follicular dilatations. During the period of practice, one is apt to mistake the degenerated follicles, the segmented or bead-shaped terminal pedicle, or the bead-shaped funicles, for follicular dilatations. The method is undoubtedly time-consuming unless a team is available whose prime objective is age-grouping by Polovodova's method of the vector species in the various areas under investigation.

6. Dr C. P. Pant, (WHO Entomologist, Amlekhganj, Nepal)

After some practice on Anopheles subpictus using the technique described by Detinova during the London course work on age-grouping of some vector species in Nepal was undertaken. The areas of collection of mosquitos were unsprayed. The following table gives the details of the number dissected and orders of parity in the specimens dissected:

| Species | Total dissected | Nulliparous | Parous | | | | | | |
|------------------------|-----------------|-------------|----------------------------------|----|---|---|---|---|---|
| | | | Number of follicular dilatations | | | | | | |
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| <u>A. minimus</u> | 129 | 23 | 50 | 21 | 2 | 3 | - | - | 1 |
| <u>A. culicifacies</u> | 55 | 5 | 27 | 10 | 1 | 1 | - | - | - |

The dissection of 29 specimens of Anopheles minimus and 11 of Anopheles culicifacies was unsuccessful. This was because the ovaries were in fifth stage and as soon as they were dissected out all the eggs were separated leaving the follicular tubes with a sac and in most cases the whole system was untraceable. In our hands the ovaries with Stage V or advanced Stage IV eggs still present considerable difficulty in dissections.

In the above survey we were fortunate in collecting eight specimens of Anopheles minimus with sporozoite positive glands. However, six specimens had ovaries in an advanced stage of development and we could not dissect out the ovaries to count the number of follicular thickenings. The remaining two specimens had 4 and 3 thickenings. Although it is too early to make a definite appraisal of this method because the technique itself has not been completely mastered, a few comments may not be out of place: (a) ovaries in an advanced stage of development still present difficulty in dissection; (b) ovaries in a very early stage are also not so easy to work with; (c) females which have just oviposited present a sac and the follicular dilatations in these cases cannot be counted; (d) the whole method is time-consuming and this creates some difficulties in implementing other scheduled work.

Considering the above points it is felt that although the objective of the technique is the age-grouping of the population of vector species, thus showing the proportion which reaches an epidemiologically dangerous age in an area, the use of the method which distinguishes nulliparous from the parous/multiparous females has been more productive. From the proportion of parous females in Stage III ovaries

as compared to nulliparous in the same stage in any population and assumed that the value of the probability of survival through one day is fairly constant during most of the life of a female, the survival rate of Anopheles minimus and Anopheles fluviatilis have been calculated. In the case of Anopheles minimus, on the basis of 247 dissections a daily survival rate of 88-89% has been calculated in unsprayed areas using the above technique.

7. Mrs M. Spencer (Malaria Section, Department of Public Health, Port Moresby, Territory of Papua and New Guinea)

The difficulty of working on a new technique, such as that of Polovodova, in isolation has been emphasized by Mrs Spencer. In Papua where the gonotrophic cycle of A. farauti in coastal areas is generally completed in 48 hours, most parous females captured for dissection in night catches on human bait or resting in houses are found to have ovaries at Stage III or later. Mrs Spencer considers that under local conditions there is insufficient time for complete involution of the follicular tube to occur following oviposition so that "beads" are not normally seen and hence cannot be used as a reliable indication of a uniparous female.

However, this worker has paid particular attention to (i) the length of the follicular "sac" and, (ii) the definition of old follicular sites in the "sac". Dissecting in saline tinted with methylene blue, she finds that old follicular sites are "vaguely distinguishable as more or less diffuse masses of epithelial debris". She recommends the extension of phase-contrast microscopy for such studies. In order to confirm that the follicular changes in A. farauti follow the same pattern as that observed in other species such as A. maculipennis with a longer gonotrophic cycle, Mrs Spencer suggests that one or both of the following methods should be explored: (1) by slowing ovulation, e.g. by cooling and/or by protein starvation, and (2) by histological examination of the follicles at all stages of the cycle by serial section.

During a study of about 800 A. farauti, including at least 8000 follicles, Mrs Spencer was able to count "beads" in a fair proportion, the greatest number being nine in one female, but in general she relied on the criteria mentioned in paragraph 2 above. In one series of 95 parous females she records the following data:

2nd ovulation - 38 females; 3rd - 38; 4th - 16; 5th - 2; 6th - 1. She states: "Care must be taken with some uniparous female A. farauti - those which have matured only a few oocytes and are therefore most likely ahead of their real age-group. They should be counted as nulliparous."

Comparing the value of "p" obtained for A. farauti by her application of Polovodova's method during periods of stable population density, Mrs Spencer finds that this value, 0.84 - 0.85, agrees essentially with the value of "p" obtained indirectly by analysis of the parasite and sporozoite rates in this area. In addition to the obvious value of the present method in comparing A. farauti populations of sprayed and unsprayed areas, Mrs Spencer hopes to employ it as a tool in carrying out further detailed bionomic studies on biting times, house visiting, etc. in relation to mosquito age. So far she does not appear to have examined the possibilities of studying the ovarian tracheoles.

Discussion of Reports¹

Before discussing some of the points which have arisen in connexion with this preliminary survey, it would be as well to state clearly the main objective of this age-grouping in the entomology of malaria eradication. These principles have been clearly defined recently by Beklemishev, Detinova & Polovodova (1959) "Determination of Physiological Age in Anophelines and of Age Distribution in Anopheline Populations in the USSR", Bull. Wld Hlth Org. 21, 223.

"The widespread use of residual insecticides has greatly increased the importance of determination of the age of malaria vectors. Complete coverage with insecticides of all the resting places of anophelines results in such high mortality during each gonotrophic cycle that the proportion of females reaching the age at which sporozoites may appear in the salivary glands is practically insignificant so far as the transmission of the infection is concerned. Thus the main objective of insecticide treatment is to affect the longevity of the local vector population. The most direct way of checking the efficiency of imagicidal measures is the study of the age composition of the vector population treated with insecticides, and a comparison on the one hand with the age composition of untreated populations and on the other hand with the age of populations so successfully treated with DDT that malaria transmission has been interrupted."

¹ This part was written by Dr R. C. Muirhead-Thomson.

These basic principles have been amply confirmed by the very thorough studies which have been carried out on the epidemiology of A. maculipennis-borne malaria in the USSR. The intensification of this age-grouping investigation in predominantly tropical areas outside the USSR has been initiated and stimulated by the reasonable expectation that the same basic principles worked out with A. maculipennis would be applicable to most if not all of the tropical anopheline vectors of malaria.

The first practical objective of the work would be to establish on an incontrovertibly factual basis that any reduction of malaria transmission resulting from intensive house treatment with residual insecticides, was due primarily to a significant reduction in the proportion of females which reach a potentially dangerous age. As far as the present reports are concerned, however, evidence to support this logical presumption is not yet forthcoming. Work on A. stephensi mysorensis in Iran has not yet revealed any obvious difference in age-groups between treated (DDT and dieldrin) and untreated areas. The figures for A. culicifacies in Baroda show that the ratio nulliparous/parous is much the same before and after DDT spraying. In the same way, the observations in the Upper Volta Republic showing that the proportion of parous females (vector species A. gambiae and A. funestus) was always found to be lower in the DDT treated area than in the untreated control, is rather robbed of its significance by the finding that the same reduction in parous rates applied to A. coustani, a species which normally does not enter houses and whose physiological age could scarcely be affected by house treatment with residual insecticides.

Studies on the entomology of malaria eradication continue to provide so many unexpected and apparently illogical findings, that it would seem advisable at this stage to proceed with caution and not to assume too readily that the mechanism by which malaria transmission by one species is reduced as a result of house treatment with insecticides is necessarily applicable to another species. It is only too easy to become unduly preoccupied with the age factor, and to assume that the possibility of transmission must necessarily continue to exist as long as mosquitos are still capable of surviving to a potentially dangerous age. This assumption overlooks entirely the real possibility that the feeding habits of some vector

species may have been altered by residual insecticide campaigns, and that there may have been a drastic reduction in their degree of contact with man. Such species, compelled to feed to an increasing extent on animal hosts outdoors, and therefore less exposed to insecticide-treated surfaces, might well continue to show a high proportion of females of "potentially dangerous age" at a time when interruption of transmission was actually being achieved as a result of the reduced contact between mosquito and man.

In this study of age-groups and the interpretation of entomological data relevant to malaria eradication, the importance of sampling methods has been well brought out by Hamon's work in the Republic of the Upper Volta. This indeed may well prove to be one of the more important outcrops from the present age-grouping work. Entomologists are in the habit of using a variety of sampling techniques. Frequently, samples of mosquito populations in treated areas are assessed by techniques which differ from those employed in the untreated control. It seems that the age-grouping technique, whether based on the simple proportion of nulliparous to parous or on the more elaborate analysis by Polovodova's method, may be of the greatest value in estimating the validity of different sampling techniques.

At this comparatively early stage in the study of age-grouping, advantage should be taken of the fact that in many as yet untreated tropical areas high natural infection rates are still recorded among the vector species. The recording of gland-infected mosquitos can still provide valuable confirmatory evidence about the presence of female mosquitos of dangerous age. In this respect tropical workers have a great advantage over the pioneers of the age-grouping technique in the USSR, who were working mainly with a species - A. maculipennis - in which the very low natural infection rates completely precluded the use of infection rates as confirmatory evidence on the question of physiologically dangerous age. These combined studies on age-grouping and sporozoite rates appear to be particularly promising with A. gambiae and other species in Tropical Africa Republic, and with A. minimus in Nepal.

Many workers in the entomological field of malaria eradication may already be aware of the significant work that has been done on Mansonioides in Malaya in the way of carrying out parallel studies on age-grouping and on infection with natural

filaria larvae. This work revealed some puzzling discrepancies, as for example, the finding that 40% of mosquitos which were known to have lived for at least ten days since feeding on an infective host, had laid only one batch of eggs, although the gonotrophic cycle is normally 3-4 days. Wharton has pointed out that the period spent travelling from feeding ground to breeding place must be taken into account when calculating mosquito survival.

Although the age-grouping studies have not yet been able to provide the evidence required about the longevity of the female mosquito in relation to malaria eradication projects based on residual insecticides, the amount of light which has been shed on other aspects of mosquito behaviour and biology is very stimulating. This applies particularly to two parallel investigations which up till now have been pursued quite independently, namely the work on A. gambiae and other species in West Africa, and the work on A. farauti in Papua and New Guinea.

One of the fascinating, although occasionally disconcerting, aspects of work on the biology of anophelines, is that each new technical advance tends to produce, or rather to reveal, almost as many problems as it solves. This was certainly the case with the precipitin test for identifying the source of the mosquito's blood-meal. There is a real possibility that the same tendencies may spread with regard to this new technique of age-grouping. A significant example is provided by the independent report on the results obtained with A. minimus and A. vagus in Viet Nam. This work is a striking demonstration of what can be achieved when highly proficient specialists in this age-grouping technique turn their attention to a tropical problem. Certainly, with regard to A. minimus, the age composition results appear to be in close accord with the known high vectorial capacity of this species: but, unfortunately, on the same age-grouping evidence, A. vagus is also revealed as a species of potential epidemiological importance. At the moment this appears quite contrary to all established views, which indicate that this common and widespread species plays a very minor, or even a completely negligible, role in malaria transmission. Other negative evidence based on an imposing body of epidemiological observations and on dissecting records carried out over many years cannot lightly be discarded. Nevertheless, the germ of doubt has now been implanted, and until further evidence is forthcoming to support the idea or to discount it entirely, those of us who are on familiar terms with A. vagus will find it difficult to recapture the easy complacency with which we have long regarded this mosquito.

COMPARATIVE DATA ON THE BIOLOGY OF A. MINIMUS AND
A. VAGUS AT TAY-NGUEN¹

(Viet Nam Democratic Republic)

During the period January 1956 to March 1957 the Soviet malaria team carried out an entomological survey in the Province of Tay-Nguen about 80 kilometres from Hanoi. Among the 11 836 Anopheles collected, 13 species were found. They included the following: A. minimus, A. aconitus, A. jeyporiensis, A. vagus, A. hyrcanus sinensis, A. barbirostris, A. maculipalpis, A. philippinensis, A. fuliginosus, A. karwari, A. maculatus, A. tellelatus, A. kochi.

Of the total number of 9930 mosquitos found in houses, 77% were A. vagus, 3% were A. minimus and the remaining 11 species comprised 20% of the total figure. In the course of the torrid and humid period (April-September) most numerous were A. vagus, while A. minimus, absent during that time, could be encountered in the dry and cool season of the year (November-March). A. vagus bred both in the temporary and permanent water basins, filled with turbid water. On the other hand, A. minimus larvae were met with only in clear flowing waters of springs, creeks, channels and in wells.

In the case of A. minimus the minimal duration of blood digestion comprised 48 hours, at the temperature of 24-28°C, the maximal being 120 hours, at the temperature level of 16-17°C.

In Table I the physiological age of epidemiologically dangerous females of Anopheles in Viet Nam North at various temperatures is shown.

(Duration of sporogony and blood digestion is given in days, the age-grouping is based on the number of dilatations in ovarioles.)

¹ Summary of paper by Zalutskaya, L. I. (1959) Med. Parazit. (Mosk.) 28, 548.

Appendix 1

TABLE I. PHYSIOLOGICAL AGE OF EPIDEMIOLOGICALLY DANGEROUS FEMALES OF ANOPHELES IN VIET NAM NORTH AT VARIOUS TEMPERATURES

| Temperature | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
|-----------------------------|----|------|----|------|----|----|----|------|----|----|----|----|----|
| Duration of sporogony | 55 | 38.5 | 29 | 24.5 | 19 | 17 | 15 | 12.5 | 11 | 10 | 9 | 8 | 7 |
| Duration of blood digestion | | 5 | 4 | 3.5 | 3 | | | 2.5 | | | 2 | | |
| Age of dangerous females | 8 | 7 | 6 | 5 | | 4 | | | | | 3 | | |

The age-composition of A. minimus and A. vagus was studied using the method of Polovodova. The 559 females of A. minimus and 5918 females of A. vagus were investigated. The results of this investigation are shown in Table II.

TABLE II. AGE-DISTRIBUTION OF A. MINIMUS AND A. VAGUS DURING 1956 AND THE FIRST QUARTER OF 1957

| Gonotrophic cycle | Month | | | | | | | | | | | | | | |
|-------------------|-------------------|----|-----|-----|-----|------|------|------|------|------|------|-----|------|----|------|
| | I | II | III | IV | V | VI | VII | VIII | IX | X | XI | XII | I | II | III |
| | <u>A. minimus</u> | | | | | | | | | | | | | | |
| 0 | 2 | 11 | 5 | 3 | - | - | - | - | 1 | 6 | 1 | - | - | 2 | 3 |
| 1 | 3 | 29 | 24 | 6 | 3 | - | - | - | 11 | 16 | 13 | 3 | - | - | 7 |
| 2 | 2 | 17 | 27 | 11 | 1 | - | - | - | 9 | 29 | 23 | 6 | 7 | 1 | 16 |
| 3 | 1 | 9 | 7 | 3 | - | - | - | - | - | 11 | 31 | 21 | 13 | 13 | 26 |
| 4 | - | - | 3 | 1 | - | - | - | - | - | 4 | 13 | 11 | 19 | 6 | 14 |
| 5 | - | - | 1 | - | - | - | - | - | - | - | 4 | 3 | 6 | 3 | 13 |
| 6 | - | - | - | - | - | - | - | - | - | - | 3 | 2 | 5 | 1 | 7 |
| 7 | - | - | - | - | - | - | - | - | - | - | 3 | 2 | 2 | - | 4 |
| 8 - 10 | - | - | - | - | - | - | - | - | - | - | - | 1 | 3 | 1 | 1 |
| No. examined | 8 | 66 | 67 | 24 | 3 | - | - | - | 21 | 66 | 88 | 47 | 55 | 33 | 91 |
| No. dangerous | 0 | 0 | 1 | 1 | 0 | - | - | - | 0 | 15 | 7 | 1 | 16 | 0 | 25 |
| % age " | - | - | 1.4 | 4.1 | - | - | - | - | - | 22.7 | 8.0 | 2.1 | 29.0 | - | 27.5 |
| | <u>A. vagus</u> | | | | | | | | | | | | | | |
| 0 | 2 | 2 | 7 | 68 | 25 | 39 | 122 | 41 | 33 | 21 | 5 | - | 1 | 0 | 1 |
| 1 | 3 | 5 | 61 | 217 | 247 | 258 | 144 | 484 | 139 | 48 | 16 | - | 2 | 0 | 0 |
| 2 | 4 | 2 | 36 | 294 | 210 | 254 | 393 | 587 | 291 | 134 | 38 | 1 | 1 | 1 | 1 |
| 3 | 1 | 1 | 8 | 108 | 45 | 81 | 108 | 324 | 199 | 148 | 55 | 9 | 4 | 0 | 1 |
| 4 | - | - | 1 | 20 | 5 | 24 | 9 | 75 | 152 | 87 | 31 | 6 | 5 | 2 | 3 |
| 5 | - | - | - | - | 3 | 16 | 5 | 14 | 14 | 39 | 17 | 6 | 2 | 4 | 2 |
| 6 | - | - | - | - | - | 5 | 1 | 3 | 2 | 14 | 8 | 2 | - | - | - |
| 7 | - | - | - | - | - | 2 | - | 1 | - | 3 | 1 | - | - | - | - |
| 8 | - | - | - | - | - | - | - | - | - | 1 | 1 | - | - | - | - |
| No. examined | 10 | 11 | 113 | 708 | 535 | 678 | 782 | 1531 | 830 | 495 | 172 | 24 | 15 | 7 | 11 |
| No. dangerous | 0 | 0 | 0 | 20 | 53 | 126 | 123 | 418 | 367 | 292 | 27 | 0 | 2 | 0 | 2 |
| % age " | - | - | - | 2.8 | 9.9 | 18.4 | 15.7 | 27.2 | 44.2 | 57.0 | 15.6 | 0 | 13.3 | 0 | 18.1 |

Potentially dangerous females of A. minimus were present during March-April and October-December 1956 and in January and March of 1959. The bulk of A. vagus females of epidemiologically potential danger made their appearance in May-October. Both species were encountered, principally, within living quarters, where they largely fed on the human blood.

The abundance of A. vagus during the time-interval comprising April-November, their constant presence in living premises, clearly pronounced disposition towards sucking of human blood and the ability of the females to survive until after reaching the age of epidemiologically potential dangers - all this makes it impossible to deny the epidemiological importance of this species.

Moreover, results of precipitin tests carried out in females of A. minimus and A. vagus are shown in Table III.

TABLE III. PRECIPITIN TESTS

| | Month | | | | | | | | | | | | Total |
|-------------------|-------|----|-----|----|---|----|-----|------|----|---|----|-----|----------|
| | I | II | III | IV | V | VI | VII | VIII | IX | X | XI | XII | |
| <u>A. minimus</u> | | | | | | | | | | | | | |
| Total | 8 | 37 | 26 | 4 | - | - | - | - | 5 | 5 | 25 | 25 | 134 |
| Human blood | 3 | 7 | 6 | 1 | - | - | - | - | 2 | 5 | 17 | 0 | 41 (30%) |
| <u>A. vagus</u> | | | | | | | | | | | | | |
| Total | 2 | 3 | 23 | 70 | 8 | 8 | 52 | 35 | 13 | 9 | 2 | 2 | 217 |
| Human blood | 1 | 1 | 2 | 4 | 0 | 2 | 10 | 11 | 4 | 3 | 1 | 0 | 39 (16%) |