

WORLD HEALTH ORGANIZATION

MONOGRAPH SERIES

No. 47

AGE-GROUPING METHODS IN DIPTERA
OF MEDICAL IMPORTANCE

With Special Reference to Some Vectors of Malaria

AGE-GROUPING METHODS IN DIPTERA OF MEDICAL IMPORTANCE

With Special Reference to Some Vectors of Malaria

T. S. DETINOVA

*Martsinovsky Institute of Medical Parasitology
and Tropical Medicine, Ministry of Health, Moscow, USSR*

With a Foreword by

W. N. BEKLEMISHEV

*Member of the Academy of Medical Sciences of the USSR
Martsinovsky Institute of Medical Parasitology
and Tropical Medicine, Ministry of Health, Moscow, USSR*

And an Annex on the Ovary and Ovarioles of Mosquitos
(with glossary) by

D. S. BERTRAM

*Professor of Entomology, London School of Hygiene
and Tropical Medicine, London, England*



WORLD HEALTH ORGANIZATION

GENEVA

1962

PRINTED IN SWITZERLAND

CONTENTS

	Page
Preface	7
Foreword, by W. N. Beklemishev	9
CHAPTER 1. General review	13
CHAPTER 2. Functional and age changes in the reproductive system of female <i>Anopheles maculipennis</i>	16
CHAPTER 3. Normal and abortive oogenesis in female <i>Anopheles</i> <i>maculipennis</i>	30
CHAPTER 4. Changes related to the excretory, digestive and repro- ductive processes of female <i>Anopheles maculipennis</i>	48
CHAPTER 5. Methods of determining the physiological age of female <i>Anopheles</i>	69
CHAPTER 6. Functional and age changes in the reproductive system of some blood-sucking and non-blood-sucking Diptera	78
CHAPTER 7. The biology of <i>Anopheles maculipennis</i> populations .	94
CHAPTER 8. Determination of the epidemiological importance of populations of <i>Anopheles maculipennis</i> by their age composition	122
CHAPTER 9. The age composition of female <i>Anopheles</i> as a criterion in estimating the effectiveness of antimosquito measures	151
Acknowledgements	169
References	173
ANNEX. The ovary and ovarioles of mosquitos, by D. S. Bertram	193
INDEX	213

PREFACE

The success of malaria eradication is to a great extent dependent on a knowledge of the longevity and age composition of vector populations. Over the past twenty years entomologists in the USSR, in their development of age-grouping techniques, have made a significant contribution to research on the bionomics of insect vectors of disease generally, and to entomological evaluation of malaria eradication in particular.

Full accounts of this work have hitherto been available only in the Russian language, and the present monograph has been published by the World Health Organization in the hope that it will make widely available, in concise form, the results of this new approach to applied entomological research.

The text is a substantially revised and expanded version of a series of lectures given by Dr Detinova during a WHO-sponsored "Course in Advanced Entomological Techniques applied to Malaria Eradication", held at the London School of Hygiene and Tropical Medicine in 1959. Professor Bertram of the Department of Entomology of the School, who helped to organize the Course, has contributed an illustrated annex on the ovary and ovarioles of mosquitos, together with a glossary of terms adapted to the new concepts.

FOREWORD

by

Professor W. N. BEKLEMISHEV

Member of the Academy of Medical Sciences of the USSR

It is well known that in campaigns against malaria no single method can be applied everywhere. The means and methods of control must always take into account the local epidemiological situation. The better we understand the epidemiological conditions of local transmission of malaria the more correct will be our choice of control measures and the more successful will be the results obtained. A particularly thorough study should be devoted to the biology of local vectors, since a knowledge of it is the basis of our understanding of the epidemiology of the malaria focus. Furthermore, it usually proves impossible to clear up an important focus of malaria without acting on the vector population. Thus, it is obvious that the progress of global malaria eradication depends on the constant improvement of our knowledge of mosquito biology. The present monograph deals with some of the most important and, until recently, little-known entomological problems—namely, the study of the age composition of mosquito populations, its effect on malaria transmission and its changes as a consequence of imagocidal measures.

As long ago as 1910, Ross realized the importance for malaria epidemiology of the age-group distribution of mortality in anopheline populations. This problem became especially important in the 1940's following the introduction of residual insecticides. During that period, research workers in the USSR devised satisfactory methods for determining the age of mosquitos and used these methods for studying the population biology of *Anopheles* and for checking the effectiveness of contact insecticides and improving their application. Dr T. S. Detinova played a leading part in all these researches and her monograph represents an original work rather than a simple review of the problem.

The ability to determine the age of any individual belonging to an animal species is a powerful method for gaining a knowledge of the biology of that species. Whenever a method is found for determining the age of a particular group of animals it becomes a starting-point for much progress in the study of the natural history of the group. It is enough to recall the stimulus given to ichthyology by the discovery at the beginning of the 20th century of a method of determining the age of fishes from

annual rings on their scales and bones. A means of determining the age of female mosquitos from the increased size of the ampullae of their oviducts was suggested in the early 1930's by G. Mer. In spite of its shortcomings this method enabled us to study a number of points concerning the natural history of anopheline populations in the USSR. Increasing experience gained during the dissection of many thousands of mosquitos gradually led us to the conclusion that not only the ampullae of the oviduct but the whole female reproductive system of the mosquito undergoes changes related to the age of the specimen. The study of these changes enabled Dr T. S. Detinova, at the beginning of the 1940's, to distinguish more accurately the changes in ovarian tracheation, and thus provided the means for achieving a clear distinction between parous and non-parous females. Later, a more precise method was evolved by V. P. Polovodova, who was able to determine exactly the physiological age of the female mosquito (the number of completed gonotrophic cycles) from the number of dilatations found in its ovarioles.

We distinguish between the calendar and physiological age of animals: calendar age is measured by the number of days or years the animal has lived, while physiological age can be assessed from the accumulation of irreversible changes in the organism as a consequence of its normal life. Thus, the physiological age of a mosquito larva is assessed from the number of completed ecdyses, regardless of the duration of each of the preceding larval instars. The physiological age of an adult gonoactive female mosquito is just as naturally determined by counting the number of gonotrophic cycles completed.

In warm-blooded animals the rate at which internal processes take place bears comparatively little relation to environmental conditions, and it is not so necessary, therefore, to distinguish between physiological and calendar age. In cold-blooded animals, on the other hand, the speed of all the vital processes depends very largely on the temperature of the external environment, and for such animals the establishment of biological laws is often more easily effected by the determination of physiological age than by the determination of calendar age. A knowledge of the temperature relationship between physiological processes and the outside temperature under which the given individual has been living makes it possible to calculate an animal's calendar age from its physiological age.

The precise correlation between the process of digestion and the maturation of eggs is termed "gonotrophic concordance" (after Swellengrebel). This gonotrophic concordance is characteristic of a number of the oldest groups of insects which suck blood periodically. In the blood-sucking Diptera (Orthorrhapha) the gonotrophic rhythm governs not only the process of digestion and oogenesis, but also the endocrine secretion, the metabolism, the activity of the Malpighian tubules—in fact most of the vital functions of the insect. The alternation of consecutive gonotrophic cycles

leaves irreversible traces not only in the structure of the female reproductive system and some other organs, but also in the physiological functions of the animal. With the increase of the physiological age, the fertility, the general intensity of metabolism and the activity of the Malpighian tubules are reduced, the enzyme activity falls, and in some arthropods (argasid ticks) (G. S. Balashov) the ability to withstand long fasting is decreased. Thus, the number of gonotrophic cycles completed by the female is really a measure of its physiological age.

Determination of the number of completed gonotrophic cycles is of still greater importance for an understanding of the natural history of an insect population. For the vectors of infectious diseases the chances of being infected and of infecting are repeated in each gonotrophic cycle. Thus, the calculation of the physiological age of a vector, from the number of completed gonotrophic cycles, is indispensable to a study of the epidemiology of all diseases transmitted by blood-sucking insects belonging to the lower Diptera. Knowledge of the physiological age of vectors and the age composition of the population is of particular value in estimating the effectiveness of antimalaria measures based on the use of residual insecticides.

Determination of the physiological age of insects from the number of dilatations remaining in the ovarioles after oviposition is not only applicable to mosquitos, and its importance is not limited to the problem of malaria eradication. It has been shown to be possible to determine the physiological age of females of many blood-sucking Diptera and other insects by similar means. Thus the method can be used in studying the epidemiology of a very wide group of infections. There is no doubt but that a similar method could also be evolved for a number of agricultural pests, and that it might thus take on an importance going far beyond malariology and medical entomology.

As far as malaria eradication is concerned, it is hoped that the methods described in this monograph will serve as a starting-point in all countries for an extensive study of the age composition of mosquitos in connexion with the epidemiology of this disease. I am convinced that international co-operation in this sphere will lead to the further development of the study of vector biology and thus to the speeding-up of global malaria eradication.

*Martsinovsky Institute of Medical Parasitology and
Tropical Medicine, Ministry of Health, Moscow, USSR*

Chapter 1

GENERAL REVIEW

For almost half a century entomologists have been working on problems connected with the determination of the physiological age of insect vectors of disease. It is understandable why this particular group of insects was the first to attract the attention of research workers. The connexion between the epidemiological effectiveness of the vectors and the duration of their blood-sucking activity was clear. Obviously, this connexion is based on the fact that each blood-meal affords the possibility either of the arthropod being infected from a host or of the host being infected from the relevant vector.

Since the end of the 1930's, entomologists in the USSR have undertaken the task of working out the best possible methods for discovering the epidemiological importance of vector populations and for assessing the effectiveness of control measures against various Diptera injurious to human health. All these investigations have been carried out by entomologists trained by Professor W. N. Beklemishev and working under his leadership.

The changes which take place in the reproductive organs of the disease vectors have been used as a basis in working out all the methods suggested. Careful study of the reproductive system of a considerable number of female Diptera led to the conclusion that all its parts undergo considerable changes connected with age or function.

The functional changes include those which are repeated during the development of each batch of eggs. They amount, in the main, to a considerable increase in the over-all dimensions of the ovaries caused by the development of the follicles, and to a subsequent sharp decrease of those dimensions after ovulation. There is also an increase in the size of the internal oviducts caused by the passage of the developed eggs, and an increase in the size of the accessory glands during the development of the ovaries, followed by a decrease after the laying of the mature eggs. Functional changes have also been noted in the dimensions of the ampullae of female *Anopheles*.

Conversely, the changes due to age consist of gradually accumulating irreversible alterations in the reproductive organs connected with the

functional activity of the ovaries. Such changes occur in various sections of the organs and afford an opportunity of determining the physiological age of the female with varying degrees of accuracy. Some changes, as will be shown, make it possible to determine whether the females are parous or nulliparous; others allow for distinguishing between nulliparous, pauciparous and multiparous females, and there are some which permit an accurate determination of the number of ovipositions performed by each female.

Functional changes and age changes in the reproductive system of the females are closely interconnected and consequently some functional changes may appear as age changes in the course of the life of the female insects.

All members of the blood-sucking Diptera (Orthorrhapha), i. e., Culicidae (Anophelini and Culicini), Simuliidae, Heleidae, Tabanidae and *Phlebotomus*, are characterized by "gonotrophic concordance", a term which was first proposed by Swellengrebel (1929) and which refers to the close connexion between the process of blood digestion and the development of the ovaries. In anophelines this connexion was first pointed out by Kozhevnikov in 1903. In cases of gonotrophic concordance, a full blood-meal is necessary for the maturing of one batch of eggs. The whole period from the beginning of one blood-meal to the next includes three phases: (1) the search for a host and the obtaining of the blood-meal; (2) digestion of the blood and egg formation; and (3) the search for breeding-places and ovipositions. These phases comprise one gonotrophic cycle. The number of gonotrophic cycles undergone by each female represents her physiological age. The ability to determine the duration of each gonotrophic cycle makes it possible to assess also the calendar age of the vector, i. e., the number of days it has lived.

Where gonotrophic concordance exists in blood-sucking Diptera, the determination of the physiological age of the females enables us at the same time to establish the number of blood-meals the females have taken, i. e., to assess their epidemiological importance.

In those blood-sucking insects which do not show gonotrophic concordance (Stomoxydinae, Hippoboscidae, *Glossina*, Aphaniptera, Anoplura, etc.) it is not possible to determine the number of blood-meals from the conditions of the reproductive system. In all these insects the number of blood-meals is always considerably greater than the number of ovipositions they have performed or the number of pre-pupae they have deposited. However, data on the physiological age of these females, obtainable with a fair degree of accuracy (the relevant details will be given later), enable us to determine the longevity of blood-sucking insects.

For disease vectors among the Diptera which do not suck blood (house-flies) the number of ovipositions indicates the approximate age of the females and their sexual activity.

The determination of the physiological age of female blood-sucking Diptera is of both practical and theoretical interest. The practical importance of the question lies in the fact that data on the physiological age of females give information on the mean age of the population, and make it possible not only to determine the causes of changes which occur in the composition of the adult portion of the population, but also to establish the epidemiological significance of the vectors.

The reliability of all these quantitative data depends on the degree of accuracy with which the physiological age of the females and the age composition of the whole population are determined. In past years when age determination depended on most primitive methods, the data obtained on the biology, ecology and epidemiological importance of the population and the assessment of the effectiveness of control measures were far from accurate. At the present time, the possibility of determining with accuracy the physiological age of many blood-sucking Diptera places in our hands the key to the solution of many hitherto obscure problems of the biology of these insects and allows for correct assessment of the effectiveness of control measures (Detinova, 1952b, 1957; Beklemishev, Detinova & Polovodova, 1959).

Moreover, on the basis of functional and age changes in the reproductive system of female Diptera, it has been possible to obtain facts concerning the comparative anatomy of various representatives of this order and to discover a number of physiological features dependent on age. The latter point has been investigated in *Anopheles maculipennis* and will be considered later.

Unfortunately, entomologists have not so far tackled the study of age changes in the reproductive system of insect pests of field crops, forests, orchards, stored grain, etc. If they had, their research would have provided the necessary facts on which to base the correct organization of control measures and the accurate assessment of their effectiveness.

Among all the blood-sucking Diptera, the malaria mosquitos are of great importance as vectors of a vicious and widespread disease; consequently they were among the first insects in which age changes in the female reproductive system underwent a careful study. A number of the latest studies by entomologists in the USSR on methods of determining the physiological age of female mosquitos and the age composition of populations were translated by Gillies, who later (1958) wrote a review of work carried out in this connexion in the USSR.

Chapter 2

FUNCTIONAL AND AGE CHANGES IN THE REPRODUCTIVE SYSTEM OF FEMALE *ANOPHELES MACULIPENNIS*

The structure of the organs of reproduction in females of *Anopheles maculipennis* and other mosquitos has been thoroughly studied and dealt with in great detail by Kulagin (1901),¹ Kozhevnikov (1903, 1905), Christophers (1911, 1923), Nicholson (1921), Mer (1932), Beklemishev (1944), Polovodova (1941, 1947, 1949), Detinova (1949) and others.

Structure of the Female Reproductive System

The reproductive system of female mosquitos consists of two ovaries, two external paired oviducts, an unpaired oviduct with its ampullae, a vagina, a spermatheca and an accessory gland. The small ovaries in the newly emerged female are situated in the area of the fourth and fifth abdominal segments. As each batch of eggs matures the ovaries increase in size and, on completion of development, take up the whole abdominal cavity from the first to the seventh segment. Each ovary is covered by an external membrane which ends proximally in a terminal process and continues distally over the external paired and unpaired ducts. Inside each ovary there is an internal oviduct into which ovarioles run radially. The number of ovarioles is not constant. Each ovariole consists of a growth zone or germarium, situated in its proximal portion, and, in the case of gonoactive females, usually of two follicles; it is always only the follicle lying nearest to the internal oviduct which develops completely after a blood-meal, provided that the female was gonoactive.

In blood-sucking mosquitos, as in all Diptera, the follicles are of the polytrophic type: the nurse cells are contained in a single egg chamber with the oocyte. A follicle contains one oocyte and seven nurse cells.

¹ One of the earliest papers on the oogeny and the detailed internal anatomy of the female reproductive system of *Culex pipiens* is that by Lécaillon (1900). Giles (1900) also described the finer anatomy of the ovary and of the oviduct of mosquitos. Kulagin's important work (1901) dealt with the oogeny of *Culex pipiens* and *Anopheles bifurcatus*; he also corrected the mistake made by Giles, who thought that the spermatheca was merely a secreting gland and not a "receptaculum seminis".—ED.

Only in follicles which have just separated from the germarium or in follicles of non-gonoactive females is the oocyte undifferentiated from the nurse cells and all its eight cells appear alike. Each follicle is surrounded by a follicular epithelium, which is formed when the follicle comes away from the germarium. The epithelium of the newly separated follicle is always less developed than in a mature follicle. It is well known that the developing follicle passes through a number of phases, first described by Christophers (1911), who designated them stages I, II, III, IV and V. Mer (1936) distinguished two further separate stages associated with the initial period of the follicle and called them N and I-II. The stages of follicular development are classified according to the following characteristics (Fig. 1):

N—a follicle consisting of eight undifferentiated cells. The follicle is spherical and the follicular cells compose a regular cubical epithelium.

I—one oocyte, situated in the distal portion of the follicle, is clearly visible. Above the oocyte lie seven nurse cells. The follicle either retains its spherical shape or becomes slightly oval.

I-II—a crown of one or two rows of yolk granules appears round the nucleus in the oocyte protoplasm. The follicle takes on an oval shape.

II—larger and more numerous yolk granules are seen in the protoplasm of the oocyte around the egg. The egg grows, becomes considerably larger than the nurse cells and takes up about half the follicle.

III—the egg gradually increases its share of the follicle space from one half to three-quarters. Its nucleus is no longer visible through the mass of yolk. The follicle becomes somewhat elongated.

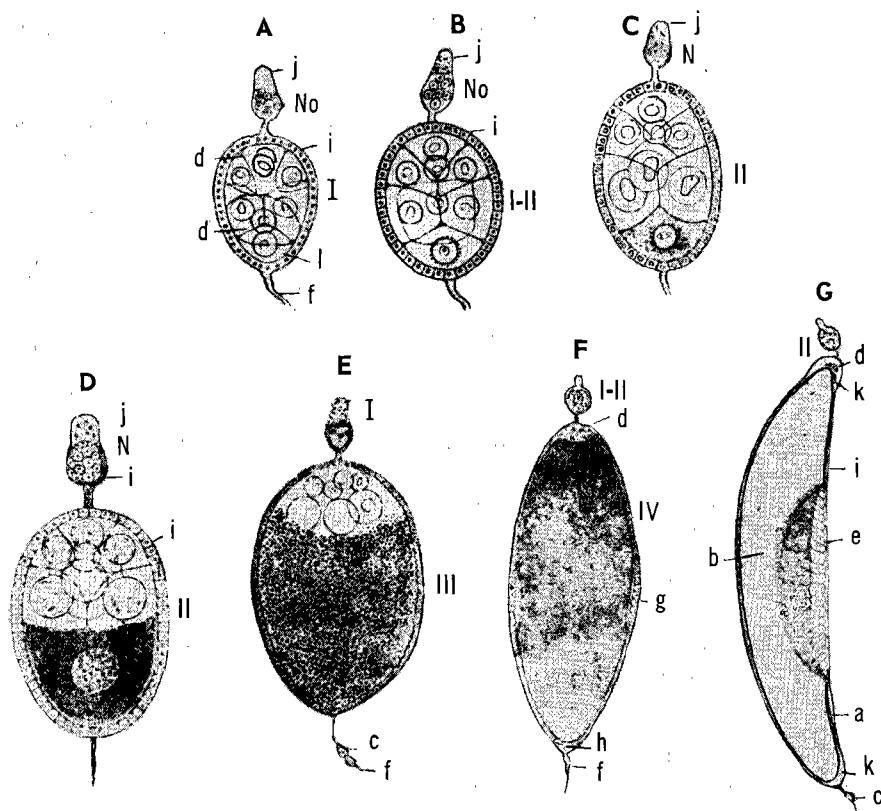
IV—the follicle becomes longer and the nurse cells occupy only the uppermost part of it. The oogonium, full of yolk, is well developed and occupies more than nine-tenths of the follicle.

V—the chorion covers the whole egg. The remains of the nurse cells are found at the proximal end of the follicle. The floats appear on the egg, which is now ready for laying.

Nicholson (1921) studied the development of the eggs on histological sections. He observed the accumulation of yolk granules first of all around the nucleus of the oocyte (or rather the oogonium), which in its gradual development pushes the nurse cells up to the proximal end of the follicle. In later stages the follicle grows longer and the oogonium, filled with yolk granules, becomes considerably bigger and opaque.

The follicle is transparent only at the top, where the nurse cells lie. The relative size of this portion decreases in every succeeding phase and at the end of the development the nurse cells are outside the egg chorion.

FIG. 1. MATURATION OF EGGS IN *ANOPHELES MACULIPENNIS MESSEAE*:
THE APPEARANCE OF INDIVIDUAL OVARIOLES*



- A First follicle in stage I, second follicle in stage N₀
- B First follicle in stage I-II, second follicle in stage N₀
- C First follicle at the beginning of stage II, second follicle at the beginning of stage N
- D First follicle at the end of stage II, second follicle in stage N
- E First follicle at the end of stage III, second follicle in stage I-II
- F First follicle at the end of stage IV, second follicle in stage I-II
- G First follicle at the end of stage V, second follicle in stage II

All drawings are based on live ovaries in saline; drawings E and G refer to parous females; in the distal portion of the ovariole corpora lutea are visible (original); a, frill; b, chorion; c, dilation; d, nurse cells; e, floats; f, distal portion of ovariole; g, cells of follicular epithelium—float formers; h, cells forming micropyle system; i, follicular epithelium; j, germarium; k, papillae of micropyle; l, oocyte; N₀, N, I, I-II, III, IV, V, follicles in the corresponding stages of development.

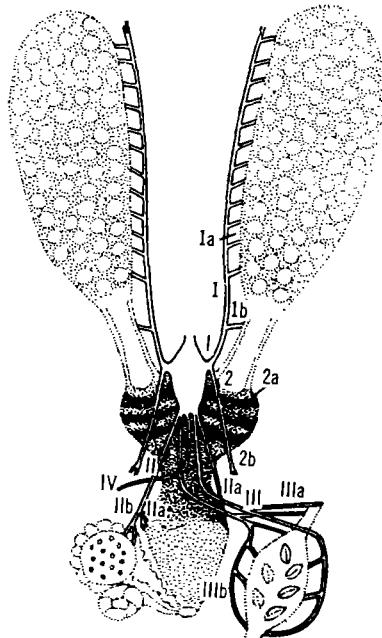
* After Beklemishev (1944).

Nicholson showed that the inner surface of the follicular epithelium secretes a gelatinous substance which gives rise to the endochorion and that the chorion as a whole is formed afterwards. In the centro-lateral part of the follicle the cells of the follicular epithelium, arranged in two symmetrical groups, become larger and give rise to the floats. In the portion previously

occupied by the nurse cells a micropyle forms, i.e., the structure through which the sperm penetrates into the egg during insemination. The fate of the nurse cells which remain outside the chorion when egg development is completed was not investigated by Nicholson but he suggested that these cells are used up during the formation of the micropyle and disappear without trace. He showed that the whole of the ovariole is enclosed in an internal membrane or intima closely adhering to the germarium and the follicles. At its distal end the intima forms the efferent portion of the ovariole. The present author proposed that this efferent portion be called the "terminal pedicle" (Detinova, 1949). This terminal pedicle consists of a fine hollow tubule with scarcely perceptible constrictions, of which the form and dimensions do not change until the end of the first gonotrophic cycle.

In addition to the intima every ovariole is surrounded, according to Kulagin (1901), by an external membrane made of sparse connective tissue and muscular fibres. During oviposition a rhythmic contraction of the ovarian membrane and of the external membranes of the ovarioles takes place, enabling the fully developed eggs to be carried forward. The innervation of the reproductive system of female *Anopheles* was described by Polovodova in 1953 (Fig. 2). She demonstrated that a pair of nerves have their origin in the anterior half of the last ganglionic centre of the ventral cord, situated in the seventh abdominal segment. Almost immediately, each of these nerves divides into two branches, the ovarian nerve running anteriad and the ampullo-parietal nerve running posteriad.

FIG. 2. NERVOUS SYSTEM OF THE REPRODUCTIVE ORGANS OF A FEMALE *ANOPHELES MACULIPENNIS*. SCHEMATIC DRAWING BASED ON OBSERVATION OF LIVE SPECIMENS *



- I Seventh abdominal segment nerve
- 1 Ovarian nerve
- 1a Lateral branch of the ovarian nerve
- 1b Oviduct branch of the ovarian nerve
- 2 Ampullo-parietal nerve
- 2a Ampullary branch of the ampullo-parietal nerve
- 2b Parietal branch of the ampullo-parietal nerve
- II Eighth abdominal segment nerve
- IIa Vaginal branch of the eighth abdominal segment nerve
- IIb Spermathecal branch of the eighth abdominal segment nerve
- III Intestinal nerve of the last abdominal segment
- IIIa Colonic branch of the intestinal abdominal nerve
- IIIb Rectal branch of the intestinal abdominal nerve
- IV Odd abdominal nerve of the last segment

* After Polovodova (1953).

The ovarian nerve again splits into two branches: one to the ovary (1a) and one to the oviduct (1b). The branch to the ovary runs along the medial side of the ovary and gives off numerous metamerically arranged branchlets. The ampullo-parietal nerves each give off a branch to the ampullae and run on to the muscles and integuments of their segment. Two pairs of nerves and one unpaired nerve have their origin in the posterior portion of the same ganglionic centre which corresponds to the ganglia of the eighth, ninth and tenth segments. The first pair of these nerves (II), the vaginal nerves, run to the vagina, one of them, the first, sending a branch to the spermatheca (IIb). This branch divides into two and approaches the wall of the spermatheca from both sides. Polovodova considers that morphologically the vaginal nerves belong to the eighth abdominal segment. The second pair of nerves, leaving the posterior portion of the ganglionic centre (III), are the nerves of the hind intestine. Each of them splits into two branches, one going to the hind intestine (IIIa), the other (IIIb) to the rectum. The rectal branch in turn sends off smaller branches to the rectal glands.

The whole process of oogenesis, including the seven phases of oogonial development mentioned above, can easily be subdivided into three successive physiological periods. The first period is marked by a new follicle leaving the germarium. During this period, instead of the undifferentiated cells, a group of two and then four and eight cells appears in the lower portion of the germarium. Simultaneously with this increase in the number of cells, follicular epithelium begins to be laid down around the newly formed follicle. When this process is finished the follicle separates from the germarium and acquires the dimensions normal for its stage of development. The first follicle breaks away from the germarium during the pre-imaginal development of the female.

According to Imms (1908) the ovaries are rudimentary in the larval stage and the follicles form only in the pupa.

At the moment of the first flight, each ovariole usually consists of one follicle, above which the germarium is situated. Follicles continue to break away in turn in all the ovarioles throughout the active life of the female, this phenomenon being co-ordinated with the stages of each gono-trophic cycle. The total number of follicles which break away from the germarium is determined by the sexual activity and calendar age of the gonoactive female. During the first period the follicles are in stage N or I.

The second period of oogenesis is marked by the appearance of yolk round the nucleus of the oocyte. The follicles reach stage I-II or II. This development may occur in newly emerged females either by their using the nutritive substances accumulated during the larval life or by their taking a carbohydrate-meal or the first blood-meal. By the time the first follicle has passed into the second period of oogenesis, a second follicle is already breaking away from the germarium.

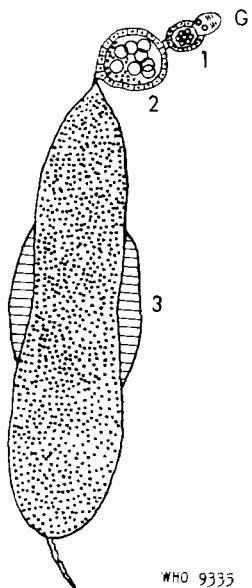
The third period of oogenesis is marked by a considerable increase in the quantity of yolk round the nucleus of the oocyte. The egg gradually passes into stages III, IV and V of development. In the female *Anopheles maculipennis* this third period occurs only when blood has been taken and digested. It ends with the full development and subsequent laying of the mature egg.

In each successive gonotrophic cycle following a blood-meal, as one follicle attains full development, the one lying above it passes through the second period and a third follicle breaks away from the germarium. Thus, during each gonotrophic cycle it is possible to observe all three periods of oogenesis occurring at the same time in follicles lying one behind the other in the ovariole (Fig. 3).

As was indicated, there are internal oviducts inside each ovary which form paired external oviducts outside the ovaries. Mer (1932) was the first to show that the ampullae are a bifurcated part of the unpaired oviduct, which has taken on a two-horned shape because of the medial invagination of its proximal end. A contrary opinion concerning the nature of the ampullae was expressed by Perfiliev (1930). He considered that the paired external oviducts expand before merging and form ampullae. Polovodova (1941, 1947) confirmed Mer's opinion on the real nature of ampullae by means of a series of histological sections. She showed that the epithelium of the unpaired common oviduct and of the ampullae is of glandular type and that during gonotrophic cycles a secretion accumulates in it; this distinguishes it sharply from the epithelium of the paired oviducts. As was pointed out by Kulagin (1901) and Perfiliev (1930) the paired oviducts can be distinguished from the internal oviducts by their well-developed musculature. Peristalsis of the muscles of the paired oviducts carries the eggs forward through them during oviposition.

The unpaired oviduct leads into the vagina, which ends in a genital opening situated ventrally not far in front of the anus. Two divisions are distinguished in the vagina: a wide proximal division and a narrow efferent one (Polovodova, 1947). In the narrow division there is a powerful sphincter consisting of transversely striped muscle fibres. The vaginal epithelium is not glandular.

FIG. 3. THREE CYCLES
OF OOGENESIS IN THE
OVARIOLE OF A NULLI-
PAROUS FEMALE OF
ANOPHELES
MACULIPENNIS



- 1 First cycle of oogenesis
- 2 Second cycle of oogenesis
- 3 Third cycle of oogenesis
- G Growth zone (germarium)

During copulation the semen passes into the seminal receptacle. This receptacle, or spermatheca, is a small globular structure; female Culicini possess two or three of them. On the outside the spermatheca is covered with cells lined with a thick brown cuticle. The cuticle is pierced with openings or pores. The spermatheca is surrounded by glandular cells, and Polovodova (1947) observed that plasm strands pass from these cells into the openings of the capsules. She considers that the secretion of these cells is necessary to maintain the viability of spermatozoa during their long storage in the body of the female. It is known, for example, that overwintering females are fertilized in the autumn and the spermatozoa remain active in the spermatheca until the end of the life of the females, i. e., until May or June of the following year. A duct runs from the spermatheca (in the Culicini there are two or three such channels) inside which, according to Kulagin's observations (1901) of female *Culex pipiens*, there is a fine cuticular lining with spiral thickenings characteristic of the cuticular linings of the fine ducts of insects. Perfiliev (1930) confirmed the presence of a similar cuticular lining also in the spermathecal duct of female *Anopheles*. In the epithelium of the upper half of the duct, there are large cells of circular or irregular shape, while in the lower portion of the duct there are no such cells. According to Polovodova (1947) the secretion from these cells also sustains the viability of the spermatozoa; it may also possess the property of attracting spermatozoa and directing them into the spermathecal duct for fertilization. According to the data obtained by Kulagin (1901) and Polovodova (1947) the spermathecal duct runs into the vagina at the genital opening itself. However, according to Perfiliev (1930) and to my own observations, the canal opens into a separate aperture next to the vagina.

The accessory gland is also situated in direct proximity to the spermatheca. Polovodova considers that the duct of the gland opens through a separate aperture into the dorsal portion of the vagina next to the spermathecal aperture. According to Perfiliev's data, with which my own observations fully concur, the duct of the accessory gland has a common opening with that of the efferent spermathecal canal. The dimensions of the accessory gland are not constant and depend, as will be shown below, on the physiological condition of the female. The gland is circular or oval in shape and consists of a number of large cuboid cells covered by a fine membrane. In the centre of the gland there is a lumen whence the efferent duct takes its origin. The length of this duct is somewhat shorter than in the spermatheca and according to Perfiliev there is no spiral cuticular lining inside it. The accessory gland functions simultaneously with the spermatheca and discharges its secretion during ovulation and probably during insemination of the egg, which takes place on its passage past the opening of the spermatheca. During ovulation the egg maintains a constant position and moves with the lower end foremost. The micro-

pyle, the opening through which the spermatozoa penetrate into the egg, lies in the upper portion of the egg. During oviposition the egg moves at first only half-way out of the genital aperture and after an interval of 20 or 30 seconds is ejected by the female. The retention of the egg in the genital opening is related to the moment of its insemination (Polovodova, 1947). By placing fully mature ovaries in physiological salt solution, Polovodova showed that mature eggs leave the various ovaries in turn; thus both ovaries are cleared simultaneously. However, among a large number of dissected *Anopheles maculipennis* I have found a small number of individuals in which one ovary was completely cleared while the other was still full of normally developed mature eggs. In such cases a disturbance of the process of oviposition can be said to have occurred and these females are probably doomed to an early death.

Functional Changes in the Reproductive System

During the oviposition, as has already been stated, the internal and external paired oviducts of the ovaries and the unpaired oviduct are very greatly distended. On completion of oviposition the oviducts and the ovaries contract considerably but do not revert to their original size.

Mer (1932) was the first to show that in females of *A. m. sacharovi* the dimensions of the ampullae vary greatly with the age of the mosquito. Later it was shown by other research workers that functional as well as age changes occur in the ampullae. Almazova (1935) showed that the increase in size of the ampulla is not due to the mechanical distension during oviposition but is connected with the glandular function of the epithelium. She found that during the most active period of ovarian growth, between stages II and V, there is a constant increase in the size of the ampullae. A decrease in the size of the ampulla due to the discharge of the secretion was noted during oviposition.

On the basis of histological study, Polovodova (1941) came to the conclusion that changes occur in the ampullae during the first gonotrophic cycle and that they are due to accumulation of secretion in the ampullary epithelium. During oviposition the secretion is discharged and the epithelium decreases in thickness although not to its original size. In all subsequent cycles only the accumulation of secretion and its subsequent discharge take place. At the same time a certain increase in the thickness of the epithelium, and consequently in the diameter of the ampullae, also occurs, at least as the result of the two subsequent cycles, but the increase is smaller each time and by the fourth cycle it is not observable at all.

In 1947 Polovodova described the functional changes which take place in the spermathecal gland of the female *A. maculipennis*. In newly emerged females the spermathecal gland consists of a number of cuboid cells in the

basal portion of which are situated large nuclei. This portion of the cell stains most intensively with haematoxylin-eosin. The proximal part of the cells, facing the duct and filled with secretion, stains less intensively. By the middle of the gonotrophic cycle the glands increase in size one-and-a-half times; the basophilic portions of the cells and the nuclei decrease in size but the quantity of secretion in the cells increases. After oviposition the glands become smaller but after a new blood-meal the cells again fill up with secretion during the development of the ovaries.

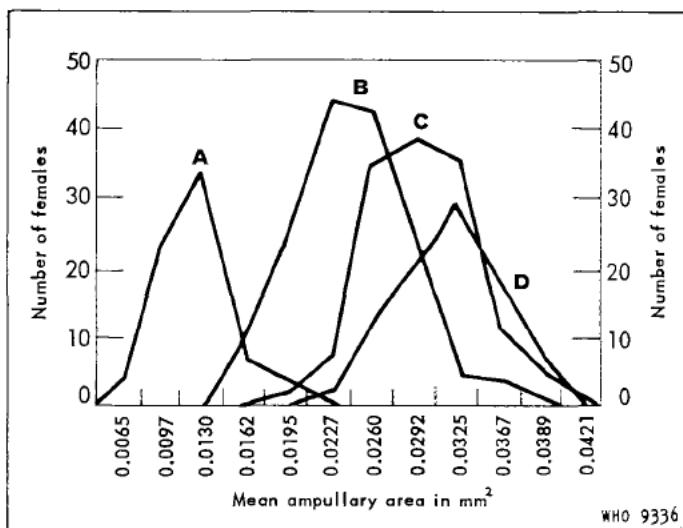
Age Changes in the Reproductive System

The study of age changes in the reproductive system of females is very important. Polovodova (1941) confirmed experimentally not only the qualitative functional changes mentioned above but also the quantitative age changes which take place in the ampullae during several gonotrophic cycles. The investigation was conducted with laboratory-reared females in which the number of completed gonotrophic cycles was accurately recorded. She showed that there is considerable variability in the dimensions of the ampullae in females of identical physiological age. This can be fully explained by the great variability in the over-all size of female mosquitos. Thus the size of the ampullae in large, newly emerged females may be equal to the size of the ampullae in small, multiparous females. Fig. 4 gives a graph of the variation in the size of the ampullae in successive gonotrophic cycles.

The increase in size occurs only during the first three gonotrophic cycles. In subsequent cycles it is difficult, if not impossible, to differentiate a multiparous female from one which has oviposited only three times. At the same time, Polovodova drew attention to the occurrence of morphological changes in the ampullae by which, she suggested, the physiological age of females should be determined. As already stated, the ampullae in newly emerged females are transparent and their walls can be distinguished from the walls of the paired oviducts only by the presence of transverse folds. By the end of the first gonotrophic cycle the walls of the ampullae take on a brownish colour and the folds appear swollen. After two ovipositions the walls of the ampullae swell still further but often they maintain their regular transverse folds. In multiparous females which have oviposited three or more times, the folds in the walls of the ampullae swell even more and become irregularly contorted.

The most obvious physiological and age changes are to be found in the ovarioles and these are now used to determine the physiological age of every dissected female.

FIG. 4. FREQUENCY DISTRIBUTION CURVES
OF AMPULLARY MEASUREMENTS OF FEMALE ANOPHELES ATROPARVUS
HAVING PASSED THROUGH A KNOWN NUMBER
OF GONOTROPHIC CYCLES IN THE LABORATORY*



- A Newly emerged females
- B Females with one oviposition
- C Females with two ovipositions
- D Females with three ovipositions

* After Polovodova (1941).

Polovodova (1947, 1949) demonstrated that irreversible processes connected with the passage of the mature eggs take place in the ovarioles and that it was possible to determine from these changes the physiological age of the females, i. e., the number of ovipositions they had performed. Polovodova showed that after oviposition there remain in the ovariole dilatations which, in her opinion, are formed by the retention of remnants of the nurse cells. By analogy with other animals and insects, she called these remnants "corpora lutea", a term which, strictly speaking, is not correct.

As far back as the last century, Müller (1825), Mechnikov (1867) and Brandt (1876, 1878) indicated the presence of "yellow bodies" in the ovaries of a number of insects. Mechnikov (1866, 1867), Verhein (1921) and Derbenieva-Ukhova (1935) showed that during the development of the egg follicles the nurse cells gradually decrease in size, contract and turn into a so-called corpus luteum, easily distinguishable by its yellowish or brownish colour. Shteinberg (1932) reported the presence of corpora lutea in the ovaries of *Loxostege sticticalis* L., and Ivanov & Meshcherskaya (1935) in the Blattodea.

Verhein observed and Derbenieva-Ukhova confirmed that in *Musca domestica* the remnants of the nurse cells after the formation of the chorion are not resorbed but only contract. After the laying of the mature eggs they lie in the form of a round yellow body in the proximal portion of the ovariole underneath the developing primary follicle. What happens to these corpora lutea afterwards was not investigated. None of the authors mentioned expressed the opinion that the corpora lutea could be used as a sign to distinguish nulliparous from parous females.

The question of the possible determination of the physiological age of each dissected female was first raised in our laboratory by Kuzina (1942) during the study of age changes in the reproductive system of *Stomoxys calcitrans* L. She proposed to use for this purpose the remnants of the nurse cells—the corpora lutea—which are retained in the end of the ovarioles after ovulation. Having established that in some females two corpora lutea were present, Kuzina suggested that if it could be proved that these bodies are retained in the ovarioles their number could be used to determine the number of ovipositions by the relevant female.

Beklemishev (1944) showed that in *A. maculipennis* after ovulation the remains of the nurse cells are seen in the ovariole as yellowish droplets and that "their presence is one of the signs distinguishing a female which has just laid its eggs from a nulliparous female". On Beklemishev's advice this method was used by Yaguzhinskaya (1945) in the study of the biology of *A. sacharovi* and *A. superpictus* near Samarkand.

Later, I studied in somewhat greater detail all the changes which take place in the ovarioles of *A. maculipennis* and confirmed the occurrence and nature of changes in the ovarioles of *Aëdes* and *Culex* mosquitos not only during the development of the follicles, but also after ovulation (Detinova, 1949). All these observations established the actual nature of the dilatations which appear in the ovarioles.

Laboratory-reared females of *A. m. atroparvus* and *A. m. messeae*, and wild *A. m. messeae* served for the following investigation. After the mosquitos had taken and digested a carbohydrate-meal, they were blood fed on myself or on rabbits. All the experimental females were given the opportunity to oviposit a number of times. The time of each egg-laying was recorded and the number of eggs in each batch was counted. When the females were dissected the number of gonotrophic cycles they had completed, the number of eggs laid at each oviposition, and hence the total number of eggs developed during the whole life of each female, were accurately known. I then dissected all their ovarioles, counted their total number and the number of dilatations in each. On the basis of these data the numerical relationship was determined between the number of eggs in the first oviposition and the number of ovarioles functioning at that time. I also determined in this way the relationship between the number of ovipositions completed by the female and the number of dilata-

tations of each ovariole, the ratio between the numbers of ovarioles functioning and not functioning, the occurrence and the number of ovarioles in which degeneration of the follicle had occurred, or which had retained eggs. The wild females were dissected either immediately after being caught, or after being kept in the laboratory until the ovaries had matured, in order to ascertain the condition of the ovarioles and the changes which had taken place in them before, during, and at various periods after oviposition. Examination was always carried out in a drop of physiological saline under the binocular dissecting microscope and further more detailed observation of particular phases was done under the compound microscope.

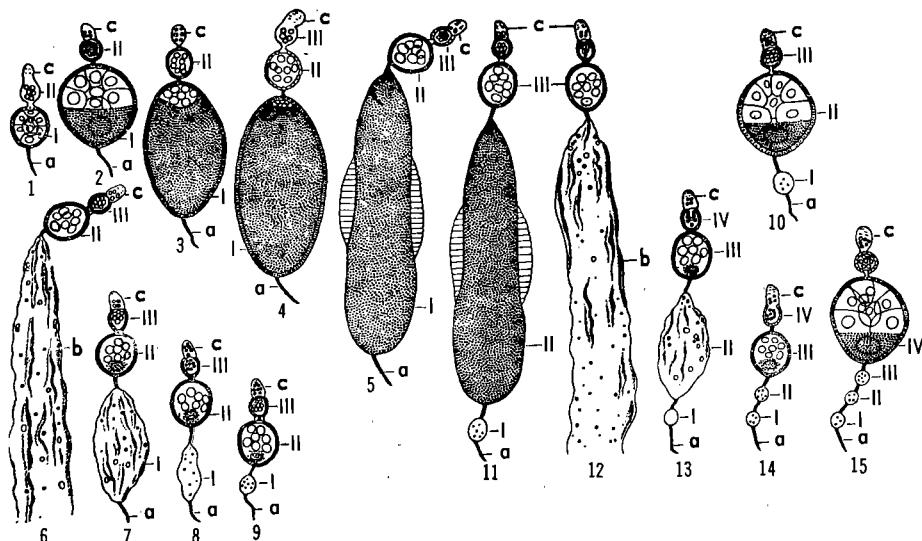
As was pointed out, each ovariole is covered by a closely adhering intima, which forms distally the efferent portion ending in the terminal pedicle, leading into the internal oviduct. As the follicle grows, the intima surrounding it is greatly stretched and takes on the same shape and the same dimensions as the whole ovariole.

The changes which take place in the ovariole during ovulation of the mature egg are as follows (Fig. 5). Under the effect of the contraction of the ovarian muscles the egg begins to move forward through the distal end of the ovariole. The terminal pedicle is distended considerably, up to the diameter of the egg, and the egg passes through it into the internal oviduct. After the egress of the mature egg, there remains at the point of its development and in the terminal pedicle the distended intima, which forms a thin-walled folded sac. The length of this sac, immediately after ovulation, corresponds to the combined length of the egg and of the terminal pedicle. Inclusions inside the sac consist of the remnants of nurse cells and follicular epithelium. Immediately after ovulation the intima begins slowly to contract; subsequently this process gathers speed and is completed in 22-24 hours. At the beginning the whole sac contracts. Then the terminal pedicle separates out, and this is followed by more intense contraction of the sac, leaving only the local dilatation. During the first hours after ovulation the walls of the sac can be slightly distended mechanically by means of entomological needles. During contraction of the intima the sac gradually rids itself of the majority of the inclusions, which pass into the internal oviducts, and then, as a result of constant ovarian contraction, both during oviposition and later, are ejected from the insect's body. When the contraction of the intima is complete, instead of a big sac-like enlargement there remains a small dilatation corresponding in size to the follicle which has just parted from the germarium. Thus the intima regains its original dimensions, contracting in length more than twenty-fold. Usually a small number of light-refracting inclusions remain in the dilated portion. The number and size of such inclusions are not the same in the ovarioles, and it is impossible to say whether they are the remains of nurse cells or of the follicular epithelium. In uniparous

females the dilatation indicates the place in which the follicle of the first series developed. Above the dilatation lies the follicle which will develop during the next gonotrophic cycle and below it is the terminal pedicle.

When the second batch of eggs develops, i. e., during the second gonotrophic cycle, the whole process is repeated. As the second follicle grows

FIG. 5. FORMATION OF DILATATIONS IN OVARIOLES



- 1-5 Development of the follicle
- 6 Ovariole after the first ovulation: the wall of the ovariole is distended in the shape of a sac
- 7-9 Contraction of the sac and formation of the dilatation
- 10-11 Beginning and end of the development of the follicle during the second cycle
- 12 Ovariole after the second ovulation
- 13 Formation of the second dilatation
- 14-15 Ovarioles of females after the second and third gonotrophic cycle
- I The first developing follicle and the place left after its discharge
- II The second follicle and the place left after its discharge
- III The third follicle and the place left after its discharge
- IV The fourth developing follicle
- a Terminal pedicle of the ovariole
- b The intima dilated after the passage of the mature egg
- c Growth zone

the intima surrounding it is stretched. The difference lies only in the fact that during the second ovulation, in each ovariole in which egg development has taken place, it is not only the pedicle which is stretched by the egg passing into the oviduct, but also the dilatation which occurred after the first ovulation. Egress of the second mature egg results again in the formation of a large thin-walled sac, which subsequently contracts gradually. During contraction, as after the first oviposition, the terminal filament separates out first, followed by the dilatation which occurred after the first oviposition; then a clearly visible dilatation is formed where the second follicle developed. Thus, after the second oviposition there

are two dilatations in the distal portion of the ovariole where eggs have been developed. The lower dilatation situated nearer to the terminal filament is the place where the first egg developed; the upper one is the place where the second developed. Subsequent gonotrophic cycles lead to the development of further follicles and each time after ovulation a sac is formed which on contraction leaves yet another dilatation in the distal portion of the ovariole. The dilatation which lies nearest to the newly developing follicle is the place where the egg of the last batch developed. After each gonotrophic cycle the length of the distal portion of the ovariole increases correspondingly, by the diameter of the dilatation plus that of the free part of the tube, which at first lies between the follicles and then remains between the dilatations. The sizes of individual dilatations vary, and this means that the length of the distal portion of the ovarioles in females of identical physiological age may also vary somewhat. The mature egg, in passing out through the dilated distal part of the ovariole, pushes forward the inclusions left at the sites of development of previous follicles. Therefore the greatest number of inclusions is always found in the uppermost dilatation, formed as a result of the development of the egg belonging to the last gonotrophic cycle. The egg developing in the next cycle pushes the whole terminal portion of the ovariole against the internal oviduct and it is therefore impossible to see it in ovarioles dissected out whole.

On the basis of all these observations I concluded that the dilatations found after ovulation occur in the ovarioles not because of the retention of a yellow body, as Polovodova suggested, but as a result of the ovulation of the egg, of which the intimal site of development is marked. The remnants of the nurse cells, known as yellow bodies, are inside this intima and are gradually extruded, partially or completely. It is therefore incorrect in my opinion to give to the dilatations the name of yellow bodies (*corpora lutea*).

The presence of dilatations in follicular tubes of parous females of *Mansonioides* was described by Bertram & Samarawickrema (1958).

Chapter 3

NORMAL AND ABORTIVE OOGENESIS IN FEMALE *ANOPHELES MACULIPENNIS*

Detailed study of female *A. maculipennis* has shown that not every blood-meal ensures the development of the ovaries, and not in every female. It has been established that the development of the ovaries is determined by a number of physiological characteristics of the female and can occur after a blood-meal only if:

- (1) at the beginning of the meal the female is in a gonoactive state, this being determined by the condition of its neurosecretory system;
- (2) the amount of blood ingested weighs at least as much as the body of the female; and
- (3) the first follicles in the ovarioles are in stage II, or at least I-II.

The absence of any one of the above factors is sufficient to interfere with the gonotrophic cycle. This shows the very close relationship between these factors and the complexity of the processes which occur in the body of the female during gonotrophic cycles. It should be added that even when all these factors are present, the gonotrophic cycle can take place only when the females are in favourable microclimatic conditions. Consequently the occurrence of gonotrophic cycles is determined by the internal readiness of the organism and by the effect of external factors.

Neurosecretory Regulation of Gonotrophic Cycles in Female *Anopheles maculipennis*

It has been established that the development of sexual products of a large number of insects depends on the activity of the corpora allata, which are endocrine glands. I have succeeded in establishing the connexion between the condition of the corpora allata and the gonoactivity of female *A. maculipennis* (Detinova, 1945a; Detinova (& Lozgacheva), 1953). The existence of this connexion was proved by histological examination of the

retro-cerebral glands in females in various physiological conditions, by observation of females which had been ligatured and by the study of the relationship between the degree of distension of the stomach and the beginning of ovarian development.

Functional changes in the corpora allata

The set of retro-cerebral glands in adult females of *A. maculipennis* consists of the corpora allata and the corpora cardiaca. The corpora allata take the form of a paired organ situated behind the brain, where the neck joins the thorax. The paired structure of the corpora allata is a primitive form and among the Diptera studied it is characteristic only of Orthorrhapha and Nematocera. Orthorrhapha and Brachycera, such as the Tabanidae, possess one unpaired corpus allatum (Mednikova, 1952), which is also characteristic for the higher flies (Cyclorrhapha). In *Anopheles* the corpora allata lie above the oesophagus along the sides of the aorta.

The glands of the newly emerged female are considerably smaller; their protoplasm is always intensely basophilic and the nuclei stand out well from the surrounding protoplasm. In the first days of imaginal life the number of nuclei in the glands and the size of the glands themselves increase in both gonoactive and diapausing females. In gonoactive females they reach their maximum size when the blood-meal is taken; subsequently they gradually become smaller, probably as a result of the discharge of the hormone into the body cavity. By the end of the gonotrophic cycle the glands have again increased in size. The cyclic nature of these changes, connected with the development and laying of eggs, indicates the close link between the two processes.

The glands of diapausing females are oxyphilic. This change in the taking of stain is probably due to the absence of secretion in the glands. In diapausing females a blood-meal does not bring about changes in the staining properties of the protoplasm of the corpora allata. The appearance of the ability of the protoplasm to stain intensively with haematoxylin indicates that certain new processes occur in the corpora allata of females after completion of diapause and the taking of a blood-meal. In all probability the change in the staining properties of the glands is due to the transformation of their secretion-precursor into secretion. Clements (1956) also demonstrated changes in the corpora allata of diapausing female *Culex pipiens*.

The part played by the corpora allata in the establishment of gonotrophic concordance

The fact that the glands produce a secretion which assists the development of the sexual products in the female *A. m. messeae* was confirmed

by experiments carried out by means of ligatures. A fine human hair was used for the ligature and controls were set up for all experiments. The ligature was put on in two different ways. In some females it was applied immediately behind the head so that the glands were left connected with the rest of the body. In other females it was placed between the first and second, or second and third, pairs of legs, so that the glands were shut off from communication with the ovaries. The following results were obtained.

A ligature applied closely behind the head, i.e., in front of the corpora allata, immediately after a blood-meal or later, did not hinder the normal development of the ovaries. If the ligature was applied immediately after a blood-meal the ovaries never developed, although the blood was always digested normally. The ovaries failed to develop even if the ligature was applied within the first five or six hours after the blood-meal. This means that the quantity of hormone secreted within that period is not adequate to activate the ovaries. When a ligature was applied after more than six hours had elapsed then the ovaries developed, which means that an adequate quantity of hormone had been secreted by that time. Similar results were obtained in the bed-bug *Cimex lectularius* by Mellanby (1939). A ligature applied three hours after the blood-meal did not permit development of the eggs, although the blood which passed into the stomach was digested with the speed characteristic of the unfertilized female. A ligature applied 24 hours after the blood-meal did not prevent the development of the eggs. The author came to the conclusion that the feeding of a fertilized female of *C. lectularius* stimulates the corpora allata to secrete a hormone which in turn causes the ovaries to develop. During the first three hours after feeding, there is too little hormone to develop the ovaries, but the gland produces the required amount within 24 hours.

Gillett (1955a,b, 1956a,b, 1957b, 1958), in observations of *Aedes aegypti* females, obtained identical data demonstrating that hormonal action was necessary before the oocytes would begin to grow. He established the length of the period during which the hormone necessary for the normal course of the gonotrophic cycle in individuals of this species was secreted into the body cavity. He called this period the critical period and demonstrated that the hormone which promotes the development of the ovaries is produced in the head and enters the blood during eight to fourteen hours; hence in *Aedes aegypti* the critical period is longer than in *Anopheles maculipennis messeae*. Gillett considers that the neurosecretory cells of the brain are the main source of the hormone.

As is known, in all the higher insects the secretion from these cells passes along the nerves of the corpora cardiaca into those bodies, whence it passes into the blood. In a number of instances the secretion passes into the blood also from the corpora allata. Numerous data (see the review by Novak, 1959) are now making it more and more obvious that

for normal maturation of the ovaries in the majority of insects not only the hormone secreted by the neurosecretory cells but also the special hormone of the corpora allata is necessary. Gillett demonstrated that the decapitation of mosquitos before the critical period hinders the development of the ovaries, whereas after the onset of the critical period the ovaries remain capable of reaching maturity.

Of particularly great interest were the experiments by Gillett (1958) on the transfusion of haemolymph from mosquitos in which the critical period had begun into mosquitos in which it had not begun. By these experiments he reliably demonstrated the existence of a hormone in the body cavity of females which is necessary for the development of the ovaries.

Clements (1956) established, by decapitation of *Culex pipiens v. molestus* and the application of ligatures, that in autogenous females on emergence the hormone which promotes ovarian development is not present in the body cavity, but accumulates there within a period of five to seven hours. In non-autogenous females of *Culex pipiens berbericus*, and also in *Aedes aegypti* and *Anopheles labranchiae atroparvus*, ligature does not prevent the development of the ovaries even if applied immediately after a blood-meal. He demonstrated, in contrast to this, that in *Anopheles stephensi* the accumulation of the hormone in the body cavity takes place in the course of nine hours.

Connexion between the degree of distension of the stomach and the onset of ovarian development

Certain data have been obtained which make it possible to state that the secretion of the hormone of the corpora allata in *A. m. messeae* females after a blood-meal occurs under the influence of nerve impulses from the walls of the stomach (Detinova (& Lozgacheva), 1953). In view of the fact that the walls of the whole abdomen, and particularly of the pleura, are distended at the same time as the stomach, it seems not impossible that nerve impulses originate also in those places. The degrees of distension of the stomach may differ, and depend on the amount of blood ingested by the female. Special experiments, which will be described later, showed that the development of ovaries in *A. maculipennis* can occur only after a complete blood-meal, i.e., when the mass of the blood ingested is equal to, or greater than, the weight of the body of the female mosquito. It has already been pointed out that the follicular development can begin only when the hormone secreted by the corpora allata is present in the haemolymph. The mechanism of the process which causes the discharge of the hormone into the body cavity may, in my opinion, be summarized as follows. When a full blood-meal is taken the terminal nerves in the

stomach "register", as it were, the fullness of ingestion. This stimulus originates a reflex to the corpora allata, which secrete the hormone, assisting with the development of the ovaries. If the blood-meal is incomplete the stomach is insufficiently distended and the nerve impulse does not reach the glands; consequently the hormone does not pass into the haemolymph. That is why the ovaries fail to develop when an incomplete blood-meal is taken. Thus it can be assumed that the beginning of the development of the ovaries is conditioned by a neurohumoral mechanism and that the impetus for the beginning of oogenesis is given by a reflex passing to the gonadotrophic endocrine gland from the distended stomach.

I first came to this conclusion after a study of wild females of *A. maculipennis* whose stomachs were infested with Microsporidia, resulting in the disturbance of their gonotrophic concordance; results of specially conducted experiments with laboratory-reared females confirmed this hypothesis.

In the 1949 season in the south of the USSR I discovered female *A. m. messeae* with stomachs showing considerable changes in shape and colour. The epithelium in the changed part of the stomach was considerably altered and thickened. Histological study showed that the epithelium was infested with Microsporidia. Kudo (1924, 1929, 1930) describes two forms of Microsporidia infesting *A. quadrimaculatus* and belonging to the genera *Thelohania* and *Nosema*. He found both these genera in larvae and adults. *Thelohania* was present in the fat-body of the larvae and adults and *Nosema* in the epithelial cells of the stomach of females and larvae and in the fat-body of the females. Missiroli (1929) describes *T. grassi* from the ovaries and fat-body of female *A. maculipennis* which, in his opinion, are similar to *T. legeri*. The form we found, however, differs from those described by Kudo and Missiroli and is apparently new.

Females in various physiological conditions and of various physiological ages are occasionally found with stomachs infested with Microsporidia. The occurrence of newly emerged females with such infestations indicates that they are infected in the larval stage, probably during feeding. Females in the first gonotrophic cycle and also females in later cycles were found to be infested. It is probable that bodies of water become contaminated with Microsporidia when infected females oviposit, the female excreting the parasites present in the hindgut and rectum at the same time as it lays the eggs.

The thickened epithelium, full of Microsporidia, projects into the gastric cavity, filling a considerable part of it. That is why the blood-meal taken by these females is always less than a full meal taken by normal females; the more the gastric epithelium is infected with Microsporidia, the smaller the blood-meal which can be taken. In the microphotographs (Fig. 6 and 7) the difference in the condition of the gastric epithelium of healthy females and females infested with Microsporidia can be seen.

FIG. 6. LONGITUDINAL SECTION THROUGH THE STOMACH OF A HEALTHY *ANOPHELES MACULIPENNIS MESSEAE* FEMALE*

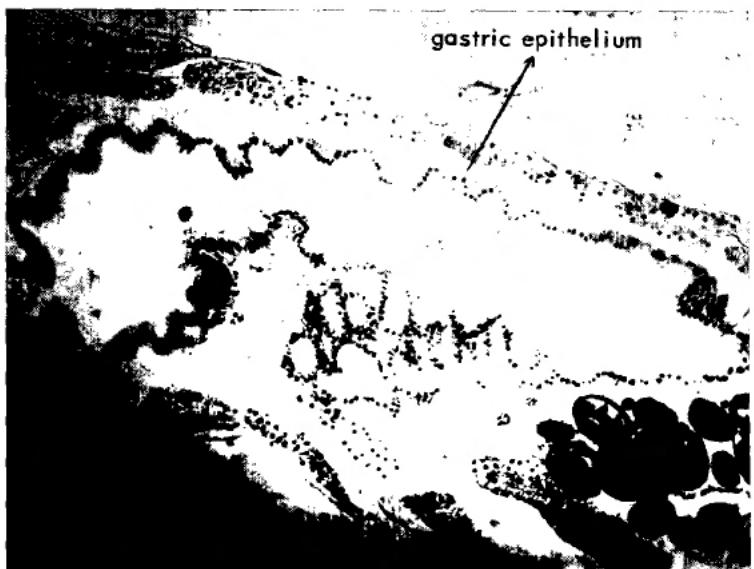
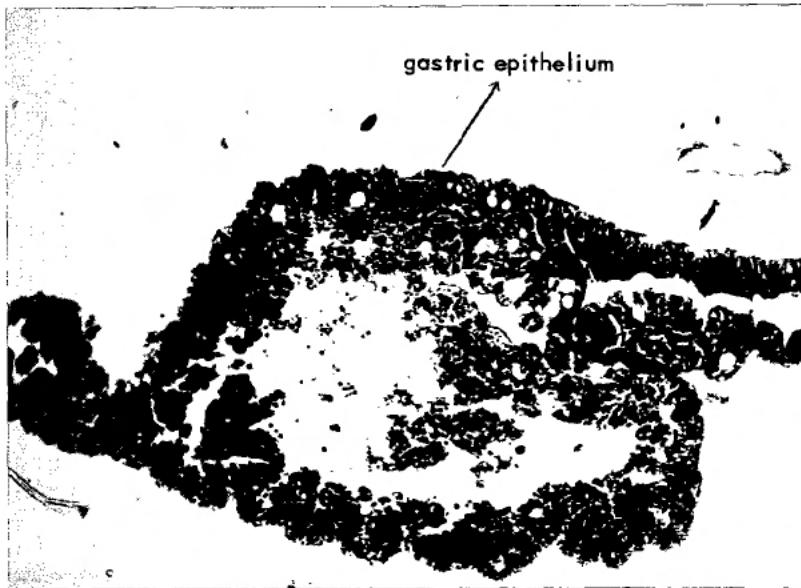


FIG. 7. LONGITUDINAL SECTION THROUGH THE STOMACH OF AN *ANOPHELES MACULIPENNIS MESSEAE* FEMALE INFESTED WITH MICROSPORIDIA*



* MBI-3, Objective 10, ocular 7.

A direct relationship was noted between the degree of infestation of the gastric epithelium with Microsporidia and the number of ovarioles which produce degenerated follicles. On the basis of the fact that in all females with Microsporidia-infested stomachs mass degeneration of the follicles was discovered, it has been suggested that degeneration is a consequence of taking an incomplete blood-meal. A number of experiments arranged to test this hypothesis are described below.

Connexion between the Amount of Blood Ingested by the Female and Ovarian Development

By weighing gonoactive females before and after a blood-meal the amount of the ingested blood was established. The relationship of the weight of ingested blood to the weight of the mosquito made it possible to estimate the completeness of the meal. It was established that the ovaries developed only in those female *A. maculipennis* in which the weight of the blood-meal equalled or exceeded the body-weight of the mosquito. Such meals were considered to be complete. The completeness of the blood-meal can easily be told by observing the degree of external distension of the stomach.

Out of 101 mosquitos which took an incomplete meal not one showed the development of the ovaries. The follicles did not develop beyond stage II even when the weight of the ingested blood was only just under the body-weight of the female and amounted to 80% or 90% of that weight. In the experimental females the follicles did not develop beyond stage II, even when the females were given several consecutive incomplete blood-meals, after blood digestion was complete. On the other hand, when a complete meal was taken the ovaries developed in all the experimental females. These observations made it possible to conclude that a full blood-meal is necessary for the development of the ovaries of *A. maculipennis*.

To elucidate the reasons why follicles begin to develop and then degenerate in females which, as a result of microsporidial infestation, have taken an incomplete blood-meal, observations were carried out on healthy laboratory-reared females. We fed the females through an animal membrane. They were given blood mixed with a solution of table-salt in varying concentrations. The dilution of the blood reduced the amount of nutritive substances taken by the females, but the volume of the ingested liquid corresponded to the volume of a full blood-meal. When females were fed with blood mixed with a salt solution in a concentration of 0.8% the liquid was quickly absorbed in the stomach and excreted through the

Malpighian tubules; as a result of this the stomach soon recovered the shape it possesses during an incomplete meal. In no case did the ovaries develop in such females. On the other hand, when females were fed with blood mixed with hypertonic salt solution the stomach kept its distended form after the completed meal. In all, 220 females were fed, of which only 14 nulliparous females and 62 parous females ingested a full meal. Of the first 14 females the follicles in three cases developed to Christophers' stage III. Of 62 parous females, ovarian development was noted in 22. In 12 females of that group the follicles developed to stage V, with subsequent oviposition. In the remaining 10 the follicles developed only as far as stage III. Examination of the ovaries of all these females established that in only one nulliparous female did all the first follicles begin to develop. In the others, different sections of the follicles began to develop while the rest degenerated in stage II or in earlier stages of development.

These experiments confirm the existence of neurohumoral regulation of the gonotrophic cycle; the shift in the development of the ovaries occurred only in those females whose stomachs were completely distended.

Gillett (1957b) suggested that in female *Aëdes aegypti* the hormone which assists the development of the ovaries is normally set free and enters the body cavity from the neurosecretory cells in response to the distension of the stomach or of the abdomen by the blood-meal. The author also indicated that partial distension of the stomach filled with liquid coming from the diverticulum may stimulate the premature secretion of the hormone and cause it to be used up before blood is ingested.

Wigglesworth (1939, 1940, 1947) established that the moulting process in *Rhodnius* is conditioned by a neurohumoral mechanism. He showed the existence of a close connexion between the degree of distension of the stomach after a blood-meal and the process of moulting under the effect of a secretion produced by endocrine glands. Similar results were obtained by Sergiev for *Cimex lectularius*.

According to Cunliffe's data (1921) the nymph of *Ornithodoros moubata* Murray moults only when the quantity of blood drunk exceeds its body-weight.

It is a well-known fact that females of a number of blood-sucking mosquitos such as *Anopheles bifurcatus* (Markovich, 1938, 1939, 1941; Melnikova, 1943; Denisova, 1946), *Anopheles hyrcanus* (Enikolopov, 1950; Detinova & Butenko, 1955), *Anopheles plumbeus* (Krivonosova, 1946, quoted by Enikolopov, 1950), *Culex molestus* (Kraan, 1956; Möllring, 1956), *Culex pipiens* (Roubaud, 1929; Huff, 1929; Roubaud & Mazger, 1934; Tate & Vincent, 1936; Spielman, 1957; Déduit, 1957), *Aëdes aegypti* (Hovanitz, 1947), *Aëdes detritus* (Vermeil, 1953), some species of Simuliidae (Hocking & Pickering, 1954), some species of Heleidae (Nichlas, 1953, quoted by Krivosheina, 1947; Glukhova, 1958) and some *Phlebotomus papatasii* populations (Dolmatova, 1946), are characterized by what is

known as autogenous ovarian development, i. e., the maturation of the first batch of eggs without a blood-meal. The first to draw attention to this phenomenon in anophelines was Markovich in her study of *A. bifurcatus*.

It is not yet clear what sort of neurohumoral mechanism causes the development of eggs in these blood-sucking insects without a previous blood-meal. However, data obtained by Mednikova (1952) concerning the condition of the corpora allata in newly emerged females of *A. bifurcatus* indicate that they probably begin to secrete the hormone at the same time as the female begins to use its internal reserves (the fat and proteins accumulated during the larval stages of development), which are needed for the development of the follicles. This exceptionally large difference in the mechanism of secretion of the hormone necessary for ovarian development in autogenous and non-autogenous races of some species of blood-sucking insect is of great biological interest.

For the time being it is not clear what neurohumoral factors play a part in the oogenesis in some species of such blood-sucking Diptera as *Aëdes*, *Phlebotomus*, Simuliidae, Heleidae and Tabanidae, in which ovarian development can occur even when an incomplete blood-meal is taken and digested (Roy, 1936; Rubtzov, 1936, 1955, 1956; Woke, 1937; Detinova, 1942; Dolmatova, 1942; Chagin, 1943; Amosova, 1956; Duke, Creawe & Beesley, 1956; Krivosheina, 1957; Prokofieva, 1957; Glukhova, 1958). In these insects a close connexion has been noted between the amount of ingested blood and the number of developing eggs. This suggests that they possess a higher degree of gonotrophic concordance than those blood-sucking insects, such as *A. maculipennis*, in which egg development can take place only when a full blood-meal has been taken and digested.

It is possible that in these cases the reflex passes from the nerve endings in the stomach to the corpora allata not only when the stomach is completely distended, but even when a small amount of blood enters it.

Beklemishev (1957c) recently suggested that there might be a close link between the amount of blood ingested and the number of developing follicles in female *A. maculipennis*. In these females the number of developing eggs varies considerably. In Beklemishev's opinion the difference between the two types of gonotrophic concordance which can occur, either only after a full blood-meal has been taken (in *Anopheles*), or after the taking of an incomplete blood-meal (in *Aëdes*), is merely due to the relative size of the quantities of blood necessary for the gonotrophic cycles to begin and follow their course. While in the case of *A. maculipennis* the necessary minimum would appear to be an amount of blood equal to or exceeding their body-weight, any increase in the amount of blood may also increase the proportion of follicles which begin and complete their development. In the case of *Aëdes* this initial minimum for the development of at least a proportion of the eggs may be considerably smaller. So far, we possess no experimental data to confirm this hypothesis, but observations of

exceptionally large variations in the number of eggs developing in groups of female *Anopheles* of the same age (a fact which will be discussed later) tend to support it. The amount of blood ingested by female *Anopheles* varies greatly and observations have shown that the maximum blood-meal may be as much as twice the weight of the female.

Degree of Development of the First Follicles as a Necessary Condition for Gonotrophic Concordance

Mer (1932) showed for the first time in the case of females of *Anopheles m. sacharovi* (a fact that was confirmed by Shlenova (1938) for females of *A. m. messeae* and by myself (Detinova, 1940) for *A. m. atroparvus*) that the law of gonotrophic concordance applies only to those females whose ovaries are in stage II at the time of the blood-meal.

Dissection of newly emerged females of *A. m. atroparvus* and *A. m. messeae* proved that about 98% emerge with ovaries of which the first follicles are in stages N or I and that only about 2% of them have ovaries in stages I-II or II (Detinova, 1944). Such ovaries are characteristic of females of rather larger size. For the ovaries to develop as far as stage II, the females must take either a carbohydrate- or a blood-meal. However, in a considerable proportion of these females the first follicles complete their development by using the internal reserves accumulated during the larval instars. This was confirmed by the dissection of females kept without food, but given water, for one and a half to two days.

Since simultaneously with the complete development of the first follicle the second follicle develops into stage II and is already prepared for further development (Shlenova, 1933), there is no need in subsequent gonotrophic cycles for supplementary feeding of the females, and each successive blood-meal leads to the normal development of the ovaries followed by oviposition. Only in late autumn, among physiologically old females, were individuals discovered in which the follicles were in stages N or I. What happens to these females is not known.

It is known that mosquitos under natural conditions feed on nectar and plant juices (Litvinova, 1941, 1949; Vinogradskaya, 1948; Twinn, Hocking, McDuffie & Cross, 1948; Grinfeld, 1955). It is probable that under natural conditions the follicles develop to stage I or II mainly after having ingested this type of food. During this period of their life the overwhelming majority of newly emerged females do not feed on blood. In the case of females not characterized by autogenous development the follicles do not develop to the later phases on a diet of fruit juices (Davis, 1928).

The necessity of a preliminary maturation of the ovaries of newly emerged females (as a result of carbohydrate- or blood-meals or by the use of internal reserves of the organism) has been proved in the case of a large number of mosquito species such as *Aedes* (Detinova, 1942; Chagin, 1943; Monchadsky, 1956), in Tabanidae (Olsufiev, 1940) and in Heleidae (Amosova, 1956; Glukhova, 1958). Glukhova considers from the example of *Culicoides griseascens* that the ovaries cannot complete their preliminary development by using internal reserves. According to the data provided by Lewis (1957a) the overwhelming majority of attacking females of *Simulium damnosum* had ovaries in stage I of development. Prokofieva (1957) showed that on carbohydrate food (plant juices) follicles do not develop beyond stage I.

Abortive Oogenesis

Until now mention has been made only of the process of normal oogenesis and of the conditions which appear necessary for it to occur. Side by side with a normal process of oogenesis, abortive oogenesis can almost constantly be seen in some ovarioles. This ends in the degeneration of the follicle in one of its intermediate phases.

Follicular degeneration is a frequent phenomenon among blood-sucking Diptera. It has been described in *A. maculipennis* by Nicholson (1921), Polovodova (1947), Detinova (1949) and Markovich (1951); in *Aedes* by Detinova (1942); in *Phlebotomus* by Dolmatova (1949b); in Heleidae by Amosova (1956); in Simuliidae by Prokofieva (1957); in non-blood-sucking flies, such as *Musca domestica*, by Lineva (1955) and in cockroaches by Ivanov & Meshcherskaya (1935). The degeneration of follicles has been most thoroughly studied in *A. maculipennis*.

Since follicular degeneration is the outcome of abortive oogenesis, it can naturally take place only in the follicles of gonoactive females. The abortive oogenesis of a follicle in individual ovarioles begins simultaneously with normally progressing oogenesis in the follicles of other ovarioles; thus the same conditions are required for it as are necessary for the normal process of oogenesis, i.e., the female must be in a gonoactive condition, it must have taken a full blood-meal and the majority of the first follicles must be in stages I-II or II of development. An exception is shown only by the fact that degeneration may occur not only in follicles in which the accumulation of yolk is in progress but also in follicles at earlier stages of development (N and I).

The process of follicular degeneration in *A. maculipennis* was first described by Nicholson (1921). During the following years it has been

possible to study this process in more detail (Detinova, 1942, 1949; Polovodova, 1947; Markovich, 1951).

Degeneration consists of granular decomposition of the protoplasm of the oogonium, the nurse cells and the follicular epithelium, and, at later phases in the development of the follicle, also of the yolk accumulated in the oogonium. Follicles degenerate most frequently in the first stages of development (N-II). When degeneration sets in, in addition to the granular decomposition of the follicular epithelium, the shape of the follicle begins to change and its outline becomes irregular. In cases where degeneration sets in during stages I-II or II, the yolk, which before degeneration was evenly distributed around the nucleus of the oogonium, either spreads throughout the follicle or concentrates in its central portion, showing as a darker spot. At the same time the cells of the follicular epithelium are shed inside the follicle. If degeneration begins in stage III, IV, or sometimes even V, then the distribution of the yolk in the follicle becomes irregular, and (where there is little of it) it seems friable; by the end of the gonotrophic cycle it takes on a yellow, raspberry-coloured, orange or brown tinge. So far neither the cause of discoloration nor that of the differences in the colour of the degenerated yolk has been established. The colour may differ in the follicles of one and the same ovary. This observation does not agree with the findings of Colless (1958), who points to the existence of a species variability in the coloration and size of the pigmented granules lying inside the ovary underneath its membrane. He states that they are brown in *Culex tritaeniorhynchus* and pale green in *Culex fatigans*. These granules probably represent follicular degeneration.

The size of each degenerated follicle depends on the phase of follicular development at which degeneration started. On the surface of the yolk the shed cells of follicular epithelium are easily visible.

Apart from ovarioles in which follicular degeneration sets in after the beginning of growth (the end of stage N and later), in practically all *A. maculipennis* there are ovarioles in which, just as in normal ones, new follicles break off from the germarium but do not even begin to grow or develop further. These ovarioles take on the shape of a fine, beaded chain, the length of which increases with each successive gonotrophic cycle by one small dilatation, formed not by the development and ovulation of the mature egg but by an incompletely developed follicle breaking off from the germarium. Such ovarioles were described in *A. bifurcatus* by Markovich (1951). This is also one of the manifestations of degeneration.

The new follicles break off from the germarium simultaneously in all ovarioles in a given gonotrophic cycle, but what happens to each follicle is decided during the subsequent cycle. Either the next follicle develops completely or it begins to develop and then degenerates at one of the growth stages (accumulation of yolk) or degeneration ensues in a follicle in which growth and yolk accumulation have not even begun.

According to Prokofieva (1957), the picture of degeneration of follicles in the Simuliidae is complicated and varied:

"It begins with the degeneration of epithelial cells in the proximal section of the follicle. The cells break off inside it and together with the nurse cells, which still keep their outline, lie confusedly in the follicle. At this stage of degeneration the follicle is deep yellow. Subsequently the cell membranes lose their outline and their contents lie freely inside the follicle in the shape of small and large lumps of an intense orange colour."

Several hypotheses have been put forward to explain the degeneration of follicles. Some of them have been confirmed by the large amount of collected factual material. Analysis of all available data shows that in general it is impossible to explain degeneration by the influence of any single factor acting on the organism, since it can be due to the most varied causes.

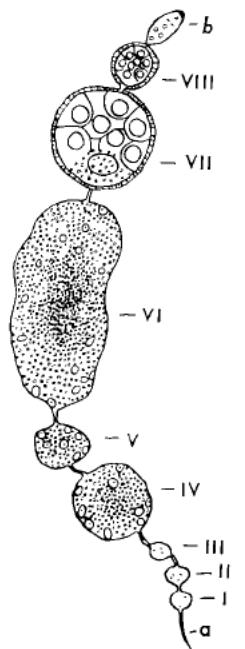
One of the main causes of follicular degeneration in *A. maculipennis* is probably the ingestion by the female of an inadequate amount of blood. As has been stated, oogenesis in the females of this species can begin only after a complete blood-meal, but the size of a complete blood-meal varies from 100% to 200% (or more) of the body-weight of the mosquito. It is quite probable that the minimum complete meal is not always adequate for the full development of the whole batch of eggs and therefore only those ovarioles begin to develop whose threshold of sensitivity to the stimuli which cause the beginning of oogenesis is lowest. If afterwards the nutrient material for the full maturing of all the developing follicles is still not sufficient, some of them may begin to degenerate at later stages—at stage III or even IV. It is probably reasonable to suppose that degeneration is a supplementary regulatory process which enables the remaining follicles to complete development. This concurs to a certain degree with the conclusion reached by Nicholson (1921), who considers that the degenerative process is even useful, since more room is left in the ovaries for the normally developing follicles.

A confirmation of the hypothesis that a link exists between the follicular degeneration and an insufficient blood-meal was found in females whose stomachs were infested with Microsporidia and in whose ovaries large-scale or even total degeneration of follicles was noted during a series of consecutive gonotrophic cycles. As has already been said, stomachs in which the epithelium is infested with Microsporidia have enough capacity for only a partial blood-meal. Despite this, however, development of the ovaries begins, since the stomach is completely distended and therefore the corpora allata proceed to produce the hormone. However, as there is insufficient food the follicles develop only to stage III or more rarely to stage IV. The next blood-meal does not cause, as might have been expected, the completion of development in follicles which have already begun to develop. Instead the overlying follicles begin

to develop. However, since the second blood-meal is also incomplete the second follicles develop only up to one of the intermediate stages. At the same time as these follicles develop, the first ones, and also a proportion of the second, degenerate. The next (third), also partial, blood-meal leads to the incomplete development of the third follicle and the simultaneous degeneration of the second and a proportion of the third. The same picture can be seen after each consecutive blood-meal. Thus the degenerated follicles in these Microsporidia-infested females are not ejected from the ovaries because they do not develop completely; this causes the failure of the overlying follicles to be discharged so that each ovariole in such females takes the shape of a chain consisting of a number of degenerated follicles; the maximum number which we saw was five. Massive degeneration shows that these females were gonoactive and reacted normally to a blood-meal and that the endocrine glands functioned normally but that the amount of blood ingested could not ensure the completion of follicular development. The ovaries of such females increase considerably in size and the large accumulation of pigment causes them to take on a general pinkish-brown coloration easily visible with the naked eye. Among females with Microsporidia-infested stomachs several individuals were of special interest. In the first, one to three follicular dilatations were of the ordinary light-coloured small type, which represents the traces of the passage of mature eggs. Subsequent dilatations (numbers four to six) were filled with a degenerated mass which, judging from its size, came from follicles which had developed to one of the stages when yolk is accumulated (Fig. 8).

This phenomenon can probably be explained as follows. At the beginning of adult life the degree of infestation of the stomachs of these females with Microsporidia was not very high and this enabled them to take a blood-meal sufficient for the development of the follicles followed by oviposition. Later the growing number of Microsporidia prevented the taking of a full blood-meal and thus the processes described above led to the degeneration of subsequent follicles. It is necessary to point

FIG. 8. OVARIOLE WITH DEGENERATED FOLLICLE IN THE 4th-6th GONOTROPHIC CYCLE



- a Terminal pedicle
- b Growth zone
- I-III Dilatations left by three previous gonotrophic cycles
- IV Degenerated fourth follicle
- V Degenerated fifth follicle
- VI Degenerated sixth follicle
- VII Developing seventh follicle
- VIII Separated eighth follicle

out that in all females whose stomachs were infested a considerable variation in follicular development was observed. Only a proportion of the follicles began to develop and reached stage III or IV; all the rest degenerated at earlier phases of development. Degeneration occurred also in follicles which had only just broken away, and then in their place there remained only a small, light, thicker portion such as is found in healthy females. Among those infested with Microsporidia some females were found in whose ovaries individual follicles developed as far as the mature egg stage.

According to Hosoi (1954a), when *Culex pipiens pallens* are fed with casein a considerable number of follicles develop to stage II, but the majority of them degenerate afterwards. The reason for degeneration may be due to the shortage of nutritive substances necessary for full development of the follicles.

The hypothesis that the cause of degeneration is the inadequacy of the blood-meal was confirmed by me. In females with artificially distended stomachs but which did not take full blood-meals the follicles began to develop but the development was not completed because of lack of nutriment.

The mechanism of gonotrophic concordance prevents the female *Anopheles* from losing oocytes unproductively, since in ordinary females, not infested with Microsporidia, an incomplete blood-meal never causes the onset of either normal or abortive oogenesis.

During the study of fertility of females at different ages (this will be described in detail in Chapter 4), I came to the conclusion that there exists a direct relationship between the age of the females and the number of ovarioles which have not produced normally developing eggs but have shown degeneration of the follicle. This increase in the proportion of degenerated follicles is responsible for the drop in fertility with increasing age. Examination of the ovarioles in dissected females of varying ages led to the conclusion that in physiologically young females degeneration rarely repeats itself in one and the same ovariole in several successive gonotrophic cycles. In ovarioles in which degeneration is repeated, two and sometimes three degenerated follicles are clearly visible. The total length of such ovarioles is considerably greater than of those in which degeneration has not taken place and in which normal follicular development and subsequent oviposition have occurred. The total length of an ovariole in which a degenerated mass remains is the greater the later the phase of follicular development at which degeneration takes place. If in any ovariole the next follicle after a degenerated one develops normally, then the subsequently developing mature egg, in its usual outward passage, dilates the whole distal portion of the ovariole and pushes before it the products of degeneration of the previous follicle into the internal oviduct, from which they are subsequently ejected.

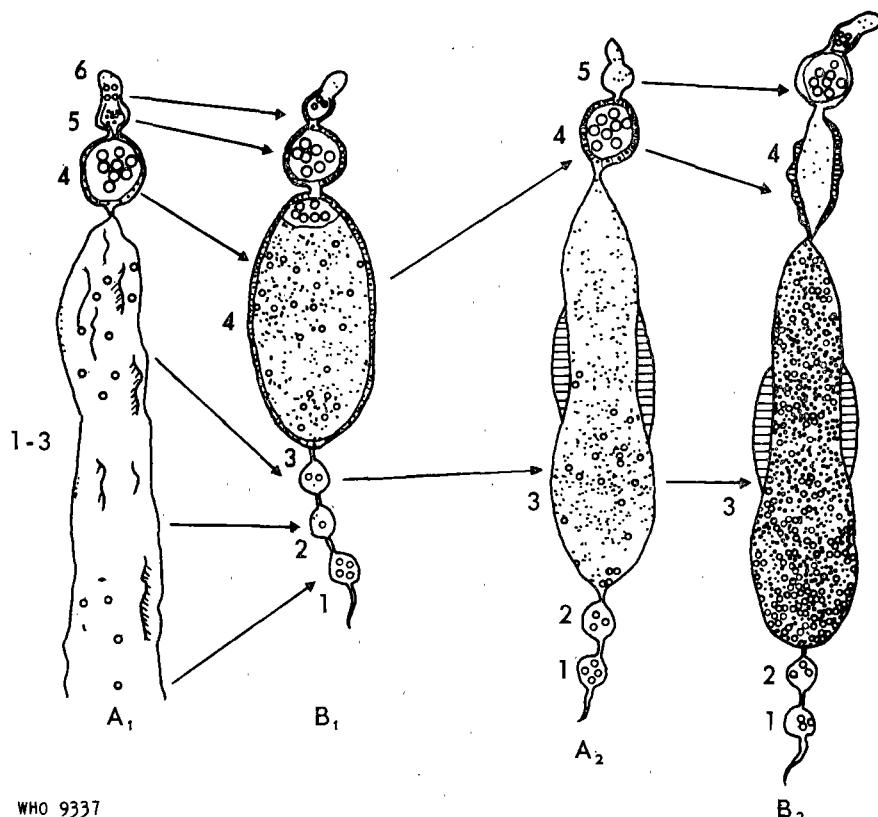
According to Dolmatova (1949b) an increase in the percentage of degenerating follicles occurs as a result of unfavourable temperature acting directly on female *Phlebotomus* during a particular cycle of oogenesis. It is quite probable that this factor is most important in the case of *Phlebotomus*, although to deny a connexion between the physiological age of the females and the proportion of the follicles which degenerate is unthinkable at the moment, since there are as yet no data on the exact physiological age of female *Phlebotomus*.

So far there have been no observations of the effect of unfavourable conditions during oogenesis in *A. maculipennis*. However, if it is remembered that in mosquitos at unfavourable temperatures the amount of food used for metabolism during the maturation of the eggs increases (Shlenova, 1938), it is very possible that a similar correlation will be found also in this case, in addition to the age factor.

It has been established that one of the causes of the degeneration of follicles is the presence in the ovarioles of mature eggs from the previous gonotrophic cycle. On dissecting females immediately after oviposition (Detinova, 1953a), I noted that the ovarioles in which mature eggs were retained have a completely normal appearance, i.e., behind the germarium from which a new follicle is usually already breaking away there follows a follicle in one of the early stages of development (usually I-II or II) and finally a mature egg. Changes in these ovarioles begin after the female takes another blood-meal. The follicle lying above the retained egg at first changes its shape and then shows all the signs of degeneration which are characteristic of that particular stage of development (Fig. 9). Thus the presence of a retained mature egg in an ovariole causes the degeneration of the follicle lying above it, which otherwise could have developed normally during the gonotrophic cycle concerned. Later, if during the next oviposition the egg which was retained from the previous cycle is actually laid, there will be the same number of dilatations in the terminal portion of the ovariole as in an ovariole which has functioned normally during each oogenesis. I have seen mature eggs in a state of decomposition retained in an ovariole. Probably, decomposition of the yolk in these eggs takes place when the egg does not pass out of the ovariole on completion of the subsequent gonotrophic cycle.

An interesting observation was made by Lineva (1955), who found that sublethal doses of DDT produce in females of *Musca domestica* a series of functional changes in their ovaries followed by morphological alterations of developing ovarioles and follicular tubes. Lineva showed that in these females the motile function of ovaries is affected; this leads to the holding up of mature eggs, which eventually degenerate. This is followed by an interference with the normal development of later follicles, which degenerate in one of the intermediate developmental stages. The above-mentioned author observed that the early development of a group of

FIG. 9. OVARIOLE WITH A RETAINED MATURE EGG
OF THE THIRD GONOTROPHIC CYCLE AND WITH A DEGENERATED NEXT FOLLICLE



A₁ Ovariole of a female having completed three gonotrophic cycles, immediately after the passage of a mature egg

A₂ Ovariole of a female having completed three gonotrophic cycles, with a retained mature egg

B₁ Ovariole of a female in the fourth gonotrophic cycle after the passage of a mature egg of the third cycle

B₂ Ovariole of a female in the fourth gonotrophic cycle, with a retained mature egg of the third cycle causing the degeneration of the fourth follicle

Note: the numbers refer to the respective gonotrophic cycles.

adjoining follicles is also affected and that some follicles are changed into long sacs filled with a gelatinous, homogeneous and congealed substance.

It follows that the factors which cause degeneration of the follicles in *A. maculipennis*, and probably in a number of other blood-sucking Diptera,

may include an insufficiency of food, the physiological age of the females, the effect of unfavourable climatic conditions acting on the female and the presence of retained eggs in the ovarioles.

The example of *Musca domestica* shows that follicles can also degenerate under the action of sublethal doses of DDT.

Chapter 4

CHANGES RELATED TO THE EXCRETORY, DIGESTIVE AND REPRODUCTIVE PROCESSES OF FEMALE *ANOPHELES MACULIPENNIS*

In addition to the functional and age changes noted in the reproductive system of female Anophelini and Culicini, important changes have been noted in the tracheal system, in the speed of the excretory and digestive processes and in the degree of fertility of the females.

There is a large amount of published material (reviews and individual articles) dealing with the general arrangement of the tracheae in the insect body, various types of tracheation, the structure of the tracheae and tracheoles, the position and number of the spiracles in various insects, and other questions connected with the morphology and anatomy of the respiratory system (Kholodkovski (1912), Shvanich (1949), Wigglesworth (1930a, 1930b, 1938, 1950a, 1950b, 1959), Chauvin (1953), Webb (1945); Glenn Richards & Korda (1950), Vinogradskaya (1960), Day (1951), Lee (1927), Le Faucheut (1956), and others). However, so far, very little information has been published on the functional changes which take place in the tracheal system in connexion with the functioning of other organs.

I have established the occurrence of changes in the tracheal system of the ovaries during the first cycle of oogenesis and in the tracheal system of the stomach at the moment when the female takes its first full blood-meal (Detinova, 1942, 1945b). The stomach and ovaries in newly emerged females are very small. These vitally important organs require a large oxygen supply to ensure the intensive metabolism which accompanies their activity.

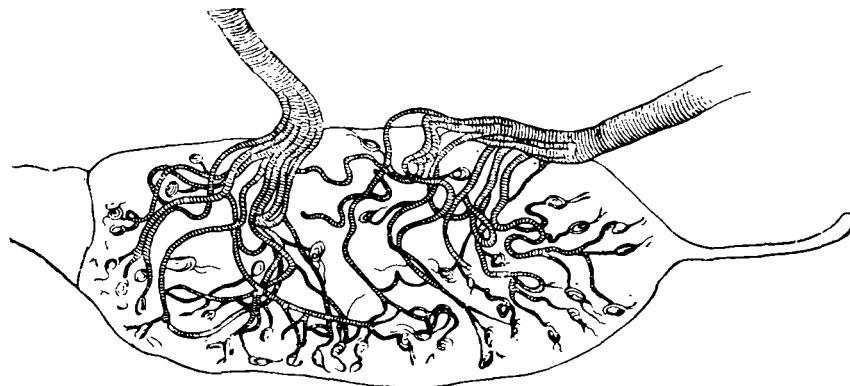
Structure and Functional Changes of the Tracheae Supplying the Ovaries

In each ovary of a female *Anopheles*, *Aëdes* or *Culex* two tracheal trunks approach from the lateral directions. They originate in the main longitudinal trunk, where this joins the anterior spiracular chambers of the fifth and sixth abdominal segments. Just before reaching the wall of the ovary the trunks begin to split into pairs of branches, each producing

four to six pairs, the most common figure being five. The total number of branches probably depends on the size of the ovary in the newly emerged female.

The separate pairs of trunks which derive from the main one are distributed along the ovary at a certain distance from each other, and thus, as a rule, the ovary is equally supplied with tracheae along its whole length. In their course the trunks describe various zigzags and loops. The branches of each pair of trunks enter the ovary and curve round the walls of the internal oviducts. Each of these branches forms further pairs of smaller branches and these run in a centrifugal direction between the ovaries and ovarioles. The small tracheae and tracheoles pass either between the

FIG. 10. TRACHEOLES OF THE OVARY OF A NULLIPAROUS FEMALE
ANOPHELES MACULIPENNIS MESSEAE



follicles or directly over their surface. In the ovaries of a newly emerged female the main branches of the tracheae are very close together. However, since the ovaries increase considerably in size during the first cycle of oogenesis the tracheal branches gradually move further apart. After oviposition the ovaries again become smaller but do not revert to their original size, so that in comparison with newly emerged females the large tracheal branches remain at a greater distance from each other.

The most characteristic feature of the tracheal system of the ovaries is the presence of skeins, which are formed at the ends of the fine tracheae and consist of closely wound, very fine tracheae and tracheoles (Fig. 10). The length of the skeins varies from 0.017 to 0.031 mm and their width from 0.007 to 0.02 mm. They take various forms—some are round, most are elongated, some resemble a figure of eight. From each skein arise the long terminal filaments of the tracheoles (usually one or two) which penetrate still deeper between the follicles and reach the outer membrane of the ovary. It seems that these terminal tracheolar fila-

ments attach themselves to the surface of the terminal portions of the ovarioles (the germarium). I have not carried out any research into the relationship between the number of skeins and the number of ovarioles in the ovaries. It is easy to see, however, that in some cases the skein supplies only one ovariole while in other cases the terminal filaments of the tracheoles from one skein supply two ovarioles.

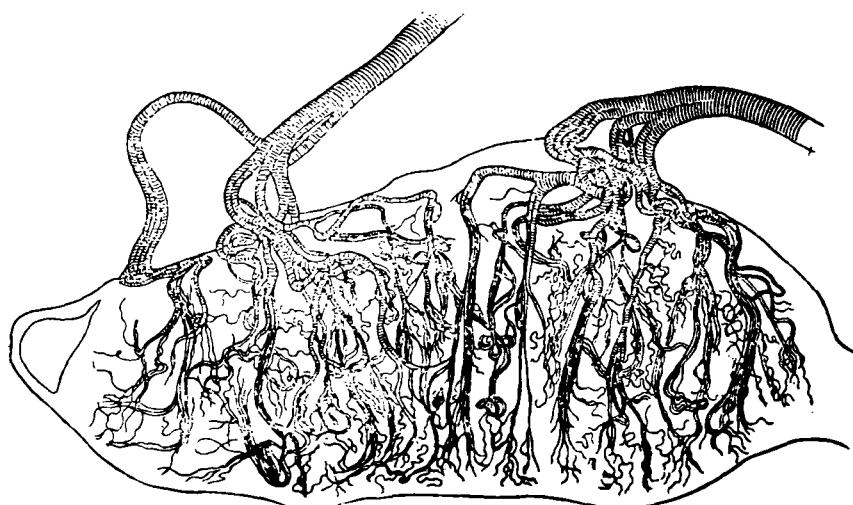
Nicholson (1921) first drew attention to the presence of small coiled tracheae in the ovaries of female *Anopheles*, but he did not ascribe this condition to any particular physiological age and did not mention the occurrence of any changes in the tracheal system supplying the ovaries.

Study of a large number of dissections has enabled me to prove that the presence of skeins is a feature of all newly emerged females and in general of all nulliparous females, including overwintered insects, no matter what their calendar age. In nulliparous females the ovarian tracheal branches described numerous loops and zigzags, which provide a reserve for distributing tracheae over the whole ovary when it increases in size, so that an even supply of oxygen can be ensured to all the developing follicles. No essential changes in the tracheal system of the ovaries have been observed before the taking of the first blood-meal. It is possible that the distance between the main tracheal trunks increases somewhat during the initial development of the ovaries, but I have observed no changes in the dimensions of the skeins and terminal filaments during that process. After the blood-meal, in addition to the growth of the ovaries, important changes begin to take place in their tracheal system. First of all, the distance between the ovary and the tracheal trunk (which branches off the main trunk on each side) decreases because of the increase in the size of the ovary; the afferent tracheal branches seem to flatten out on its surface. The loops and zigzags of the smaller tracheae straighten out and stretch as the total volume of the ovary increases. The most important changes during the first gonotrophic cycle take place in the terminal portions of the tracheae. As the follicles gradually increase in size, the terminal filaments begin to increase in length. The filaments become longer by the unwinding of the skeins, thus gradually reducing their size; by the end of the first oogenesis the skeins are completely unwound. Increasing in length, the terminal filament gradually stretches itself along the whole of the greatly distended ovariole. As result of this process all the ovarioles become enclosed in a network of fine tracheae and tracheoles. When the mature eggs have passed out of the ovary there are no skeins or loops left in the tracheal branches: they have all been stretched out during the growth of the ovaries (Fig. 11).

The process of unwinding the skeins seems to be irreversible. Examination of a large number of ovaries has shown that in parous *A. maculipennis* unchanged solitary skeins are very rare among the unravelled tracheoles. Their presence indicates the failure of some of the follicles to develop.

Markovich (1951) stated that in some parous females of *A. bifurcatus* 5-10 or, in some instances, even 18-25 skeins can be found. She explains this by the degeneration, during the first cycle, of a considerable number of the follicles due for development. It should be pointed out in this connexion that when a follicle degenerates in the early phases its dimensions do not increase and consequently the skeins attached to the respective ovarioles are not unravelled.

FIG. 11. TRACHEOLES OF THE OVARY OF A PAROUS FEMALE
ANOPHELES MACULIPENNIS MESSEAE



It follows from the above that it is possible to distinguish with certainty nulliparous from parous females by the condition of the tracheal system which supplies the ovaries.

Structure and Functional Changes of the Tracheae Supplying the Stomach

While the changes in the tracheal system of the ovaries take place gradually during the whole of the first cycle of oogenesis, the changes in the tracheal system of the stomach occur immediately after the first blood-meal, when the stomach becomes distended. In newly emerged females that have not taken any blood-meal the stomach is small. Its enlarged part is situated in the area of the fourth and fifth abdominal segments. The enlarged portion of the stomach is, as a rule, transparent and cylindrical in shape. After the first blood-meal the stomach increases greatly in size and takes up almost the whole abdominal cavity from the first to the sixth or seventh segment. By the end of the blood-meal the stomach is pear-shaped. On completion of the digestive process its size

decreases sharply but does not revert to its original dimensions and folds appear in its walls.

On each side of the stomach there are four tracheal branches originating in the longitudinal trunk at the level of the third, fourth, fifth and sixth abdominal segments. Each of these primary branches divides further, creating a tracheal tree which supplies a particular part of the stomach. Those which arise in the spiracles of the fourth and fifth segments are the most highly branched; but there is some variability in the tracheal branching of individual females.

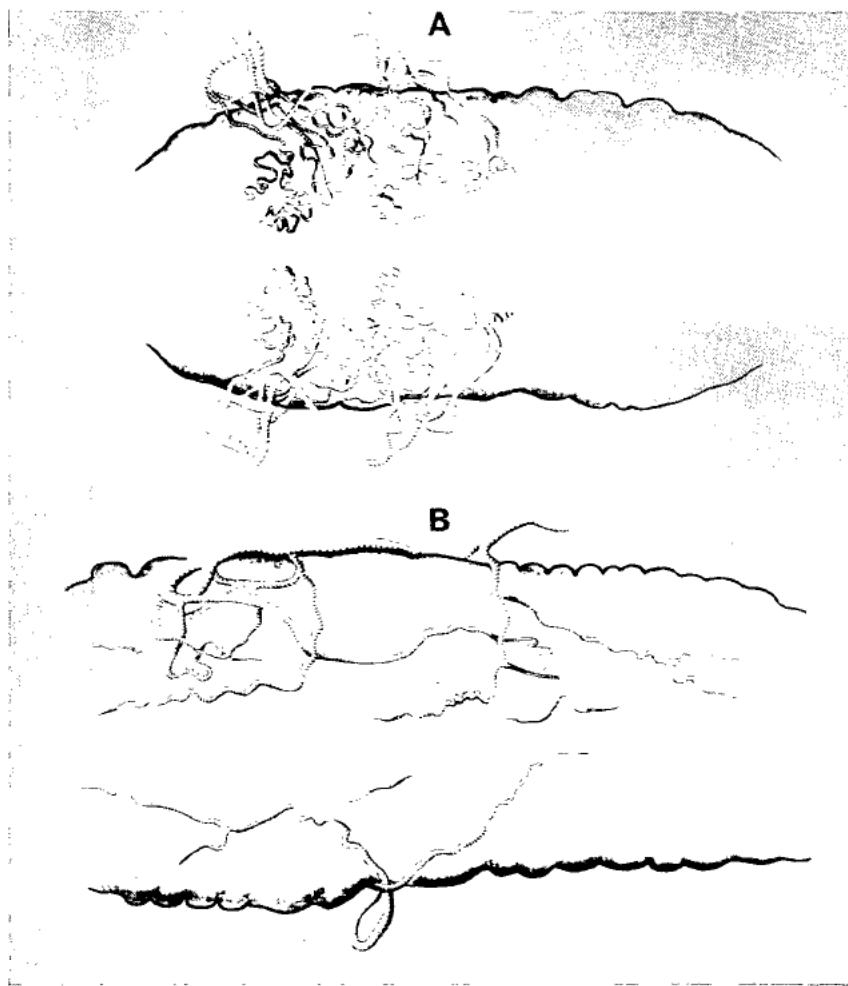
The branches of the tracheae on each side do not reach the midline of the stomach, and therefore on its dorsal and ventral aspects there remain thin portions free of them. Unlike the ovarian tracheae, the gastric tracheae do not penetrate inside the organ but pass along its walls, while the finest tracheae and tracheoles are attached to the outer membrane of the organ.

In all females which have not taken a blood-meal the tracheae form throughout the whole length of the stomach a varying number of bends while the tracheoles form coils and tight spirals, often reminiscent of those seen in the ovary (Fig. 12 A). All these bends and coils represent a reserve length of tracheae sufficient to ensure an even supply of oxygen to the distended organ. When the blood-meal is taken the stomach distends and the tracheal system simultaneously stretches out. On completion of digestion the tracheae do not coil up again but lie along the stomach in the shape of fine, slightly curved branches (Fig. 12 B). The tracheal branches which serve the stomach are capable of considerable extension. On completion of digestion the stomach resumes its original position but the tracheae do not contract and are therefore considerably longer in all previously blood-fed females in comparison with newly emerged females.

We have found no mention in the literature of functional changes of the gastric tracheae in any other insect. In larvae of the clothes-moth, Day (1951) noted that the midgut was very poorly supplied with tracheae. This, in his opinion, is explained by the composition of the food, which needs no oxygen for its digestion.

In examining the females of *Culex pipiens* and some species of *Aëdes* I have found that the changes which occur in the gastric tracheae after a blood-meal are the same as in *A. maculipennis*.

Thus, the appearance of the tracheal system connected with changes in the volume of the ovaries and the stomach during imaginal life is due to the stretching or straightening out of the tracheal trunks of various sizes and of the tracheoles, which in newly emerged females are coiled. Since the changes in the tracheal system are irreversible, it is possible to distinguish parous from nulliparous females by examining the tracheal system of the ovary and to distinguish by the changes of the tracheal system of the stomach those females which have taken a blood-meal from those which have not.

FIG. 12. TRACHEAL SYSTEM OF THE MIDGUT OF A FEMALE *ANOPHELES MACULIPENNIS*

A Before the first blood-meal

B After the first blood-meal

Age Changes in the Excretory Processes of Female *Anopheles maculipennis*

Until comparatively recently, in the absence of an accurate technique for determining physiological age, it was impossible to study age changes in the organism of female mosquitos. During the last few years the example of *A. maculipennis* facilitated an attempt to study age changes in the metabolic processes which occur in the female insects. The changes in

the speed of excretion and in blood digestion have been particularly investigated.

I shall not dwell on the well-known structure of the Malpighian tubes, but shall deal with certain features of their excretory processes. These processes may be classified as follows:

(1) the process of charging the epithelium with excreted material (the accumulation of waste products in the epithelial cells), and

(2) the process of discharge (the transfer of the excreted material from the epithelium into the duct and its subsequent ejection from the organism).

When there is no excreted material in the ducts or only a comparatively small quantity of it in the cells, the presence of excreta in the hindgut shows that evacuation has taken place not long before dissection. If the hindgut is full of excreta it indicates that the evacuatory process has occurred. The processes occurring in the Malpighian tubes of live females (Beklemishev & Detinova, 1940) can be assessed by the number, colour and order of appearance of the droplets or granules which may be the result of the elimination of excess water from the blood-meal (aqueous droplets) or the evacuation of excreta (yellowish granules) or of excreta mixed with haematin (brown granules) or of haematin itself (black granules). In the case of *A. superpictus* and *A. maculipennis*, the charging of the epithelium with excreted material appears to progress slowly and continuously throughout the whole of the female's life. In connexion with the discharging process, we noted certain rhythms in the clearing of the epithelium (Beklemishev & Detinova, 1940; Denisova, 1940). The basic rhythm as was noted by Missiroli (1925, 1927) and Falleroni (1926) coincides with the rhythm of the gonotrophic cycles. An outline may be given of the processes which occur in the Malpighian tubes during the gonotrophic cycles. In empty females a process of epithelial charging is noted. From the moment when the female sucks bloods the Malpighian tubes begin to expel in large quantities the water which passes from the stomach into the body cavity. The quantity of excreta in the cells does not change at first and ducts filled with aqueous matter become visible at this time. After elimination of the excess water, the process of discharging the excreted material from the epithelium begins and sometimes continues until the end of blood digestion. The epithelium is rarely completely cleared or even cleared to any considerable degree, since in the majority of females there occurs a simultaneous process of recharging the epithelium. When the new gonotrophic cycle begins, the epithelium is again full of excreted material, the quantity of which increases when the female is fasting.

There is evidence that in many other blood-sucking Diptera which show gonotrophic concordance a definite and related cycle occurs in the Malpighian tubes. Thus, Dolmatova (1942) studied the Malpighian-tube cycle in female *Phlebotomus papatasii* and Prokofieva (1957) mentioned

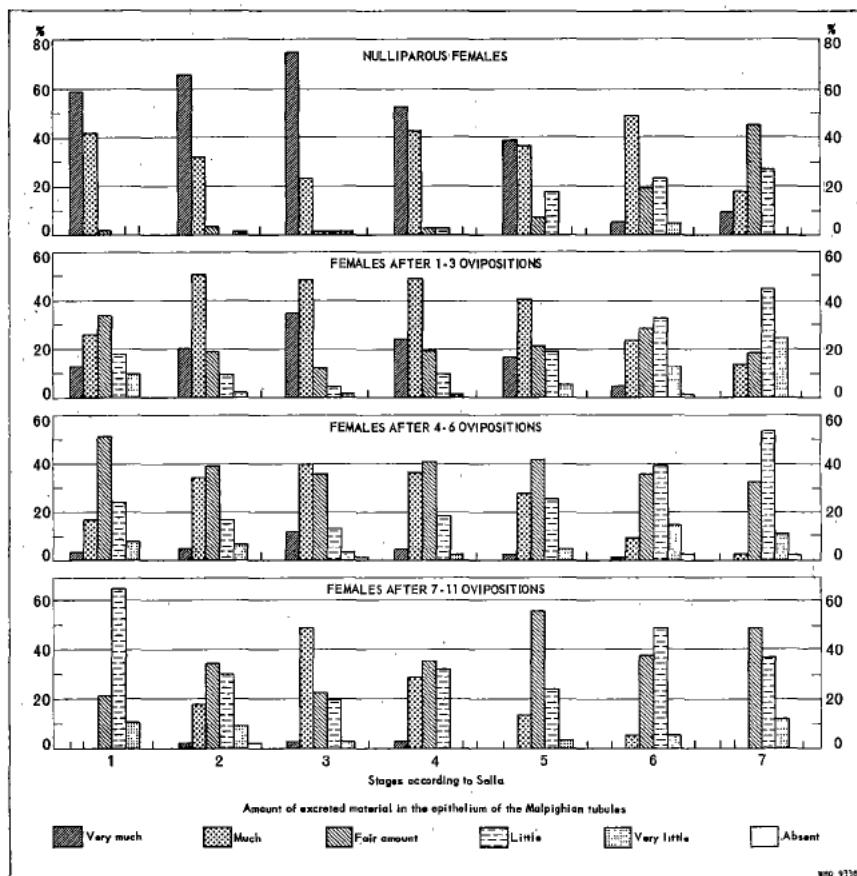
the occurrence of a definite cycle during the gonotrophic cycle in female Simuliidae, while Glukhova (1958) made the same observation in Heleidae. The general picture of the changes which take place in the Malpighian tubes of sandflies, Heleidae and black-flies is similar to that found in mosquitos.

The occurrence of considerable individual variations in the condition of the Malpighian tubes in female *A. maculipennis* at the same stage of blood digestion suggested that age differences might occur in the excretory processes. In the seasons of 1951 and 1952 when females were dissected to determine their physiological age the condition of their Malpighian tubes was examined at the same time to prove this hypothesis. More than 4500 females were examined.

The first mention of age differences in the condition of the Malpighian tubes in malaria mosquitos was made by Sella (1920a), who stated that the Malpighian tubes of old females differ from those in young mosquitos; they are narrower, the excreted material is distributed unevenly and the tubes themselves appear compressed. However, the age of the females mentioned was unknown. Falleroni also mentioned the existence of age changes in the Malpighian-tube cycle in female *Anopheles*. This author stated that the occurrence in empty females of a small quantity of excreted material is characteristic of old females (such as overwintered individuals after oviposition) and that the absence of any material in the ducts and the transparency of the cells of the tubes were signs of exhaustion. Lewis (1957a) observed that in unfertilized females of *Simulium damnosum* the Malpighian tubes were not transparent and were full of excreted material as a result of active metabolism in the pupal stage of development. In fertilized females the clear outlines of the cells were visible and in females infested with *Onchocerca* the tubes were transparent. All females infected with *Onchocerca* could be confidently considered to have oviposited a number of times.

However, none of these authors had any facts to offer concerning the actual physiological age of the females and therefore the results of their research could only lead to the general conclusion that considerable changes take place in the Malpighian-tube cycle, depending on the age of the females.

Having collected a large number of observations on the condition of the Malpighian tubes in female *A. maculipennis* of different physiological ages (from 1 to 13), I was able to study the gradual changes in the excretory processes which occur in females as they grow older (Fig. 13). I noted a gradual reduction in the intensity of metabolism of older females. While there is always a very large amount of excreted material in the cells of the Malpighian tubes in young empty females, such material was completely lacking in the Malpighian tubes of physiologically old females. As the age of the population increases there is a considerable increase in

FIG. 13. STATE OF MALPIGHIAN TUBULES IN FEMALES OF *ANOPHELES MACULIPENNIS* IN RELATION TO THE PHYSIOLOGICAL AGE

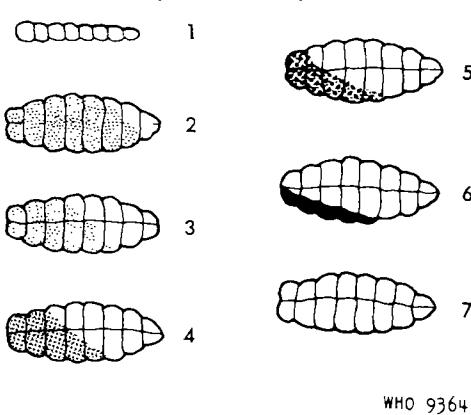
the proportion of females with little excreted material in the cells of the Malpighian tubes. It has also been established that in physiologically old females the discharge of excreted material from the epithelium still continues on completion of digestion, whereas in young females it usually ends at the end of the digestive process. This indicates that in physiologically old females both the discharge and the charging process are slowed down.

The appearance of the Malpighian tubes in physiologically old females differs considerably from that of the tubes of young females. The former tubes are always of smaller diameter, they appear dehydrated and the distribution of excreted material in the cells (even in the same tube) may be very uneven compared with the distribution in young females. As

already stated, Sella characterized the condition of the Malpighian tubes by similar signs, although he judged the age of the females only by indirect data.

Age and Functional Features in the Digestive Processes of Female *Anopheles maculipennis*

FIG. 14. STAGES OF BLOOD DIGESTION
AND OVARY DEVELOPMENT*



WHO 9364

- 1 Empty female, ovaries not developed
- 2 Freshly blood-fed female, ovaries not developed
- 3 Blood-meal darker, area of 2-2½ sternites and 4-5 tergites free of blood
- 4 Blood very dark, area of 2½-3 sternites and 5-6 tergites free of blood
- 5 Blood black, area of 2½-3½ sternites and 6½-7½ tergites free of blood
- 6 Remaining blood black, only on the ventral side, the rest of the abdomen occupied by developing ovaries
- 7 No blood visible, eggs fully developed

Note: the drawings represent the lateral view of the abdomen.

* After Sella (1920a); redrawn from Beklemishev (1949).

The work of many authors (Kozhevnikov, 1903; Sella, 1920 a, b; de Buck, Schouute & Swellengrebel, 1932; Hecht, 1933; Beklemishev, Vinogradskaya & Mitrofanova, 1934; Shlenova, 1938; West & Eligh, 1952; Detinova, 1953b,d; Kalmykov, 1959, and others) has demonstrated the existence of a direct relationship between the duration of blood digestion, the development of the ovaries and the meteorological conditions in the mosquito's external environment. If the temperature rises to the optimum the speed of the processes increases, but at temperatures above the optimum they slow down. Atmospheric humidity also affects the speed of these processes.

Sella (1920a) in his remarkable monograph on the ecology of *Anopheles* suggested that the whole process of blood digestion and ovarian development in female *Anopheles* be divided into seven stages. This suggestion has been widely adopted in practical entomological research. The degree to which the stomach is filled with blood and the development of the ovaries in females at these various stages are shown in Fig. 14. The stages may be briefly described as follows:

- (1) Empty female, stomach without blood, ovaries undeveloped.
- (2) A freshly fed female. The stomach is filled with red blood. About the last two sternites and the last three tergites are free of blood.
- (3) The blood still retains its reddish tinge; 2-2½ sternites and 4-5 tergites are free of it.

(4) The blood in the stomach is black, the last 2½-3 sternites and 5-6 tergites are free of it.

(5) The blood in the stomach is black, the last 2½-3½ sternites and 6½-7½ tergites are free of it.

(6) The blood in the stomach is black and is only visible on the ventral side.

(7) The stomach is free of blood. The abdomen is full of mature eggs.

Since the blood in the stomach and the developing ovaries are clearly visible through the fine abdominal integument of the live female, it is easily possible to observe the duration not only of the whole process of blood digestion and ovarian development, but also of each of Sella's stages. Nowadays these stages which Sella worked out for *Anopheles* are also used for all other, non-malarial, mosquitos and also for *Phlebotomus* (Dolmatova, 1942), Tabanidae and Heleidae (Glukhova, 1958). Great importance is attached to the duration of digestion, since the rate of digestion affects the frequency of biting and hence the ability of the insect to transmit disease.

When females are fed in the laboratory, and kept in crowded containers, the ovaries of a high proportion of mosquitos do not develop even after a number of consecutive meals. Accurate recording of the time taken by the digestive process showed that even in identical conditions variations occur in the length of the time needed for the digestion of blood by the same female. More time is necessary to digest the first blood-meal than the subsequent meals; while the average digestion time for the first meal is 65-68 hours, it will be about 45-55 hours for the sixth and seventh meals. The reason for this difference is not yet established and it may only be supposed that the increased speed of digestion after the first blood-meal is due to a gradual intensification of the functions of the digestive glands.

We also established the time necessary for blood digestion and for simultaneous development of the ovaries in relation to the number of blood-meals already taken by the female. It was proved that the process of blood digestion and development of the ovaries was faster the greater the number of blood-meals which had been taken before the beginning of the first gonotrophic cycle. The process was slowest after the first blood-meal. Thus, digestion of blood and maturation of ovaries lasted 97 hours after the first blood-meal, 85 hours after two consecutive blood-meals and 69 hours after four meals. This relationship can also be partly explained by the speeding-up of the digestive process of females taking subsequent blood-meals.

Observations carried out on the age differences in the speed of blood digestion and ovarian development did not give any clear-cut results.

There is only a slight decrease in the speed of the process as the females grow older. It is probable that the absence of any significant relationship between the duration of the processes and the age of the females can be explained by the fact that the two processes tend to cancel each other out: on the one hand, digestion speeds up when the second blood-meal is taken, while on the other, there is a general slowing down of the metabolism as the insect grows older, and this may have an effect on the digestive processes.

It is well known that female *A. maculipennis* show considerable individual variations in the speed of blood digestion. One of the probable causes of this variability is the physiological condition of the individual insect, i.e., the age and functional differences in the speed with which the digestive processes are completed (Detinova, 1953b). We were able to subdivide all females into three groups according to their physiological condition:

- (1) those in which the ovaries had developed after a blood-meal;
- (2) those which had previously oviposited but whose ovaries did not develop after a new blood-meal; and
- (3) nulliparous females whose ovaries did not develop after a blood-meal.

There is a considerable difference in the speed of blood digestion by females in these three groups. Thus, while at temperatures ranging from 20°C to 26°C the average duration of blood digestion with simultaneous development of the ovaries was between 73.4 and 87.1 hours, the digestion in females whose ovaries were not developing after this blood-meal lasted 55-65 hours, and in females whose ovaries had never developed, 57.7-60 hours.

This indicates that the process of ovarian development slows down the speed of digestion. Neurohumoral regulation of the duration of the digestive process may therefore be postulated for mosquitos.

Fertility of Female *Anopheles maculipennis* in Relation to Age

The question of the fertility of malaria mosquitos has been quite fully described and many opinions have been advanced regarding the determining causes of fertility. Thus, Martini (1922a,b), Weidling (1928), Roy (1931), Ustinov (1941), Beklemishev (1944), Markovich (1951), Hosoi (1954b), Detinova (1955b), Bar-Zeev (1957) and Déduit (1957) mention the existence of a link between the over-all size of the female and its degree of fertility. The work of Pokrovsky (1935), Detinova (1936),

Kalita (1937), Vainshtein (1938, 1940), Naumov (1940), and Holstein (1954) shows that there are seasonal fluctuations in fertility. Kalita and Pokrovsky indicate the existence of different degrees of fertility in various subspecies and species (Saliternik, 1955), while Roubaud (1934), Vainshtein (1940), Danilova (unpublished material), Detinova (1949, 1955b), Lachmajorova (1950), Love (1954) and many other authors describe a connexion between the physiological age of females and their fertility. In view of the fact that almost all this work was carried out before it was possible to determine the physiological age of females, the conclusions as to the factors affecting fertility did not take age into account. The ability to determine the physiological age of females with accuracy, and at the same time to ascertain their fertility in one oviposition, made it possible to assess and to study the main causes for differences in fertility.

The relationship between the physiological age of female *Anopheles maculipennis messeae* and their fertility

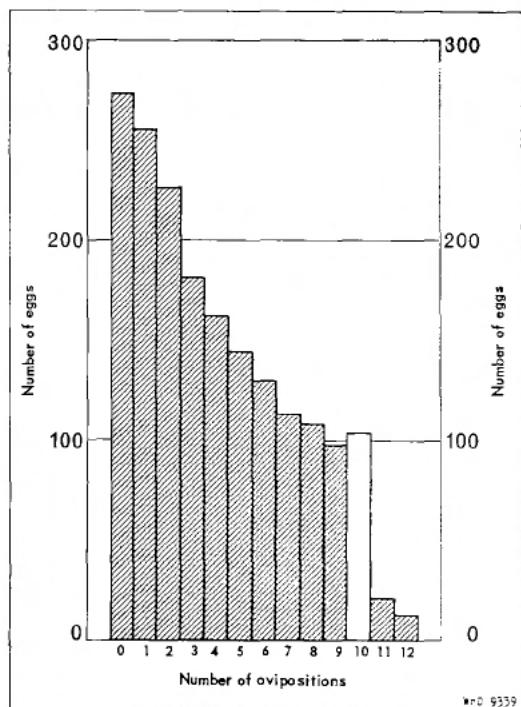
This work was carried out in the Moscow Oblast and in the south of the USSR. Wild females were dissected, their physiological age was determined, while at the same time the number of developing follicles was counted. This was done on females in stages 4-7 according to Sella's classification. The dissected females were of all the consecutive physiological ages from 1 to 13 inclusive. In the Moscow Oblast fertility in one oviposition was determined for 1400 females and in the south for 317.

The processing of the available material showed a direct connexion between the age of the female and the number of developing eggs (Fig. 15). Thus, while the average number of developing eggs in the first gonotrophic cycle was 276.5 in the females of the Moscow district, in subsequent ovipositions a gradual reduction in the number of such eggs was noted, and in a female in its thirteenth gonotrophic cycle only 12 eggs were found. The same relationship was noted in the females in the south. This fall in fertility, proceeding side by side with physiological aging, can be explained by the fact that with increasing age there is a reduction in the proportion of ovarioles which produce a normally developed egg while the number of ovarioles producing degenerated follicles increases. An increase in the number of such follicles occurs not by reason of their gradual accumulation in the ovaries of parous females, but by a reduction in the proportion of normally developing follicles in each consecutive cycle. The fact that ovarioles which produce a degenerate follicle in one of the cycles are capable of producing a normally developed follicle in subsequent cycles is confirmed firstly by the finding of ovarioles in which normal development of the next follicle was taking place despite the degeneration which had occurred in a previous gonotrophic cycle, and secondly by the

occurrence of individuals in which, under laboratory conditions, the number of eggs laid in certain ovipositions was greater than in previous ones. In every age group considerable individual variations were noted in the number of developing eggs.

Many workers (Roubaud, 1934; Roy, 1931; Vainshtein, 1938, 1940; Love, 1954; Lachmajorova, 1950; Holstein, 1954) observed the reduction in the number of eggs laid in consecutive ovipositions by female *Anopheles* living under laboratory conditions.

FIG. 15. MEAN FERTILITY OF FEMALE ANOPHELES MACULIPENNIS IN RELATION TO THEIR AGE



Relationship between the age of the females and their fertility has been established for a number of other Diptera, e.g., for *Musca domestica* by Lineva (1950) and Dunn (1923), and for horse-flies by Olsufiev (1938).

It is quite probable that the connexion between the increase in the physiological age of females and the decrease in their fertility which has been noted in several Diptera is characteristic also of other multiparous insects.

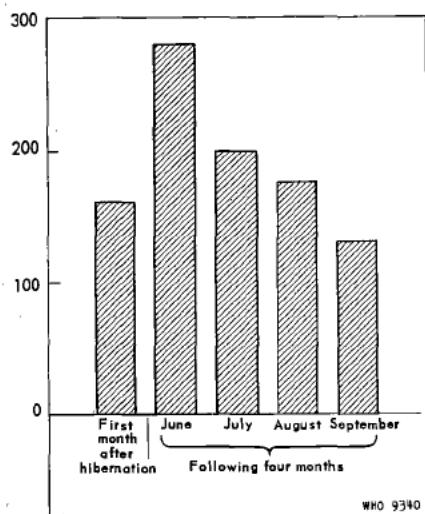
Seasonal changes in fertility

Observations carried out by a number of research workers on the average fertility of females dissected, at various times during the season, regardless of their age, showed that there is a seasonal variation in the size of ovipositions (Mer, 1931; Pokrovsky, 1935; Detinova, 1936; Vainshtein, 1938, 1940; Ustinov, 1941; Saliternik, 1955). The greatest fertility was noted in females in the early summer months. No explanation was given for this phenomenon. The material collected by me throughout the whole season on the fertility of females in one single oviposition threw some light on the problem; the mean monthly fertility rate of summer females fell gradually from month to month (Fig. 16 and 17). Thus, while

the mean fertility of the females was 282 eggs in June, the respective figure for July was 235, for August 180, and for September 132. The fertility among overwintered females also fell in the spring; there were 195 eggs per oviposition in April, 172 in May and 149 in June. We established that this reduction in fertility was caused, among overwintered females, by changes in the age composition of the parous female population, and among summer females by changes in age composition at various periods during the season, as also by a seasonal variation in the fertility of groups of females of the same age. I should like to dwell

briefly on these figures. After overwintering during the year of our observations (1952), the first flight occurred at the beginning of April. Because of low temperatures in that month the gonotrophic cycles proceeded more slowly and this resulted in only single specimens, among the investigated insects, which were in their second or third gonotrophic cycle; all the others were in their first, i.e., most prolific, cycle. In May the group of investigated females consisted mainly of parous females, most of which had completed two and three ovipositions. In June all the overwintered females were already multiparous and consequently their fertility, as has

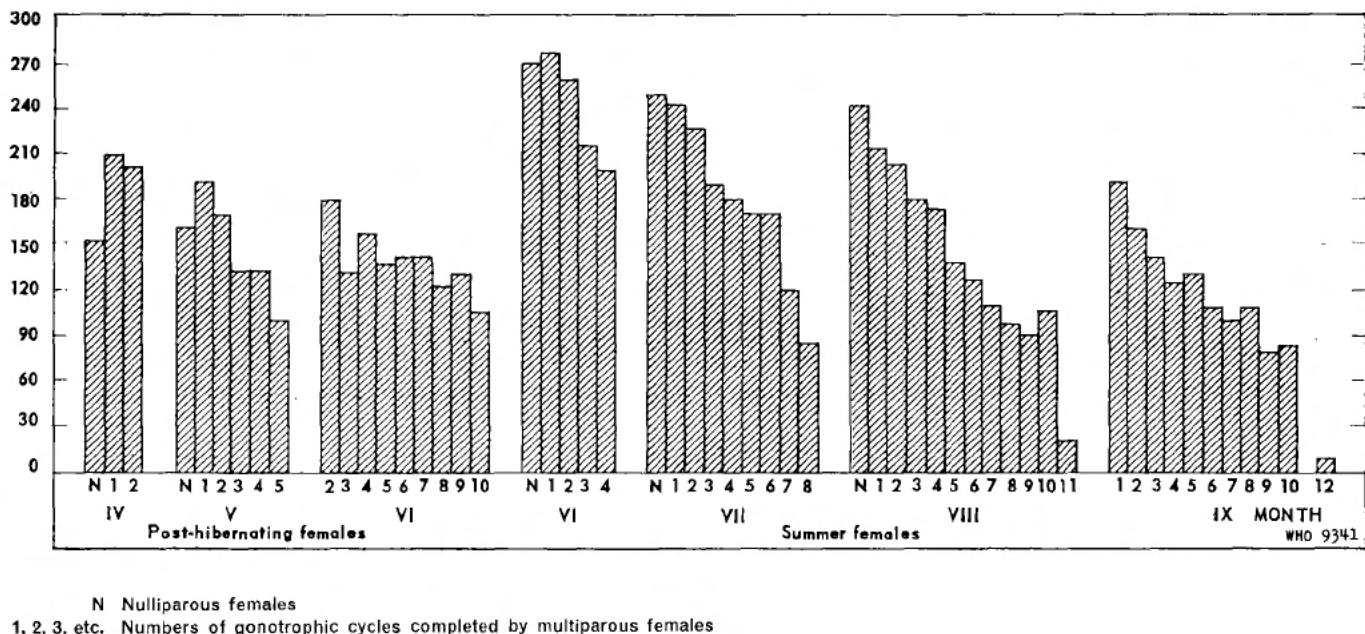
FIG. 16. MEAN MONTHLY FERTILITY OF
FEMALE
ANOPHELES MACULIPENNIS MESSEAE



already been stated, was considerably lower than among the pauciparous females.

The emergence of the first generation took place on 4 June. Among the June females pauciparous and nulliparous insects predominated; as a result of this the average fertility was high among all insects dissected at this time. In the subsequent summer months the reduction in fertility can be explained firstly by the fact that a considerable proportion of those dissected were already multiparous, and secondly, as already stated, by the occurrence of seasonal variations in the fertility of females of the same age. We established that from month to month there is almost always a gradual reduction in the average number of developing eggs in females of the same physiological age. Thus, for example, the average fertility of females in their first gonotrophic cycle was equivalent in June to 289.2 eggs per oviposition, in July to 263, in August to 255, and in September to

FIG. 17. RELATIONSHIP OF FERTILITY TO THE MEAN AGE OF FEMALE *ANOPHELES MACULIPENNIS MESSEAE*
DURING THE SHORT INTERVALS OF THE SEASON



180. For females in their second gonotrophic cycle the corresponding figures were 294, 255, 226 and 205. The same relationship was noted for females of all the other physiological ages. Despite the seasonal reduction in fertility in females of all ages the basic relationship between age and fertility is preserved.

It is impossible to explain by any other factor than reduction of initial fertility the occurrence of a seasonal reduction in the number of eggs laid by females of the same age. By initial fertility is meant the total number of ovarioles in the ovaries at the first flight of the individual female. Since the fertility of females falls with age, the higher the initial fertility the larger is the number of eggs which develop in females in each consecutive gonotrophic cycle, and this is actually noted in groups of females of the same age in different months. The occurrence of the same relationship in females studied by me in the south of the USSR leads to the conclusion that this phenomenon is general for the species and perhaps for the whole genus.

An analysis of the data, according to the conditions in which development of each dissected female has taken place, shows that the most probable reason for the degree of initial fertility is the length of daylight during pre-imaginal development. The seasonal changes in body size and fertility coincide with the seasonal changes in the length of daylight, whereas there is no such coincidence with the temperature.

The period of development of each individual was determined from the total number of gonotrophic cycles completed and from the duration of each cycle at corresponding temperatures. The method of this calculation will be described later.

For *A. gambiae* Holstein (1954) showed that the fertility of the females varies in the dry and wet seasons. Thus, the average number of eggs per oviposition laid by the female in the dry season was 128.1 and in the wet season 206. The maximum number of eggs laid by a single female in four consecutive ovipositions in the dry season was 480 and in the wet season 656.

The relationship between fertility and body size in female *Anopheles maculipennis messeae*

One of the interesting factors in analysing data on fertility is the establishment of a relationship between the size of the female and the number of developing eggs. The size of females was determined from the length of the femur. The fertility in one oviposition and the body size of 1026 females were determined. The existence of a direct relationship between age and fertility made it possible to evaluate the closeness of the relationship between body size and fertility only within groups of the same age.

We found that among physiologically young females, where age changes in fertility are not yet very great, a fairly close association exists between the factors mentioned. In the first gonotrophic cycle the coefficient of correlation r was equal to 0.47 ± 0.06 and in the second to 0.52 ± 0.05 . In older females in which the reduction in fertility dependent on age was more clearly marked, the relationship between these factors was less obvious and subsequently disappeared altogether. This is understandable since females of all sizes survive to physiological old age, while their fertility falls catastrophically.

Our data concerning the relationship between body size and fertility among physiologically young females fully concurred with the relevant observations by Roy (1931), Martini (1922a,b), Beklemishev (1944), Markovich (1951), Hosoi (1954b) and others whose investigations were carried out mainly on laboratory-reared uniparous or pauciparous females.

**The whole-life fertility of female *Anopheles maculipennis*
*messeae***

The data obtained with regard to changes in fertility related to age allowed us for the first time to tackle the problem of determining the fertility of females, living throughout their whole life in various periods of the season. For rough calculations we added together the average number of eggs per oviposition characteristic of the various periods of the season. These calculations established that on the average a female that completed 13 ovipositions could lay about 2000 eggs. If the maximum figures were taken the total number would be 3500. A very interesting fact was that the occurrence of seasonal variations in fertility among groups of females of the same age had an effect also on the total number of eggs laid by the female during its whole life. Thus, for example, in June, during its first four ovipositions, a female laid 1085 eggs; the respective figures were 964 in July, 890 in August and 708 in September. As already stated, the females in the south of the USSR were less fertile than those in the central areas. This meant that their whole-life fertility was also considerably lower during each part of the season.

These data also made it possible to assess the problem of determining the average sexual production of the whole population during the season. To determine this figure it is necessary to know the average total number of females in the population, their age composition, their fertility, and the number of captured females that can lay eggs during the 24-hour period concerned. An analysis of all the data showed that sexual production may vary considerably, even when the population is identical in numbers, since the age composition of parous females and their fertility varies very considerably at different periods.

**Basic Features of the Physiology of the Various Age Groups of Female
*Anopheles maculipennis***

On the basis of all the available data on functional and age changes in the organs of female *A. maculipennis* and on the influence of age on the duration and intensity of their vital processes, it was possible to reach a conclusion as to the occurrence of physiological differences between various age groups of these blood-sucking insects. A number of periods could be distinguished in the life of gonoactive females, each period characterized by a particular condition of the organism and by functional and age changes in many organ systems.

The first period, from emergence to the first blood-meal, is marked by the following changes in the body of the female:

- (1) the maturing of the corpora allata; there is an increase in the size of the glands and in the total number of their nuclei;
- (2) the maturing of the ovaries; the first follicles mature to stage I-II or II after the female has taken a carbohydrate-meal or through its use of the internal reserves accumulated during the larval or pupal stage;
- (3) the distension of the diverticulum by a carbohydrate-meal or by water;
- (4) the passage of small amounts of the contents of the diverticulum into the stomach and the simultaneous washing of the meconium out of the stomach;
- (5) the partial discharging of excreted substances from the cells of the Malpighian tubes in females which have ingested a large amount of liquid;
- (6) the fertilization of the females.

The second period stretches from the first blood-meal to the end of the first gonotrophic cycle. The following changes are noted:

- (1) at the moment when the blood is taken the walls of the stomach become fully distended and their tracheal system is stretched and uncoiled;
- (2) the blood-meal ensures that the meconium is washed out of the stomach of those insects which have not previously taken either water or a carbohydrate-meal;
- (3) during the process of blood digestion the first complete discharge of excreted material from the epithelium of the Malpighian tubes takes place and the epithelium is speedily charged again with excretory material;
- (4) for the first time the corpora allata discharge a hormone which assists with the development of the sexual products. After one discharge

of the hormone the glands decrease in size. By the end of the gonotrophic cycle they have somewhat increased once again;

(5) simultaneously with the process of blood digestion there occurs the first normal oogenesis, completed with the laying of mature eggs. During this process, the development of the first follicles occurs in all, or almost all, the ovarioles;

(6) during the growth of the follicles the tracheal skeins of the ovaries are unravelled. After the discharge of the eggs, i.e., by the end of the first gonotrophic cycle, these skeins have been transformed into a tracheal net which consists of long, almost straight, tracheal branches and elongated tracheoles;

(7) after oviposition, a sac of stretched intima first forms in the terminal portion of the ovariole; the sac then contracts to produce the first follicular dilatation;

(8) during the maturing of the eggs the ampullae increase in size, and are noticeably reduced in size at the time of oviposition;

(9) the accessory gland increases in size during the development of the ovaries and decreases after the eggs are laid.

The third period is the age of maturity of the gonotrophically active multiparous female. The following changes are noted:

(1) with each completed gonotrophic cycle the number of dilatations in the ovarioles increases;

(2) as the physiological age of the female increases, so the number of non-functioning ovarioles which produce degenerate follicles constantly increases; in consequence the fertility of the females decreases with the physiological age;

(3) as the females grow older their metabolism becomes slower, and in each new gonotrophic cycle there is a decrease in the number of females with the epithelium of the Malpighian tubes completely filled with excreted material;

(4) during the first three gonotrophic cycles there is a noticeable increase in the size of the ampullae. Cyclic changes in their size are also seen during all gonotrophic cycles, i.e., the ampullae grow bigger during the maturing of the eggs and smaller after oviposition, this being connected with the accumulation of secretion and its discharge on oviposition;

(5) at the beginning of each new gonotrophic cycle there is a certain increase in the size of the corpora allata, with a decrease after a full blood-

meal has been taken by the female. This change is caused by the accumulation and subsequent discharge of the hormone into the body cavity;

(6) in each gonotrophic cycle the size of the accessory glands increases. At the moment of ovulation the glands grow smaller again as a result of discharge of their secretion;

(7) no visible changes take place in the tracheal system supplying the ovaries and the stomach.

The fourth period begins with the eighth gonotrophic cycle. In such physiologically old females:

(1) the number of ovarioles in which follicles develop normally is sharply reduced;

(2) many cases are observed where follicles degenerate again in the same ovarioles during the consecutive gonotrophic cycles;

(3) the number of follicles degenerating during the period of yolk accumulation noticeably increases. These degenerated follicles take on a distinctive coloration giving the ovaries a barred appearance;

(4) the diameter of the Malpighian tubes decreases and the distribution of the excreted material in the cells along the length of the vessels is uneven. At each new gonotrophic cycle many females show no accumulation of excreted material.

Thus it follows that in the organism of female *A. maculipennis* there is a certain periodicity connected with age and functional changes. This periodicity is closely related to the rhythm of the gonotrophic cycles and is due to the taking of a blood-meal by the female and to the development of her ovaries.

In addition to these cyclic changes, non-cyclic irreversible changes occur during the gonoactive life of the female which lead to its aging and to the exhaustion of its vital functions.

Chapter 5

METHODS OF DETERMINING
THE PHYSIOLOGICAL
AGE OF FEMALE *ANOPHELES*

The earliest indications that characteristics existed by which it seemed possible to distinguish nulliparous from parous *Anopheles* by studying the condition of their reproductive system are found in the work of Kozhevnikov (1903). He mentioned that the "egg chamber" in the nulliparous female and in the parous female is approximately identical since, although it is swollen and elongated during the period of egg formation, it again becomes round and decreases in size after oviposition. However, the traces of the enlargement which takes place during oviposition are quite clearly preserved in the oviducts. If this work by Kozhevnikov had not been forgotten and if research in this direction had continued at that time, it would have been possible to learn much earlier how to determine the age of the females and how to establish the age composition of the population.

In addition, Kozhevnikov thought that a female which had just oviposited could be distinguished by its greatly distended but empty and translucent abdomen; he called this condition the post-partum period. This characteristic, however, cannot be considered reliable.

For a number of years the age of female *Anopheles* was determined by the method suggested by Perry (1912). This method, based on the appearance of the scaling of the wing, distinguishes four degrees of wear:

- (1) the whole scaling including the fringe of the hind edge of the wing is undamaged;
- (2) the fringe is slightly worn but the scales on the upper surface of the wing are whole;
- (3) the fringe is badly damaged, the upper surface of the wing is worn, but the spots on the wings are clearly distinguishable;
- (4) only remnants of the fringe remain, the upper surface of the wing is badly worn and the wing spots are almost indistinguishable.

Until the 1930's Perry's method was widely employed to determine the age of mosquito populations but nowadays few authors advise its use. In the USSR the method is no longer used, as it is considered that the wear of the wings is due not only to the age of the females but mainly to the conditions under which they live. As far back as 1932 Gordon, Hicks, Davey & Weston showed that there is a lack of concordance between the degree of wear on the wings of the females and the rate of infection of mosquitos. In some of the females whose wing-scaling was undamaged, sporozoites were discovered in the salivary glands.

The difficulty of applying this method is also due to the fact that examination of each female must be carried out immediately after capture. If the females are placed in test-tubes or cages, wing-scales are quickly damaged and thus the number of the late stages according to Perry's classification artificially increases.

In temperate latitudes, Perry's method may be used to determine the first appearance of individuals of the first generation, since by that time all overwintered females have their wings badly worn and are easily distinguishable from the females whose wings are undamaged.

In addition to Perry's method, a number of other indicators were used for tentative assessment of the age of female mosquitos.

(1) *Eggs retained in the ovaries.* Naturally the presence in the ovaries or in the ampullae of retained mature eggs points to the fact that the relevant female has already oviposited. However, since not all females retain eggs, it is impossible to establish from the total number of individuals with retained eggs the actual proportion of parous females among all those examined.

(2) *The presence of meconium in the stomach.* This is the best indication that the newly emerged female not only has not had a blood-meal but has not ingested any liquid at all. However, it is impossible to establish the proportion of nulliparous females from this sign alone, since the meconium may be excreted soon after flight and the female may remain nulliparous for a number of days. The absence of meconium in the stomach cannot therefore be taken as an indication that the female belongs to the parous group.

(3) *The presence in the body cavity of green-coloured fat.* Since the change in the colour of the fat occurs after a varying number of hours following the first flight of the adult, this sign cannot be used to distinguish the group of nulliparous females.

(4) *The presence in empty females of a greatly distended and translucent internal oviduct.* This sign can be used to distinguish a female which has oviposited not long before. As the next batch of eggs develops the oviduct is contracted and becomes obscured by the yolk of the follicles.

Among methods whose application is just as limited may be mentioned the recent suggestions by Gillies (1956b) and Gillett (1957a). Gillies first pointed out that in females of *A. gambiae* a secretion of the male during impregnation forms "mating plugs" after copulation. In some females two such plugs have been found, showing that they had copulated twice. Gillies suggested that the presence of the mating plug should be used to determine the age composition of the population and to assess the effectiveness of control measures.

Since the mating plug is resorbed in the course of a few hours, its presence can be used only to distinguish the group of females which have just copulated; it is impossible to determine the total proportion of nulliparous females from this sign alone. Gonoactive females may remain nulliparous for a number of days and this period is made up of (a) the time between the flight and the first blood-meal, and (b) the time required for the first development of the ovaries. Davidson (1954) established that in *A. gambiae* in tropical Africa this period lasted for five days.

Gillett suggested, from the example of *Taeniorhynchus (Mansonioides) africanus*, that the presence or absence on their bodies of larvae of the Hydrachnidae could be used for determining the age of the females. The author stated that the adult mosquitos are infested as they emerge from the pupa; therefore the presence of mites on the body of the mosquito may indicate that a particular female is nulliparous. During oviposition when the females alight on the water, the larvae of the mite, ready for moulting, fall off into the water, where they continue their development.

Entomologists in the USSR used this method previously as an auxiliary indication and tried to establish a connexion between the presence of Hydrachnidae, and particularly of *Megaluracarus globator*, on the body of the females and their physiological age. It is true that in the overwhelming majority of cases only the newly emerged females are infected. However, observations carried out on the degree of infestation of females with these mites and simultaneous determination of their physiological age led to the conclusion that mites are also found on the bodies of parous females. This finding and the fact that not all waters in which mosquitos develop are infested with Hydrachnidae, and that therefore not all females which have flown have mites on their bodies, show that it is impossible to establish the proportion of nulliparous females by this method.

From the middle of the 1930's until the beginning of the 1940's extensive use was made of the method put forward by Mer (1932) for the determination of the age composition of mosquito populations; this method was based on the changes in the size of the ampullae, which were measured with an ocular micrometer.

As physiological and age changes in the reproductive system of females were more and more thoroughly studied, methods of determining physiological age improved. The method proposed by Polovodova (1947, 1949),

based on counting the number of dilatations in the ovarioles, was a culmination to all the work in this field.

To separate out their reproductive organs, digestive system and Malpighian tubes, the females are dissected in the following way. They are first lightly anaesthetized with ether or chloroform and then immersed briefly in alcohol and placed on a glass slide in a drop of physiological saline. The internal organs are then separated out as follows: the female is held on the slide by a dissecting needle held in the left hand and piercing its thorax; another needle held slantingly in the right hand presses down and pulls asunder the two final segments. The internal organs come away with these segments. This type of dissection can be done with the naked eye or with the help of a magnifying glass. The Malpighian tubes should be examined immediately after they are dissected out, as otherwise their condition may change slightly.

Determination of the Physiological Age of Females from the Size of the Ampullae

Polovodova (1941) showed that an increase in size of the ampullae takes place only during the first three gonotrophic cycles and that using Mer's method it was impossible to distinguish the physiologically old females among all the parous females. It was established that there are great variations in size between individual females. This meant that in some instances the ampullae of physiologically old but small females were smaller than the ampullae of physiologically young but large females. However, Mer's method can be successfully used to establish the time of flight of individuals of the first and subsequent generations, since in the majority of cases it is possible to distinguish the newly emerged females by the small size of their ampullae.

As was shown by Polovodova, and later by Swellengrebel, data on the size of ampullae made it possible, to a certain extent, to judge the trend of changing age composition of the population from the increase in the proportion of females with large ampullae. It was also possible to determine the time of appearance of the diapause from the considerable increase during the autumn of the proportion of females with small ampullae. An attempt was made by Sarikian & Pölezhaev (1941) to assess the effectiveness of control measures from data on the size of ampullae but the method proved inaccurate and cumbersome.

Determination of the Physiological Age of Females from Qualitative Changes in the Ampullae

In 1941 Polovodova suggested a method of determining the physiological age of females from qualitative changes in the structure of the ampullae. She subdivided all females into three age-groups:

(1) *newly emerged females*—the ampullae are transparent and their walls differ from the walls of the paired oviducts only by the presence of transverse folds. By the end of the first cycle the walls of the ampulla take on a slightly brownish shade and its folds appear swollen;

(2) *pauciparous females*—the folds of the ampullae are more swollen but often preserve a regular transverse appearance;

(3) *multiparous females* (i.e., those which have oviposited three times or more)—the folds in the ampulla walls are very swollen and irregularly contorted.

Although it is possible from these signs to establish with a certain degree of accuracy the physiological age of females without measuring their ampullae, the work is nevertheless very cumbersome; it is difficult to differentiate between females of various age groups and the assessment of changes in the walls of the ampullae is too subjective. For these reasons the method has not been used in practice and is now discarded.

Differentiation between Parous and Nulliparous Females by Examination of the Condition of the Tracheal System of their Ovaries

Only females in stages 1 and 2 according to Sella's classification are dissected, i.e., those with follicles in which there is only a small amount of yolk, thus making the tracheae of the ovaries plainly visible. The females are dissected in a drop of pure water. The ovaries dissected out of the body cavity are carefully transferred to the edge of the drop or placed in a separate small drop of water where they dry out. In drying out, the air enters the tracheae and the whole tracheal system, including the smallest tracheal branches and tracheoles, supplying the ovaries becomes clearly visible. The presence of skeins shows that the females are nulliparous, whereas the presence of a tracheal net is the sign of a parous female. It is not advisable to dissect the ovaries in physiological saline, since the salt crystals formed on drying would cover the whole of the ovary and the tracheae would become invisible. The drying ovary adheres to the slide and can be kept in good condition for a number of years if necessary. Such specimens are not mounted; they need only to be protected from dust and rubbing. If there is dust on the ovaries a drop of clean water should be placed on the specimen and then immediately shaken off. The ovary then clears up and the tracheae become visible.

A large batch of mosquitos may be dissected at the same time and examined as the ovaries dry out. The examination of the tracheal system is done under a low-power microscope. One investigator can, without special effort, dissect and examine 300 or 400 or even more females daily. A number of females may be dissected on one glass slide, but as it is often

of interest to establish the relationship between empty and freshly fed females, insects in stages 1 and 2 of Sella's classification should preferably be dissected on separate slides. It is also possible to leave the body of the female near each ovary dissected out, to know on the examination of the ovary whether the female was parous or nulliparous, and if there was any blood in its stomach. These preparations will stand long storage and the collected material can be rechecked at any time.

Determination of the Physiological Age of Females from the Number of Dilatations in their Ovarioles

Females at any stage of blood digestion and ovarian development are used for dissection. The ovaries are examined under a binocular microscope. The most suitable magnification is obtained by using Zeiss ocular 7 and objective 8.

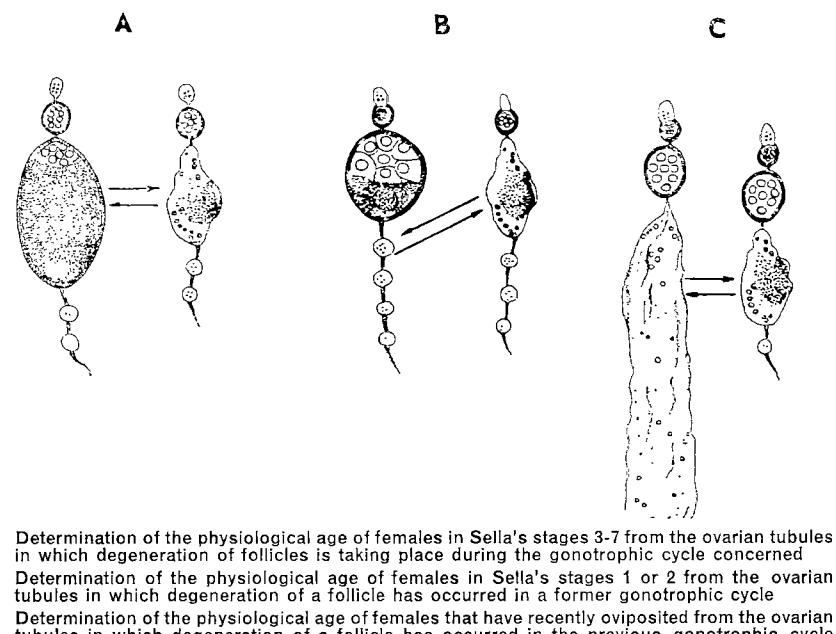
During dissection the ovaries should always be kept in a small drop of physiological saline. The ovary is moved to the edge of the drop and the ovarioles are examined in a small quantity of liquid.

The whole process of examination of the ovaries to determine the number of ovipositions consists of the following stages. One of the ovaries lying in a drop of physiological saline is immobilized on the slide by means of a needle held in the left hand; a needle held in the right hand is used to pierce the outer ovarian membrane in several places and then to remove it bit by bit. Where the membrane has been removed the ovarioles appear separated one from another. This makes further examination much easier. The internal oviduct and the ovary are then pierced with the left-hand needle to immobilize them. The ovariole being examined is carefully moved aside from the internal oviduct with the right-hand needle. At the same time the whole terminal portion of the ovariole, which up till then has been pressed up against the internal oviduct by the developing follicle, is straightened out and its dilatations become visible. It is possible to count the dilatations only when the ovariole has been preserved in its entirety and when the site of its connexion with the internal oviduct is visible. If the pressure is carelessly applied, the ovarioles are very easily torn and then the total number of dilatations cannot be determined. Only if the ovariole torn away from the internal oviduct preserves its terminal pedicle is it still possible to determine the physiological age of the female. If the terminal pedicle is missing in the isolated ovariole it is impossible to establish the age of the female, since it is not known how many dilatations were torn away. It is necessary to examine a number of ovarioles in each ovary.

The number of dilatations in the ovarioles may also be counted by another method, which is particularly recommended if the female has

fully developed eggs. When the ovarioles containing the ripe eggs are stretched the eggs usually come out of the ovarioles through tears in the intima which covers them. It is difficult to stretch the empty ovarioles by means of a dissection needle and to count the total number of dilatations in their internal portions. Therefore, when the outer membrane of the ovaries has been removed or torn in several places, the ovarioles with the developed eggs are moved to the side; between them those ovarioles

FIG. 18. DETERMINATION OF THE PHYSIOLOGICAL AGE OF FEMALE MOSQUITOS FROM OVARIOLES WITH A DEGENERATING FOLLICLE



are sought which are not functioning in the last gonotrophic cycle and of which the last follicles are degenerating. The number of dilatations in these ovarioles with the degenerating follicles are counted. The physiological age of females whose ovaries are in stages 3 and 4 can also be determined from such ovarioles, since the degeneration of follicles always occurs simultaneously with the normal development of follicles in other ovarioles. The degenerating follicle corresponds in its position in the ovariole to a normally developing follicle and the number of dilatations below it corresponds to the number of ovipositions completed by the examined female (Fig. 18A).

The age of females may also be determined from the number of "false dilatations"; these are small follicles separated out in ovarioles in which

development has never taken place. The breaking away from the germarium in each cycle of a new completely undeveloped follicle also makes it possible to calculate correctly the number of gonotrophic cycles completed by the female.

Where the examined female is in Sella's stage 1 or 2 and has ovaries still in the first phases of development, its age may be determined from the ovarioles which contain a degenerating follicle. However, in this case the process of degeneration could not have taken place in the last cycle and the degenerate follicles must be ascribed to the previous gonotrophic cycle. Thus by their position they correspond to the mature eggs deposited during the previous cycle and to the new dilatations in the terminal portions of the other ovarioles which were formed at the site of development of those eggs. Therefore, in determining the age of a female, the dilatations containing products of degeneration are also counted in addition to the total number of dilatations in the terminal portions of the ovarioles (Fig. 18B).

In determining the age of a female in Sella's stage 1 or 2 which has recently oviposited, when all the ovarioles from which mature eggs have been discharged are sac-like in appearance, it is necessary to seek out those in which degeneration has taken place in the cycle just completed. Only from such ovarioles is it possible to determine the physiological age of a female that has just oviposited. In this case the position of the degenerated follicle in the ovariole corresponds to that of a newly discharged mature egg, and in calculating the number of ovipositions completed it is necessary to count this degenerated follicle in addition to the total number of dilatations in these ovarioles (Fig. 18C).

Thus, the fact that in *Anopheles* during each gonotrophic cycle each ovariole produces a new follicle (which may either develop normally or degenerate at one of the phases of development or before the beginning of growth and the accumulation of yolk in the follicles) makes it possible to determine with accuracy the physiological age of each dissected female.

Determination of the Presence or Absence of a Previous Blood-Meal from the Appearance of the Tracheal System of the Stomach

The method of determining which females have or have not had a blood-meal is based on the changes which take place in the tracheal system of the stomach at the moment when the first portion of blood is ingested. To examine the condition of the tracheal system, the stomach is placed on an object glass in a small drop of pure tap- or well-water (but not saline, since the salt crystals which sediment out during drying hinder examination) and is then dried. During the drying process air enters the tracheae and the tracheoles, making them clearly visible under the microscope or bin-

ocular microscope. The dried stomach, which adheres to the glass, is not covered with a cover slip or processed in any way and can be kept for a long period.

In determining whether females belong to the group of those which have taken a blood-meal or not, only the stomachs of empty females are examined. The material obtained on the condition of the tracheal system makes it possible to establish the proportion of summer gonoactive females which have taken a blood-meal but whose ovaries have not developed. In this case the tracheae on the stomach are stretched, while skeins remain on the ovaries. Further, by inspection of the gastric tracheation the proportion of blood-feeding diapausing females can be established. If the female takes carbohydrate nourishment the gastric tracheae are not stretched, since the sweet liquids pass first of all into the crop and thence enter the stomach in small portions only, causing no distension. For that reason the finding of diapausing females with unravelled smaller tracheae and tracheoles on their stomachs indicates that they have taken a blood-meal. These data make it possible also to determine the epidemiological importance of diapausing females under warm autumn conditions, when, despite the completion of gonoactivity, the process of sporogony appears to be still possible as far as temperature is concerned. The method of determining the state of the gastric tracheal system is just as simple as that of examining the ovarian tracheation of female mosquitos and can be carried out by one conversant with that method.

Chapter 6

FUNCTIONAL AND AGE CHANGES IN THE REPRODUCTIVE SYSTEM OF SOME BLOOD-SUCKING AND NON-BLOOD-SUCKING DIPTERA

The study of age changes in the reproductive system of females of a large number of blood-sucking insects has been carried on by entomologists in the USSR simultaneously with the study of similar changes in *A. maculipennis*. The methods of determining the physiological age of females have a practical importance and are also of considerable theoretical interest, since they provide comparative material on the physiology of various groups of blood-sucking Diptera.

It has now been established that in the majority of females of blood-sucking Diptera (Orthorrhapha) characterized by gonotrophic concordance (Simuliidae, Heleidae, Tabanidae), the same changes take place in the ovarioles as have been noted in mosquitos. This has opened a possibility of determining with accuracy the physiological age of every female examined.

The formation of dilatations in the ovaries proved to be also a characteristic of Diptera not showing gonotrophic concordance, such as Hippoboscidae, and of those which do not suck blood (*Musca domestica*). Age changes found in the reproductive system of a number of blood-sucking Muscidae and *Phlebotomus*, made it possible to determine the physiological age of females with some degree of reliability.

Wanson (1950) established that there were three follicles in each ovariole of nulliparous female Simuliidae and hence concluded that they could pass through gonotrophic cycles. Having discovered corpora lutea in the ovarioles he concluded that the females are able to oviposit an even greater number of times since the growth zone continues to give off new follicles. In the end he came to the conclusion that female Simuliidae can oviposit a varying number of times, depending on the life-span of individuals and the possibility of finding blood-meals. Beklemishev (1957c) also suggested that these blood-sucking insects must oviposit more than once, since otherwise they could not be vectors of onchocerciasis of cattle and avian haemosporidiasis.

As the result of careful study of the physiology of female Simuliidae by Beltiukova (1953), Prokofieva (1957) and Rubtzov (1956) individuals were found in whose ovarioles one dilatation was visible—the result of a completed oviposition.

According to the findings of Davies (1955, 1957) and Lewis (1956, 1957a,b, 1958a,b) it is possible to distinguish parous female Simuliidae from nulliparous by means of a large number of characteristics, which include the general condition of the ovaries, the presence of corpora lutea in the ovarioles and the amount of fat-body in the abdominal cavity. The number of ovipositions completed by the females was not taken into account. According to Davies (1957) in nulliparous females there are no corpora lutea in the ovaries and the fat-body is strongly developed, whereas in parous females there are corpora lutea in the ovaries but all traces of fat-body have disappeared from the abdominal cavity. Lewis also indicates that in parous females there is no fat-body in the abdominal cavity, and he distinguishes the parous females not only by the presence of corpora lutea in the ovaries but also by the existence of dilated oviducts and the general loose structure of the ovaries (Lewis, 1956). In addition, the same author (1958a) noted certain differences in the condition of the accessory glands in the two groups of females examined.

In summer 1957, Detinova & Beltiukova (1958) proved that female Simuliidae can complete several gonotrophic cycles. Altogether only 147 females were dissected, belonging to various species of the family. However, even among this small number of females some were discovered which had completed up to five gonotrophic cycles. It is not impossible that individuals which had completed an even greater number of ovipositions would have been found if a greater number of insects had been dissected. Study of the reproductive system of the females showed that, as in mosquitos, after the mature eggs have been laid, the ovarioles take on the shape of a sac, which contains the remains of the follicular epithelium and corpora lutea. These sacs gradually contract, forming clearly marked dilatations. The shape and structure of these dilatations are the same as in mosquitos. Determination of the physiological age of female Simuliidae from these dilatations is more difficult than in the case of mosquitos or flies because of the smaller size of their ovarioles. The examination of individual ovarioles in female Simuliidae which had completed a varying number of ovipositions showed that they are typical for females which oviposit more than once.

The first follicle, which must develop in each gonotrophic cycle, is normal in structure and possesses a well-developed follicular epithelium. A new follicle breaks away from the germarium and this indicates that the ovariole in question is continuing to function. Each ovariole ends in a fine terminal pedicle opening into the internal oviduct.

It is possible to determine the physiological age of female Simuliidae from the number of dilatations in their ovarioles. Determination of the proportions of parous and nulliparous females among all those dissected may be done by examining the general condition of the ovaries and the shape of the terminal portions of the ovarioles. In the ovaries of empty parous females the expanded internal oviduct is quite translucent and the ovaries themselves seem to have a loose structure, as a result of the greatly expanded terminal portions of the ovarioles, whereas in nulliparous empty females the ovaries are very compact. Many years ago, Dolmatova (1942) described the same difference in the condition of the ovaries of parous and non-parous female *Phlebotomus* and used this characteristic to establish the proportions of the two groups in the population.

The proportion of parous and nulliparous females in the Simuliidae may be determined by examining the general condition of the ovarioles. While in nulliparous females there is a fine terminal pedicle beneath the first follicle, in parous females there is either a sac (immediately after passage of the mature egg) or a dilatation.

Prokofieva (1957) stated that the tracheal system supplying the ovaries of Simuliidae is very extensive and does not undergo any age or functional changes.

I shall not discuss in detail the age changes in the ovarioles of Heleidae and Tabanidae. These blood-sucking insects also oviposit more than once, but while in the Tabanidae oviposition can only take place after a full blood-meal, in Heleidae some of the eggs may develop even when an incomplete blood-meal has been taken. After the passage of the mature egg a sac is formed, the contraction of which leads to the formation of a dilatation. Glukhova (1956, 1958) and Buyanova (who carried out observations in 1947 and 1958) state that in field conditions female Heleidae completed up to four ovipositions. Bei-Benko found among wild females of various species of horse-fly individuals which had completed one or two ovipositions. According to data obtained by Olsufiev (1938) female Tabanidae are capable of as many as five ovipositions in the laboratory. In all probability, if a larger number of dissections is made in field conditions it will be possible to find physiologically older females. The structure of the individual ovarioles in Heleidae and Tabanidae indicates that females are capable of a number of ovipositions. In parous females the breaking away of new follicles from the germarium was noted and the follicle previously formed was in the stage of growth and development.

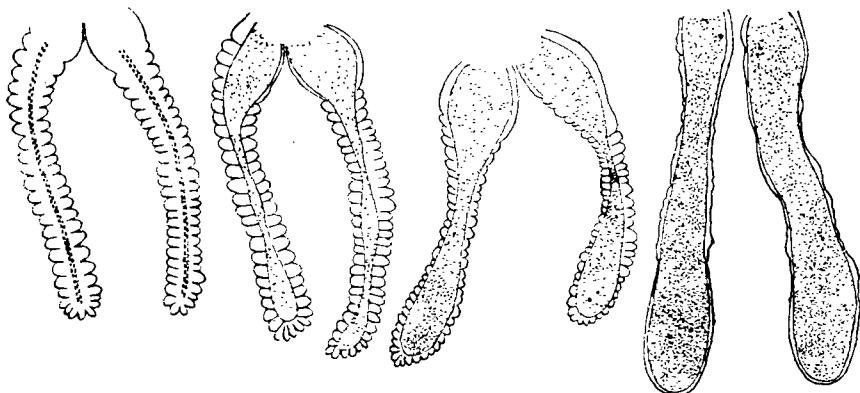
The dissection of Simuliidae and Heleidae and the examination of the ovarioles is done under the binocular microscope, but a higher magnification is used than in the case of mosquitos (for instance, a Zeiss ocular 7 or 10 and objective 10 or 12). In horse-flies the ovaries are examined under lower power. To examine the ovarioles of these insects, the outer coat of the ovary is first teased out and then gradually removed, as in

mosquitos. After this, the ovary is covered with a drop of physiological saline and steadied by means of an entomological needle held in the left hand; another needle held slantingly in the right hand is inserted under the ovariole to be examined to move it to the side so that the number of its dilatations can be counted.

Methods of determining physiological age in *Phlebotomus*, which is also characterized by gonotrophic concordance, were described by Adler & Theodor (1935) and Dolmatova (1942, 1946).

Adler & Theodor stated that the accessory glands of sandflies produce a secretion and increase in size after a blood-meal, when the ovaries begin to develop. When the eggs have matured, the glands are full to overflowing. On oviposition the secretion is discharged but a portion of it remains in the glands. The presence of the remaining secretion in the accessory glands of empty females with undeveloped ovaries shows that those females have oviposited (Fig. 19).

FIG. 19. FUNCTIONAL CHANGES IN THE ACCESSORY GLANDS OF FEMALE *PHLEBOTOMUS PAPATASI* DURING THE GONOTROPHIC CYCLE *



* After Dolmatova (1942).

Although Dolmatova showed that after the passage of a mature egg a long sac is formed in the ovariole which then gradually contracts, a method of determining age in *Phlebotomus* has not yet been worked out and it is not therefore possible to determine the exact physiological age of the females. Dolmatova proposed a method of determining whether a female was parous or nulliparous. This may be decided firstly by the structure of the ovaries in empty or freshly fed females. In nulliparous empty females, the follicles in the ovaries lie close together; the ovaries themselves are transparent and uniform in colour. In parous females the follicles in the ovaries do not lie close together; there is a "net" between them which is particularly noticeable in the centre of the ovary. This net is made up of elongated distal portions of the ovarioles, i.e., those portions where

the development of the egg takes place. The net is therefore clearly marked in females which have recently oviposited. A second characteristic of parous females used by Dolmatova is the condition of the accessory glands, as proposed by Adler & Theodor (1935). Dolmatova also showed that the tracheal system supplying the ovaries is extremely scanty and that no functional or age changes are noted in it.

Shoshina (1951), in determining the age composition of a *Phlebotomus* population, also used the condition of the accessory glands as a determining characteristic and distinguished the following age groups among the females examined:

- (1) nulliparous newly emerged females;
- (2) females in their first gonotrophic cycle;
- (3) females which had oviposited;
- (4) females in their second gonotrophic cycle.

In the case of blood-sucking flies not characterized by gonotrophic concordance, methods of determining physiological age have been worked out for *Stomoxys calcitrans*, *Haematobia stimulans* and *Lyperosia irritans* by Kuzina (1942, 1950a, 1950b) and for *Hippobosca capensis* by Detinova (1955a).

In the structure of their ovaries and the individual ovarioles, Muscidae and Hippoboscidae differ greatly from the blood-sucking Orthorrhapha which have already been discussed.

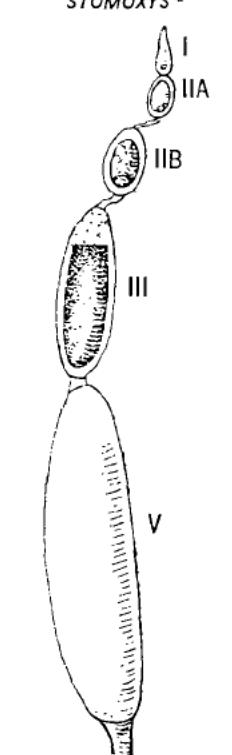
Thus in the ovarioles of *Stomoxys calcitrans*, *Haematobia stimulans* and *Lyperosia irritans* five or six follicles develop simultaneously. When the first follicle has completed its development, the second is usually already in stage III, the third at the end of stage II, etc. (Fig. 20). There is no gonotrophic concordance in blood-sucking Cyclorrhapha (Beklemishev, 1940). Each blood-meal leads to a shift in the development of the first batch of eggs and of the follicles lying above it. For complete maturation of the first batch of eggs, the female takes not less than three blood-meals

First follicle in stage V,
second in stage III, third in
stage IIB, fourth in stage
IIA, fifth in stage I.

* After Kuzina (1942).

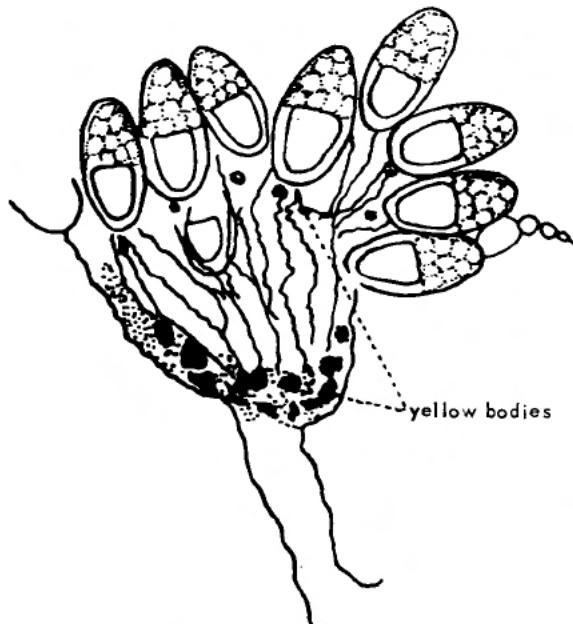
but a smaller number is required for the laying of subsequent batches, since the follicles are already in one of the end stages of development.

Kuzina showed that in the case of the three species mentioned, the main sign by which the physiological age of females can be estimated is the presence and number of the corpora lutea, which are not ejected with



the mature egg but remain at first in the ovariole and then pass down into the paired oviducts (Fig. 21). The presence in the oviducts of a large number of corpora lutea is a sign that the females concerned have

FIG. 21. OVARY OF AN OLD FEMALE *HAEMATOBIA STIMULANS* :
LARGE AGGLOMERATIONS OF YELLOW BODIES *



* After Kuzina (1950a).

oviposited many times. Kuzina suggested that according to this sign these flies should be divided into five age categories as follows:

- (1) Newly emerged females—the first follicle in stage II-A of the classification by Christophers.¹
- (2) Young nulliparous females—the first follicle may be at any stage from II-B to V. There are no yellow bodies under the first follicle.
- (3) Uniparous females—the first follicle may be in stage II, IV or V. Under these follicles there is a small yellow body usually of light yellow colour.
- (4) Females having completed two or more ovipositions—the first follicle is in the same stage as in uniparous females. The yellow bodies form more or less large accumulations according to the number of ovipositions completed. These accumulations are yellow but all differ in intensity of colour throughout their length.

¹ Characteristics of the phases of maturation of egg follicles as applied to flies were worked out by Derbenieva-Ukhova (1942, 1952).

(5) Old females with the ovaries full of yellow bodies—the first follicle as a rule is not in stage III but in II-B. Very often in these ovaries there are retained eggs, which are never found in the ovaries of females which have only completed a few ovipositions. The signs of aging include the irregular development of the follicles and granular degeneration and lack of elasticity of the ovarioles.

The method of dissecting females is as follows. They are lightly anaesthetized with ether or chloroform. Entomological scissors are used to cut the abdominal pleura. The female is placed on the slide and the reproductive system is pulled out with entomological needles and covered with a drop of saline solution. In view of the large size of the insect, dissection can be done without a magnifying glass. It is recommended that further examination should be carried out under a binocular microscope.

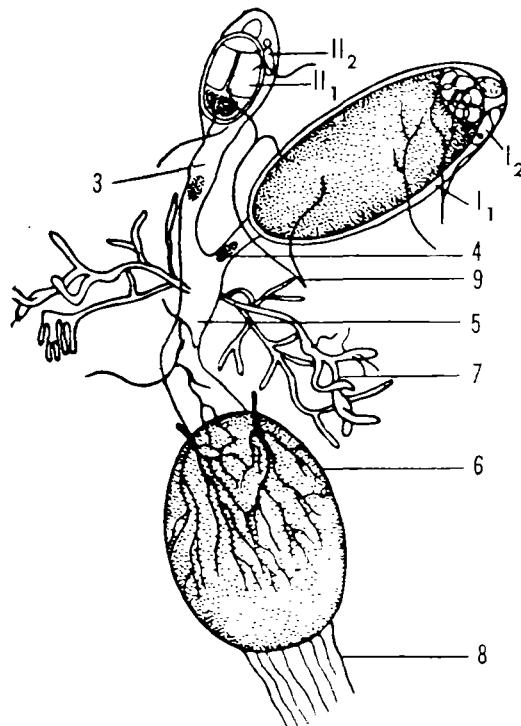
Age changes in the reproductive apparatus of Hippoboscidae, the most highly specialized blood-sucking Diptera, were described by Detinova (1955a). In this family the physiological age of females is determined by the number of pre-pupae they have deposited. It has been possible to determine this number by the changes occurring in the reproductive system.

The general outline of the structure of the reproductive system in *Hippobosca capensis* and the function of the ovarioles are not unlike those of *Musca larvipara* and *Mesembrina meridiana*, as described by Derbenieva-Ukhova (1952). The female reproductive system of *Hippobosca capensis* has two ovaries. From each ovary oviducts pass into the upper, narrow part of the uterus. The uterus opens out to form a wider portion—where the development of the larva takes place. Below this portion the uterus again contracts and ends in the genital opening. The uterus is extremely well supplied with tracheae. In the upper part of the proximal division of the uterus there are accessory glands, which begin to increase in size when the growth of the first oogonium begins. Thus, the finding of well-branched accessory glands when there is still an undeveloped pre-pupa in the uterus shows that the female is parous (Fig. 22).

Each ovary consists of two ovarioles covered by a richly tracheated common membrane. Each ovariole is invested with an external membrane, easy to remove with needles, under which there is an internal membrane or intima. The ovariole contains a follicle, above which lies the gerarium, which produces a new follicle; this follicle begins its development after the development and passage into the uterus of an egg from the first follicle. Each follicle contains eight cells: one oocyte and seven nurse cells. During the period of most intensive growth of the oogonium the nurse cells are sharply differentiated. The three cells directly adjacent to the oogonium take up the greater part of the volume of the follicle. The other four cells are small and are pushed into the proximal end of the follicle. The differentiation of the nurse cells into larger distal and smaller

proximal cells is found also in mosquitos (Beklemishev, 1944) and apparently in Muscidae (Derbenieva-Ukhova, 1952), but in *Hippobosca capensis* it is more sharply marked. The distal portion of the ovariole appears as a very long terminal pedicle, much longer than in the other Diptera

FIG. 22. PART OF REPRODUCTIVE SYSTEM OF A FEMALE
HIPPOBOSCA CAPENSIS



- I₁ and I₂: First and second ovarioles of the first ovary
- II₁ and II₂: First and second ovarioles of the second ovary
- 3: External oviducts
- 4: Collection of sperm
- 5: Narrow part of the uterus
- 6: Uterus with a developing larva
- 7: Part of accessory glands
- 8: Distal part of the uterus
- 9: Tracheae

mentioned above. All four ovarioles are always at different stages of development, during which a definite sequence is followed. The two ovaries always differ considerably in their over-all size. The final development of one follicle takes place in only one of the ovarioles, while the other three remaining ovarioles are at previous stages of development. The sequence of development of the follicles is as follows.

The follicle begins developing in the ovariole of one ovary. On reaching its final size the egg passes into the uterus. After the passage of the egg, the ovary concerned is much reduced in size and from that moment a considerable increase begins in the size of the follicle in one of the ovarioles of the second ovary, which consequently also increases in size. The development of this follicle is completed when the first pre-pupa leaves the uterus. After the passage of the pre-pupa the egg ready for development passes from the second ovary into the uterus and the next follicle, i.e., one in the second ovariole of the first ovary, begins to develop in turn. On completion of this development and after the passage of the egg into the uterus, the development begins in the second ovariole of the second ovary. Then the whole cycle can be repeated. It was found that after the passage of the mature egg, the ovariole takes on the shape of a sac, but this sac does not contract quickly. Females were found in which a fully developed larva lay in the uterus, while the ovariole, from which the previous egg and larva were produced, still had the form of a distended sac. After the final contraction of the sac, there remains in the terminal portion a dilatation containing a small amount of inclusions, probably the remnants of follicular epithelium. Among dissected females, we discovered some with one, two, three or all four ovarioles functioning alternatively. A female was also found with one dilatation in each of the four ovarioles and with the next developed egg in one of them, while a second egg was ready for development in one of the ovarioles in the other ovary. This shows that the ovarioles can function more than once. The dilatations in all the ovarioles differ in size, and this depends on the time which has elapsed after the passage of the mature egg (Fig. 23).

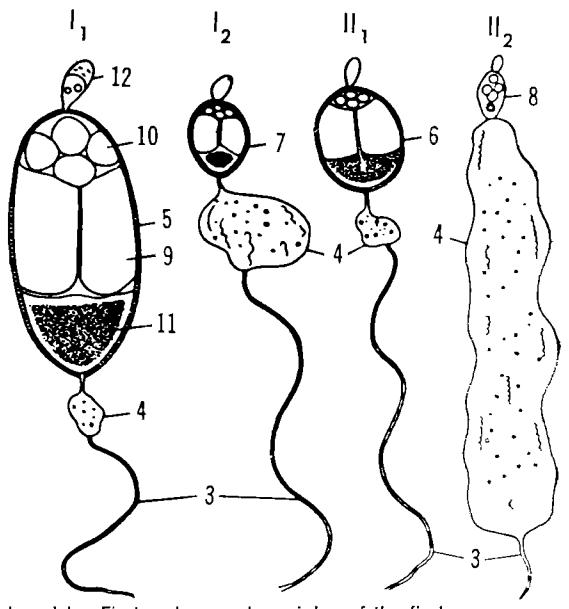
In the poultry-bitting louse *Ornithomyia aviculare* I found that the structure of the ovaries and their functioning were the same as in *Hippobosca capensis*. In a figure showing the structure of the reproductive system in the female *Musca larvipara*, Derbenieva-Ukhova (1952) has drawn a yellow body beneath the follicle of one of the ovarioles and this shows the sequence of ovarian function also in that fly.

In view of the great similarity of the structure of the reproductive system of female biting-lice and Glossinae, it may be that this method of determining physiological age can be used with success for determining the age of Glossinae.

In a recently published paper by Saunders (1960) a description is given of the structure of the ovaries in *Glossina morsitans*. He states that each ovary consists of two ovarioles and noted in 24-hour-old females the same sequence in the development of the follicles in the ovarioles as I myself noted in *Hippobosca capensis* (Detinova, 1955a).

Squire (1950, 1951) worked out some methods for determining the age composition of tsetse flies. In these flies paired scars are formed on the sternum as a result of copulation and different degrees of resulting striation

FIG. 23. STAGES OF DEVELOPMENT OF FOLLICLES AND THE APPEARANCE OF OVARIOLES OF *HIPPOBOSCA CAPENSIS*



- I₁ and I₂, First and second ovarioles of the first ovary
- II₁ and II₂, First and second ovarioles of the second ovary
- 3 Terminal pedicle of the ovariole
- 4 Dilatations left after ovulation (the size of enlargement depends on the time after oviposition)
- 5 Follicle during the period of intensive oogeny
- 6 Follicle ready for development after the passage of the mature egg from the first ovariole into the uterus
- 7 Follicles of the second ovariole of the first and second ovaries
- 8 Distal nurse cells
- 9 Proximal nurse cells
- 10 Oocyte
- 11 Growth-zone

can be distinguished; they vary from light spots in young females which have copulated once, to large dark spots in those which have copulated many times. Using this sign Squire divided the female tsetse into three age groups and considered that the percentage of unscarred, i.e., unfertilized, females is a good criterion of the rate of breeding. He also noted that in old females, larvae are more often found in the uterus. Maturing eggs and sperm in the spermatheca are encountered more rarely, and this indicates the beginning of a fall in sexual activity.

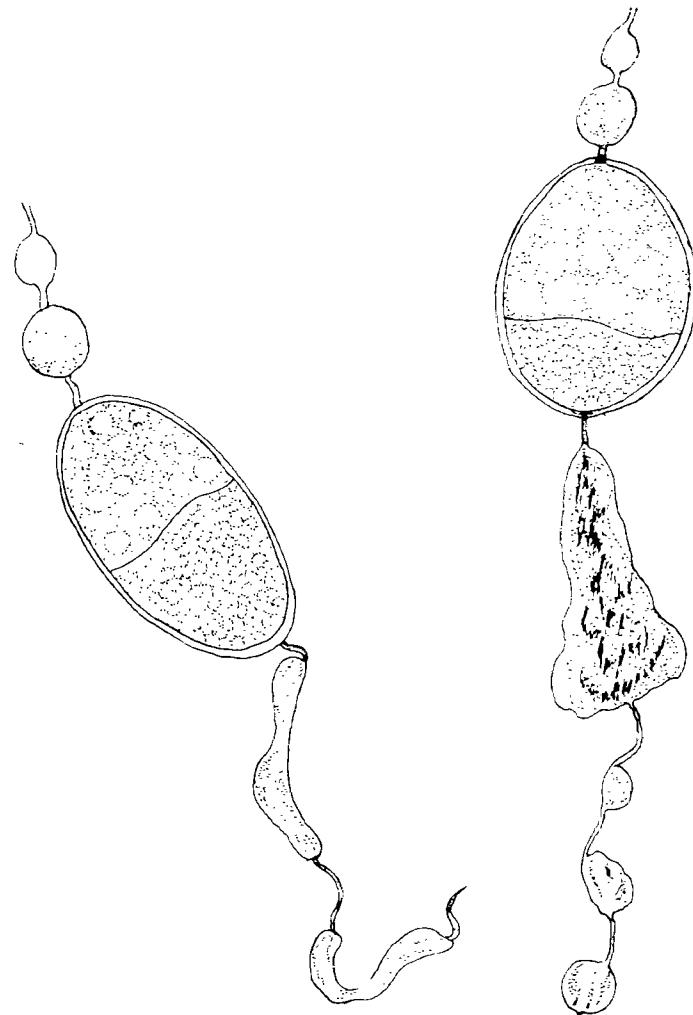
Squire suggested that the age of females can also be determined from the degree of wear on the wings and from their colour. Collected tsetse flies were classified by these signs into three age groups: the first had dark wings; the second, wings of a rusty-brown colour at the edges; the third, wings very much worn and of a rusty-brown colour throughout. The age of the tsetse fly was also determined by the degree of wear on the wings by Jackson (1946).

The method proposed by Perry (1912) for mosquitos having proved to be a disappointment, it is doubtful if it would provide reliable information on the actual age of female tsetse flies, since the degree of wear on their wings depends not only on the duration of life, but also on the conditions in which the flies have lived. It seems that it would be worth while trying on tsetse flies the method proposed by me for determining the age of biting lice. The method of dissecting *Hippobosca* and examining their ovarioles is as follows. The females are lightly anaesthetized with ether or chloroform and briefly immersed in alcohol; the insect is held by the head and thorax in the fingers of the left hand while entomological scissors held in the right hand cut the abdomen along the line of the pleura. The last segment of the abdomen is also removed. The dissected female is placed on a slide in a drop of physiological saline and the reproductive organs are dissected out by means of entomological needles. The outer sheath of the whole ovary and of the ovarioles is carefully removed, after which it becomes possible to study them in detail. They are examined under a binocular microscope (Zeiss ocular 7 and objective 8 are recommended).

Among non-blood-sucking Diptera age changes in the reproductive apparatus of the female *Musca domestica* have been carefully studied by Lineva (1950, 1953a,b). The reproductive apparatus of *Musca domestica* consists of two ovaries, each of which is connected with one of the two short paired oviducts. The joined paired oviducts form an unpaired oviduct which ends in a vagina. Just before the entrance of the oviduct into the vagina three spermathecae open into it. Side by side with the spermathecae there is a pair of tubular accessory glands, attached to the paired oviducts. The ovary has an inconstant number of ovarioles. Each ovariole consists of an apical or terminal filament, a terminal chamber—really a tube—where the development of follicles takes place, and a short duct or pedicle which separates the growing follicle from the paired oviduct. The walls of the ovariole are formed of two sheaths: an outer sheath and an inner membrane or intima. The outer sheath has a thick network of tracheoles, the terminals of which pass from one ovariole to another and closely bind them together. Both the outer sheath and the intima are greatly stretched as the follicle develops. Lineva stated that after the laying of the first batch of eggs the part of the intima which forms the pedicle and the part which covered the follicle greatly contract and are moved forward to the base of the ovariole. At the base of the ovariole in the parous female, a portion of intima may be found between the oviduct and the follicle, in the form of a dilatation inside which the previous follicle has developed. These dilatations correspond in character to those which are formed after the discharge of the mature egg in all mosquitos, black-flies, horse-flies, Heleidae and Hippoboscidae. The number of dilatations in the ovarioles enables us to determine with accuracy the number of

ovipositions completed by each female (Fig. 24). However, the dissection of the ovaries of house-flies to count the number of dilatations is technically more difficult than in the case of mosquitos and other Diptera.

FIG. 24. OVARIOLE IN *MUSCA DOMESTICA* *



* After Lineva (1953b).

The physiological age of female house-flies is determined mainly by the number, colour and position of the yellow bodies and by the condition of the tracheal system of the ovaries. Lineva noted that in a nulliparous female the nurse cells are separated from the mature egg by a chorion

and lie freely above it in the form of a small, yellowish crescent-shaped structure, closely pressed by the mature egg against the dome of the internal membrane.

Immediately after the discharge of the egg from the ovariole during oviposition, the yellow body still lies above in the dome of the internal membrane but it loses its crescent shape and its individual granules lie scattered inside the intima. Then the yellow body gradually moves downwards to the base of the ovariole. By the time the second follicle has completed its growth, a slightly yellowish-brown structure is clearly visible at the base of the ovariole; it consists mainly of remnants of nurse cells and is surrounded by the contracted portion of the intima in which the egg developed.

At this time the corpus luteum is a fairly compact structure consisting of individual granules and lumps difficult to distinguish from one another. Yellow bodies are also formed in all subsequent ovipositions. As yellow bodies accumulate, so the colouring of the whole structure becomes more intense. In females which have completed a different number of ovipositions the distribution and number of yellow bodies differ.

Lineva also studied the occurrence of changes in the tracheal system supplying the ovaries during the development of the first batch of eggs. From the changes which take place in the ovarian tracheoles it proved possible to subdivide all flies into two main groups: the physiologically young, nulliparous females and the parous females (Fig. 25 and 26).

Between 16 and 19 large tracheal trunks approach each ovary from a ventrolateral direction. The branching of the tracheae in flies does not proceed evenly and they divide into unequal branches. In most cases a main trunk remains from which small branches gradually divide off. In the ovarioles the tracheal trunks divide into finer tracheae which pass along the ovarioles to the terminal filaments. Some of them penetrate the wall of the outer membrane of the ovariole and divide inside it into the tracheoles, while others remain on the surface of the ovariole. The transition from tracheae to tracheoles may proceede gradually but sometimes a small trachea subdivides straight away into a bunch of four to six or more tracheoles. In newly emerged females this bunch of tracheoles forms a compact skein. The finest tracheolar endings do not form a part of the skein but divide off centrifugally from it.

During the growth of the follicles considerable changes take place in the tracheal system supplying the ovaries. As the follicles grow the skeins begin to unwind. Loops and zigzags both in the fine tracheae and in the thick tracheal trunks are stretched out along the growing follicle. A gradual transition takes place from a compact skein to a loose bunch of tracheoles. The sharpest changes take place during the intensive growth of the follicle. In stages II-B and II, after the discharge of the mature eggs, the tracheae and the tracheoles which were stretched along the

ovariole form zigzags and loops but the skeins are never formed again. Thus, the presence of skeins is a sign of the newly emerged female and their absence that of a parous female. The observation which we made in mosquitos has therefore proved applicable to non-blood-sucking flies.

On the basis of all the age changes occurring in the reproductive system of females, Lineva proposed to subdivide all flies into three age groups.

I. Nulliparous females.

These include:

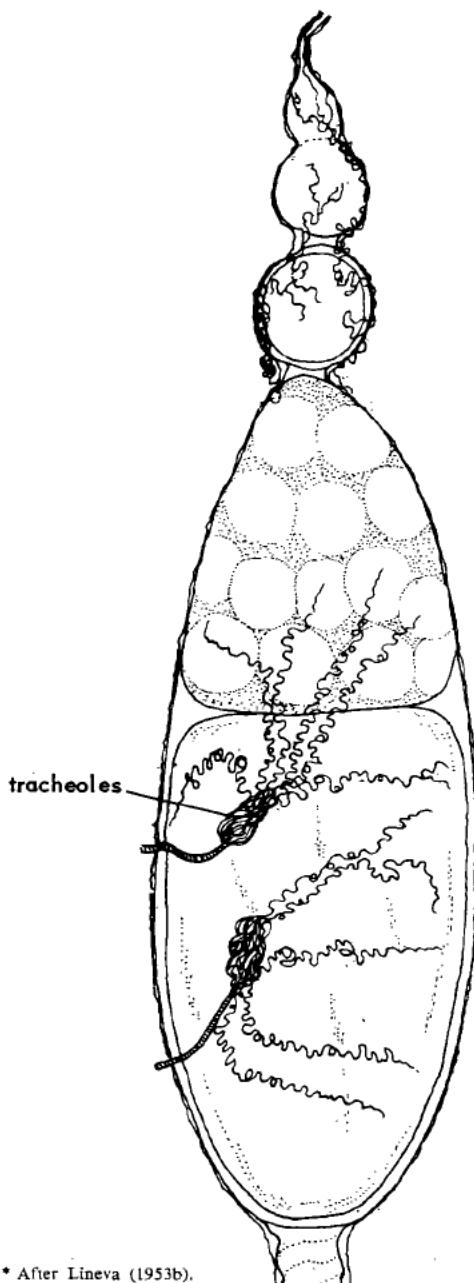
(1) all those females with ovaries at stages N₀, N, I and II-A;

(2) females in whose body cavity some remnants of histolysis still remain; and

(3) nulliparous females at stages of ovarian development later than II-A. These may be distinguished by the presence of tracheal skeins and the absence of yellow bodies.

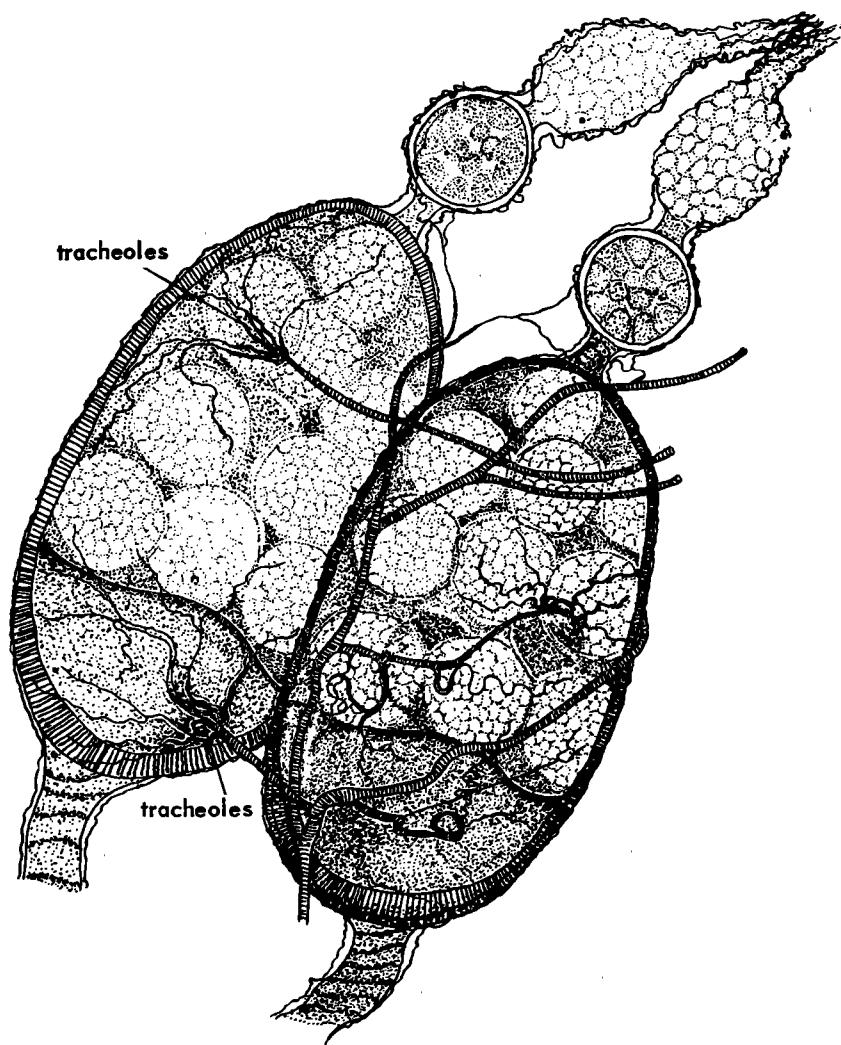
II. Pauciparous females. This group is made up of females with no tracheal skeins. Yellow bodies are found only at the base of the ovarioles. Among these females it is possible to distinguish those which have oviposited once; when the female has just laid its eggs, the yellow body is still in the upper part of the tube and there are no yellow bodies at the base of the ovariole.

FIG. 25. CHANGES IN THE APPEARANCE OF TRACHEOLES IN THE OVARY OF *MUSCA DOMESTICA*:
I. OVARIOLE OF A NULLIPAROUS FEMALE*



* After Lineva (1953b).

FIG. 26. CHANGES IN THE APPEARANCE OF TRACHEOLES
IN THE OVARY OF *MUSCA DOMESTICA*: II. OVARIOLES OF A PAROUS FEMALE*



* After Lineva (1953b).

III. *Old females.* To this group belong individuals which, as in the previous case, have no skeins, but their yellow bodies have a dense coloration and are to be found not only at the base of the ovariole but in the ovariole itself.

Females are dissected by the same method as that used for *Stomoxys*. The yellow bodies, the condition of the tracheal system of the ovaries

and the dilatations in the ovarioles are examined under the binocular microscope.

On the basis of all the changes in the reproductive system of *Musca domestica*, a method has been worked out for determining the age composition of females. This method can be used for the analysis of changes in the fly population and for the evaluation of the effectiveness of various control measures.

In view of the scientific and practical importance of the results obtained through the study of age changes in the reproductive systems of insect vectors of disease, work has now begun on a number of other insects—e.g., *Cimex lectularius* (Das Gupta & Ray, 1956), Aphaniptera (Kunitskaya, 1960) and *Blatella germanica* (Plyater-Plokhotskaya, in press).

Chapter 7

THE BIOLOGY OF *ANOPHELES MACULIPENNIS* POPULATIONS

Many publications have been devoted to the biology and ecology of malaria vectors, widely distributed throughout the world. Most of these publications deal with the main vectors, such as *A. maculipennis* (and all its subspecies), *A. quadrimaculatus*, *A. gambiae*, *A. funestus* and *A. minimus*. A number of reviews, such as those by Beklemishev (1944), Muirhead-Thomson (1951) and Martini (1952), summarize the state of our knowledge in this field. Subjects studied in detail include the geographical distribution of the main malaria vectors, the conditions under which they live in the pre-imaginal stages, the effect of various factors on the speed of their development, the types of anopheline breeding-place and some basic problems of the physiology of their cycle of development.

The main problems of the biology and ecology of adult mosquitos have been studied, the seasonal changes in the density of the various species in different parts of their habitat have been determined, research has been done on their behaviour during a 24-hour period and on the conditions which determine that activity, their food preferences and the relationship between blood-meals and reproduction. The types of resting-place, their microclimates and their relative mosquito densities have been studied together with the conditions determining the number of generations in a season; the factors which determine the diapause, its duration in various parts of the habitat, and many other subjects.

Moreover, for several decades now mosquito control measures have been worked out on the basis of the available knowledge of the biology and ecology of the vectors. Great attention has been paid in the USSR to the study of questions connected with the phenology of the main malaria vector, *A. maculipennis*. Data obtained in this connexion, apart from their scientific interest, are of great practical importance, since it has become possible to use them as a basis for determining the best times for carrying out eradication measures over the whole area of distribution of the species concerned (see the collection of papers edited by Beklemishev & Shipitsina (1957)).

Despite this extensive range of research many vital problems of the ecology of malaria vectors have remained obscure because of the lack of data on the longevity of females and on the age composition of the female populations. It has previously been possible to estimate the individual life-span of females, their number of ovipositions and hence the number of blood-meals they take, the duration of gonotrophic cycles and the survival rate in consecutive cycles, etc., only on the basis of laboratory observations or from indirect data. However, laboratory conditions do not reflect all the various factors which affect mosquitos in nature, so that only a very approximate picture of the life of mosquito populations can be obtained in this way. Moreover, the laboratory data for a particular species in a part of its habitat cannot be directly applied to populations living under different geographical and climatic conditions.

Lack of information on the calendar—and physiological—longevity of females under natural conditions made it difficult to assess the epidemiological importance of a vector population. It was also impossible to estimate the composition of the female population at various periods during the season. On the other hand, knowledge of these factors was necessary for correct timing of vector control measures and for chemotherapeutic antimalaria programmes. When reliable methods of determining the physiological age of females had been worked out it became possible to throw some light on many hitherto obscure aspects of the life of the vectors, to make a correct estimate of the epidemiological importance of a population at various periods during the transmission season, and to estimate the success of various mosquito control measures.

Moreover, the information obtained on the age composition of the females gave a different view of a number of problems of the biology and ecology of the vector which had earlier seemed quite clear. In particular, it led to a review of the question of the distribution of mosquitos in different resting-places. The data now available reveal the distribution of mosquitos in resting-places; according to age; apart from its theoretical interest, this knowledge is also of great practical significance.

By the age composition of the population we mean the ratio between the numbers of females of various physiological ages which make up that population. The age composition of populations of malaria mosquitos (the same applies to the majority of other blood-sucking Diptera) is never constant and depends, as stated by Beklemishev (1944), on the intensity of breeding, on the speed of aging of the females and on the mortality rate among females at various physiological ages.

The intensity of mosquito breeding fluctuates in accordance with the weather, with the trend of emergence of various generations and the conditions of the breeding-places. The more favourable the temperature, the faster is the pre-imaginal development and the greater the number of generations which can emerge in one season; the more favourable

and more extensive the breeding-places, the greater is the number of mosquitos which breed in them.

The speed of aging of females, that is, their passing from one physiological age to another, depends on the duration of respective gonotrophic cycles. As pointed out by Beklemishev (1940), each gonotrophic cycle consists of three main phases: (1) the search for a host and the taking of a blood-meal; (2) the blood digestion and the development of the ovaries; and (3) the search for a suitable body of water and the oviposition.

The duration of the gonotrophic cycle is constant only in tropical conditions or hot climates where temperatures and other meteorological factors vary within comparatively narrow limits. Thus under tropical conditions, according to data given by Muirhead-Thomson (1951) for *A. gambiae*, *A. minimus* and *A. melas*, and by Davidson (1954) and Mouchet & Gariou (1957) for *A. gambiae*, a gonotrophic cycle takes 48 hours. The same length of cycle in the summer was noted in the case of *A. superpictus* by Beklemishev & Detinova (1940) in Turkmenia and by Buyanova (1959) in Tadzhikistan. In all other climatic conditions its duration is not constant and depends on the environmental temperature, which primarily determines the speed of blood digestion and the ovarian development; the nearer the temperatures are to the optimum, the quicker the process is completed.

The duration of the two other phases of the gonotrophic cycle is more or less constant and we believe that it depends much less on the temperature. Only very sharp weather changes can hold back in the resting-places mosquitos with mature eggs, or discourage empty females from flying in search of a host. Observations carried out in temperate latitudes (in the central areas and the south of the USSR) have shown that on the average these two phases together last about 24 hours. The methods of establishing the duration of all three phases of the gonotrophic cycle will be described later.

From all the available data on the age composition of females and the speed of aging of the population, we have found that in places where the temperature is higher, the gonotrophic cycles proceed faster and so, from a calendar point of view, does the aging of the whole population. However, under hot climatic conditions the mortality of mosquitos, even of the relatively thermophilic species, is sometimes so high in each gonotrophic cycle that a smaller proportion of the females succeeds in surviving to physiological old age than in more temperate climatic conditions.

Mortality among females of *A. m. messeae* depends on the sum of all the external factors acting upon them and upon internal changes in the organism which accumulate as an individual ages (see Chapter 5).

The death rate among adult mosquitos under natural conditions has not yet been finally determined. Even at the present time, although it

is fully possible to determine the physiological age of females and to establish, on the basis of large-scale dissection, the numerical relationship between the various age groups in the population, which in the long run should indicate the mortality rate in each gonotrophic cycle, we can estimate that rate only approximately. The difficulty of determining the mortality from material obtained by dissecting mosquitos from settlements, as will be shown below, lies in the fact that the size and composition of that portion of the population which remains in resting-places outside the settlements remains unknown. The mortality rate could only be established if two conditions were fulfilled: (1) if consecutive gonotrophic cycles were of the same duration; and (2) if females in consecutive gonotrophic cycles were represented in the traps proportionately to their numbers. The first condition, as already stated, can be fulfilled only in the tropics or in the south at the height of summer, but the second condition can be fulfilled only if the mosquitos are extremely endophilic, when it is possible to judge the actual composition of the population from the composition of the females caught in the day resting-places or, on the other hand, when the population is exophilic and the bulk of the mosquitos are caught in their resting-places in nature.

In addition it is quite probable that the figure required could be more reliably estimated if a uniform group, e.g., empty females attacking their prey, underwent examination.

Research in the last few years, which will be described in more detail below, has shown the difference in the behaviour of female *A. m. messeae* at various ages in regard to the choice of day resting-places in the course of gonotrophic cycles. This circumstances has made it extremely difficult to determine mortality among female mosquitos.

As a result of observations carried out the whole year round on the age composition and numbers in an *A. m. messeae* population and of numerous seasonal observations of populations living in various parts of the distribution area, we have obtained a complete idea of the changes which occur in that composition.

Changes in the numbers of adult *A. m. messeae* in the most typical instance take place as follows. In early spring as soon as temperatures above zero become established, overwintered females which have already become gonoactive appear in the day resting-places. During the first few days they are few in numbers in the day resting-places, but as the places of hibernation become warmer, more females leave them and at the same time an increase is noted in the numbers of overwintered females in the day resting-places. This phenomenon is also due to the fact that microclimatic conditions in the day resting-places are not yet sufficiently favourable for swift digestion of blood. Because of this, females which have flown in and have had a blood-meal do not complete their first gonotrophic cycle for a comparatively long time and do not leave the day resting-

places for oviposition. At the same time a gradual influx continues of new batches of mosquitos from hibernating places which have been less warm during the spring. In subsequent days, as a result of the appearance of favourable conditions, oviposition begins and the mortality among the parous females increases. As a result, despite the influx of mosquitos from hibernation, the general increase in mosquito numbers in the day resting-places ceases. In the subsequent days the total numbers of this part of the population gradually fall and finally it is noted that they have completely died off. As a rule, by the time of flight of individuals of the first generation, a comparatively small number of overwintered females remain in their natural habitat. However, there are data (Beklemishev, Bryukhanova & Shipitsina, 1931) which indicate that in some localities in certain years the overwintered females begin to die off before the first generation has flown. Beklemishev (1944) later showed that the figures in question should be regarded with a certain amount of caution, since it is always very difficult to establish the complete absence of a species. From the moment of appearance of newly emerged spring-summer females a considerable increase in population numbers is noted. This increase may continue for a long period. The flight of gonoactive females belonging to various generations continues for a period whose length depends on the local climate. Thus in the south of the USSR, first flights of mosquitos may occur for five to seven months, in the north for one and a half to two months.

The summer gonoactive mosquitos take blood-meals, lay eggs and then gradually grow old and die off. However, the mortality among mosquitos in the summer is lower than the birth rate, and this means that the number of mosquitos increases so long as the breeding of gonoactive females continues on a considerable scale. It is true that in some conditions of distribution of a species considerable fluctuations in mosquito numbers are often noted. This is usually due to unfavourable meteorological factors acting over a certain period. If favourable conditions are restored numbers again increase. From the time when emergence of large numbers of gonoactive females and subsequently of single individuals ceases in the autumn, at first there is a slow and then a very swift reduction in the size of the gonoactive portion of the population. At this time the proportion of females dying in each gonotrophic cycle probably increases as a result of the onset of unfavourable meteorological conditions. Side by side with the reduction in the size of the gonoactive population there occurs at first a slow and then a sharp increase in the proportion of diapausing females. Large numbers of females depart for their winter quarters. Under favourable conditions population numbers reach their annual maximum by this time. However, owing to the absence of any addition to the population through new individuals during the lengthy late-autumn and winter period, and to deaths among these wintering mosquitos, the numbers of

insects returning from hibernating places are considerably less than those that went into them. The death rate among mosquitos during hibernation depends on the degree to which favourable conditions exist for them in the shelters concerned. It has been shown (Khelevin, 1941; Beklemishev, 1944; Pokrovsky, 1946; Pozdeev, 1957, and many others) that, in the overwhelming majority of cases, between 70% and 90% of the females die during the winter. Wintering conditions are different for the various subspecies of *A. maculipennis*. The conditions favourable for wintering mosquitos are an even and relatively low temperature, moderate humidity, the absence of draughts, and semi-obscurity. The favourite wintering places for *A. m. messeae* are those in which the temperature varies between + 2° and - 3° C (Beklemishev, 1944). At higher temperatures in the hibernating places the fat-body—which is the source of nutrition for mosquitos during their long hibernation—is quickly used up. Premature exhaustion of this fat causes the death of the individual. At lower temperatures mosquitos die of freezing. The optimum humidity for hibernation varies between 60% and 85%. At other levels of humidity there is an increased death rate among the mosquitos. The hibernating places chosen by *A. m. messeae* are very varied, but as a rule they contain no prey. The main places chosen are cellars, vegetable stores, unheated premises and shelters in nature. Hibernating places in nature were found by Sergiev (1934), Minayev & Kravchenko (1936), Khelevin (1938), Markovich & Remennikova (1939), Luppova (1940), Timrot (1941), Beklemishev (1944), Pozdeev (1957), Netski (1957), Almazova, Prokopenko, Shesterikova & Levitanskaya (1957) and many others. The main natural shelters are depressions round the roots of trees, animal burrows, piles of straw, heaps of brushwood, stacks of peat, reed-grown areas, beneath the bark of trees and many other similar places. There are almost no data available on the survival rate of mosquitos in natural winter shelters. It can be assumed that the survival rate of females in such shelters depends on the depth of snow cover. It is well known that favourable conditions for wintering plants and animals and particularly for insects are found under snow cover, where the temperature is considerably higher than that of the outer air (Zolotarev, 1936, Formozov; 1946; Naumov, 1955).

First flights of males, as of females, occur throughout the whole season. In the autumn the males fly off at the same time as the diapausing females but after copulation they all die. Thus only females leave for the hibernating places and for that reason in the spring the whole population consists of females.

The appearance of males in spring is due to the emergence of individuals of the first generation. In the Moscow Oblast (Detinova, 1946) it has been established that even summer gonoactive females fly in small numbers in the autumn to overwintering places, but they all die during the autumn and winter.

The changes in the age composition of populations of *A. maculipennis* can be outlined as follows. In the first few days after the spring flight of females from their hibernating places, only physiologically young, nulliparous females are found among those caught, but their calendar age may be as much as five to nine months, depending on the duration of the winter. All the overwintered females which fly into the daytime resting-places are gonoactive and the diapause is completed long before they leave their winter quarters. The ovaries of these females are as a rule in stage II and therefore when they take a blood-meal in the spring (when meteorological conditions are favourable) the ovaries begin to develop. From the commencement of oviposition, among the overwintered females individuals gradually begin to appear which have completed one, two, three, etc., ovipositions. In view of the lengthy period during which mosquitos fly from their places of hibernation, in addition to females which have completed a varying number of ovipositions, nulliparous and increasingly older females still continue to be encountered for a long time during the spring. The protracted period of flight from hibernation also means that shortly after its commencement the population of this group of females is of various ages. When the post-hibernation flight is finished, but before the first generation has begun to emerge, a gradual aging among the overwintered mosquitos is noted. In view of the mortality of females at each of the successive physiological ages and because of the cessation of further flight from hibernating places the total number of overwintered mosquitos decreases and the whole of this portion of the population dies off altogether. It has been shown that in the various geographical and climatic conditions encountered in the USSR a small proportion of the overwintered females can complete as many as 12 gonotrophic cycles (Detinova, 1953d; Kozhevnikova, 1953; Korzhenevskaya, 1957). From the commencement of the emergence of the first generation a rapid decrease begins in the mean age of the population. In the course of dissections, nulliparous females are first discovered and their proportion quickly increases. Later, throughout the whole season, the population is constantly supplemented with nulliparous females but their proportion varies considerably. In the days of mass emergence of each successive generation there is a considerable increase in the proportion of nulliparous females. In the intervals between these mass emergences there is simply some increase in the number of young females. Females of the first generation begin to oviposit but after a few days, since emergence is not simultaneous everywhere, females that have completed one and then two or three ovipositions become increasingly common (in addition to nulliparous females) and in subsequent days still older females are discovered. As the emergence of new individuals of all the succeeding generations and the gradual aging of the emerged mosquitos take place throughout the whole season, the population always consists of mosquitos of various ages.

In the autumn, when the flight of the summer gonoactive females has ceased, no more gonoactive nulliparous females are encountered and then pauciparous females also cease to be found. Nevertheless, the gonoactive females which emerge earlier continue to take blood-meals and to oviposit and in the end form a group of physiologically old females. At the same time, as the summer females are older and the summer gonoactive females cease to breed, the diapausing autumn females begin to appear and their proportion increases every day.

Thus in autumn the following groups of females can be found in the population: (1) diapausing, non-gonoactive females; (2) summer females, physiologically older; and (3) females which emerged in the last days of summer and have managed to complete only a small number of ovipositions.

During this time the proportion of the first group constantly increases and that of the second and third groups decreases. In the subsequent days of autumn all the gonoactive females die off and the whole population consists only of diapausing females which leave for their hibernating places.

Study of the age composition of the population by determining the exact physiological age of the females began in the USSR in 1947. In the seasons of 1952 and 1954 these observations were carried out on a very large scale under various geographical and climatic conditions. In a number of places the work was repeated in two, or even three, seasons; this allowed for studying in greater detail the changes in the composition of the population due to various meteorological factors. All the other conditions which could also have a relevant effect, such as the distance of the breeding-places, the total area of water, the layout of the settlement, etc., remained without change.

The method of establishing the age composition of the population of *A. maculipennis* was always the same and may be summarized as follows. A centre of population with a sufficient number of *Anopheles* was chosen for observation; it was well away from settlements which had been treated with residual insecticides, and its cattle or crops were untreated. Standard capture stations characteristic for the prevalent anopheline species were selected and used during the whole season. To obtain reliable data reflecting the age composition and total numbers of mosquitos in the settlement, these capture stations were widely distributed. Collecting mosquitos from a single capture station may give a completely false picture of the general composition of the population, since the effect of chance is not excluded.

As it was impossible daily to collect and dissect mosquitos from all capture stations, a certain sequence was observed throughout the whole season. In a large number of capture stations mosquitos were collected every two or three days; in this way, by capturing mosquitos from one part of the settlement every day, one obtains data on the numbers and age

composition of the mosquitos characteristic for that part, while figures for the settlement as a whole are obtained very two or three days.

To establish the age composition of the female population from all capture stations only a proportion needs to be dissected according to the time available for examination. This was chosen at random and we assumed that the age composition of the dissected sample correctly reflected the population composition in the respective capture station and that the daily dissection of females from various capture stations corresponded to the population composition of the mosquitos flying into the settlement.

When a female was dissected a note was always made of its physiological age, its stage according to Sella, the stage of development of the ovaries according to the Mer-Christophers classification, the date of dissection and the number of the relevant capture station. In special research, an additional note may be taken of the condition of the Malpighian tubes, the number of developing follicles, the condition of the tracheal system of the stomach of empty females, the condition of the fatty tissue and the presence or quantity of any ectoparasites and endoparasites. Subsequently all these observations were correlated with the physiological age of the female.

The date of dissection and the number of the capture station made it possible, after all the material had been processed, to study seasonal changes in age composition and the distribution of females of various ages in various types of resting-place and various parts of the settlement.

The proportion of females belonging to each age group was established from the results of daily dissections. Since the total number of mosquitos in each capture station was calculated at the same time, the numbers in the various age groups in the dissected sample gave the approximate numbers of each age group in the capture station concerned and also in the whole settlement. More will be said later of the methods of calculating these data.

At the beginning of our work it was supposed that from the results of dissection of female *Anopheles* collected in capture stations we should be able to characterize with complete accuracy the age composition of the whole population in the relevant locality. In fact, on dissection of females from each separate capture station, we do obtain a correct idea of the age composition of the sample of females flying into it on the day of dissection. Nevertheless, an analysis of the age composition of females found in the majority of capture stations and totalled for the whole season and for the whole settlement showed that they do not reflect accurately the real composition of the total mosquito population.

In view of the fact that gonoactive females are produced continually throughout the whole season (although at an uneven rate) and taking into account the mortality occurring during each gonotrophic cycle, one could suppose that the most numerous age group in the population would be that of the nulliparous females, i.e., those in their first gonotrophic cycle,

and that their number would decline in each consecutive older group. Naturally, in view of the uneven breeding activity, at any particular moment during the season this orderly sequence may be disturbed. For example, the proportion of nulliparous females may increase considerably on the days of mass emergence of a new generation. On the other hand, on days when breeding has completely or almost completely ceased, the numbers of nulliparous females decrease sharply, and the proportion of females in higher age groups rises considerably. We expected that the age composition of the whole population for the season would give a correct picture of the size of each age group and that on the basis of these data it would be possible to estimate the actual mortality rate of mosquitos in successive physiological age groups. The data obtained on the age composition of females, relating to a considerable portion of the local population of *A. maculipennis*, did not, however, confirm our expectations.

First of all, we discovered that in almost all house-sheltering populations studied in various parts their habitat in the USSR, the proportion of nulliparous females, in their first gonotrophic cycle, proved to be smaller than the proportion of females in their second gonotrophic cycle. This observation was termed "deficit of nulliparous females" in the capture stations. In several populations, a deficit of females having completed one gonotrophic cycle was also noted and in some even a deficit of females having completed two such cycles. In these cases the deficit of nulliparous females was large, of "uniparous" females smaller and of "biparous" females still less.

In the USSR this deficit of nulliparous females was observed in *A. superpictus* as well as in *A. maculipennis* (Ivanova, 1956; Buyanova, 1959). Observations carried out recently by Zalutskaya (1959) in Viet-Nam showed that among *A. minimus* and *A. vagus* there was a marked deficit of nulliparous females in all settlements. Thus all the available data suggest that the deficit of nulliparous females is a widespread occurrence and characteristic not only of *A. maculipennis* but probably of other *Anopheles*.

All these data suggested that the distribution of females between the indoor and outdoor shelters varies according to age.

It is a well-known fact that newly emerged females, which have not yet mated and felt the need of a blood-meal, show different preferences and behaviour patterns from those females that have commenced their gono-active life. Newly emerged females are not attracted to a host and remain in considerable numbers amid the vegetation near the water. It also appears that young females, and particularly those that are passing through their first gonotrophic cycle, seek the natural shelters more frequently than do physiologically older females; it can be considered that females in their first gonotrophic cycle are more exophilic than the physiologically older females, and represent a transition between the old and newly emerged which have not yet sought a host.

Before research had begun on the age composition of the population of *A. maculipennis* by Polovodova's method, there were no data on age differences in the behaviour of adult gonoactive females generally, and on differences in their choice of resting-places in particular. It was accepted that the behaviour of females does not depend on their physiological age. Data on the relationship between parous and nulliparous females obtained by the measurement of the ampullae or examination of the tracheal system of the ovaries did not determine the relative size of various age groups and there was therefore no basis for even suspecting the existence of age differences in the behaviour of the females.

The relative exophily of young females during the development of the first batch of eggs is probably due to the somewhat different biological and physiological demands made on the environment. In females still physiologically young, but which have completed one or two ovipositions, the physiological demands on the environment remain closer to those of nulliparous females than to those of the physiologically older females. This question still requires further study.

It follows from the data obtained that the population of gonoactive females of *A. maculipennis* can be divided into two main groups. The first includes mainly nulliparous females in their first gonotrophic cycle and some females which have oviposited once or twice; these individuals clearly gravitate towards natural shelters. The second group consists mainly of parous females, and these for the most part gravitate towards stables and houses. The latter group is of great epidemiological significance. It seems to us that the first group of females could be called more exophilic and the second class more endophilic. The size of each of these two groups in any given situation depends on the local natural conditions and on conditions inside the settlement. Thus the ratio between the numbers of mosquitos remaining in their natural surroundings and those coming into the settlement is determined by the degree of the respective suitability of natural shelters, by the natural conditions in the settlement and by the type of behaviour dependent on age of female *Anopheles*.

In a number of instances the environmental conditions are not very favourable to mosquitos. If it is too cold outside, resting-places in the settlement have undoubtedly advantages and the whole population concentrates there. If it is too hot, dry and windy outside, the character of natural shelters is equally important. When these shelters do not give enough protection, the females tend to fly into dwellings in the settlement, where favourable and more constant microclimatic conditions exist. In such cases the age composition established from dissections of females collected in daytime capture stations gives an idea of the real relationship between the various age groups of the given mosquito population. If, on the other hand, in the settlement itself or in natural shelters in its immediate vicinity, microclimatic conditions occur which are favourable

to mosquitos, then a considerable proportion of the mosquito population may remain in them. The absence of reliable methods of capturing *Anopheles* mosquitos in the field makes it impossible to calculate the total numbers remaining in nature or their age composition. In these circumstances, when part of the population shelters out of doors, the dissection of mosquitos gathered in capture stations could only give a correct idea of the age composition of the whole population if female mosquitos were distributed in different types of resting-place regardless of their physiological age. In other words, the distribution of mosquitos between indoor and outdoor shelters and the age composition of the population in each type of resting-place can be identical only if there are no age differences in the choice of the resting-place. However, the existence of a deficit indicates an uneven distribution of females of various physiological ages between the indoor and outdoor shelters, and in such cases it is impossible to gauge correctly the composition of the population attached to a particular inhabited locality.

Special observations on the age composition of females captured in natural shelters (Detinova—observations during the seasons of 1949-53; Shmelova—observations during the season of 1949; Belskaya, 1952) showed among all females dissected a preponderance of physiologically young, nulliparous females. In this group newly emerged, unfed females predominated. However, even among females which had taken a blood-meal and already had mature eggs, a clear preponderance of nulliparous females was also noted. Although the total number dissected from natural outdoor shelters was not great, nevertheless the data obtained lead to the conclusion that nulliparous females stay in natural shelters and this may be the explanation for the deficit of that group in settlements.

On the basis of data collected for the Moscow Oblast (Detinova, 1953d) it was established that the deficit of nullipars among freshly fed females was somewhat more marked than among the whole mass of females examined. It can therefore be suggested that some of the physiologically young females, although they fly into the settlement for a blood-meal, do not remain afterwards in the usual day resting-places but fly off after the meal into natural shelters nearby, joining the influx to the day resting-places at one of the intermediate stages of blood digestion.

Age differences in the behaviour of females were also shown by the fact that the composition of females was not identical in day resting-places at varying distances from the breeding-places (Detinova, 1952a, 1960; Shevchenko, 1955). According to data obtained by Kiseleva (1955), in day resting-places containing no hosts the proportion of nulliparous females was higher than that of physiologically older females, whereas there was a reversed relationship in day resting-places where hosts were present.

There are many published observations on the finding of malaria mosquitos in all sorts of resting-places in nature: on vegetation, in hollow

trees, burrows or caves, under bridges far from inhabited places, etc. The majority of authors (Grassi, 1901; Martini, 1933; Beklemishev, Vinogradskaya & Mitrofanova, 1934; Shlenova, 1941; Pravikov, 1934; Mitrofanova, 1946; Sautet, 1937; Detinova, 1952a; Gillies, 1956a; Buyanova, 1959; Kalmykov & Lysenko, 1959; Kazantsev, 1959; Kekhcher & Markovich, 1960; Markovich (& Bandin), 1960; Prokopenko, Shmeleva & Timrot, 1960, and many others) note that favourable microclimatic conditions may exist in natural shelters and that this may explain why mosquitos concentrate in them. No suggestions that there is a link between the finding of female malaria mosquitos in such shelters and age differences in their choice of shelter were made, however.

It has been customary to think that the various species of *Anopheles* are either endophilic or exophilic. The greater the proportion of mosquitos remaining during the completion of gonotrophic cycles in houses and stables, the more endophilic is the population; the greater the number remaining in natural shelters, the more exophilic is the population.

In this use the terms "endophilic" and "exophilic" are purely descriptive; also the reasons for endophily and exophily may vary; on the one hand, different species react in different ways and, on the other, the environment in which they live may differ. In the case of *A. hyrcanus*, a comparatively small percentage of females fly for a blood-meal into premises or into settlements in general, but once having done so and taken a blood-meal, they usually remain there only until dawn. When engorged they retain their positive phototaxis and usually leave the premises at dawn. Blood-fed females of *A. hyrcanus* never fly into dwellings. Females of *A. maculipennis* or *A. superpictus* are attracted by large concentrations of hosts and when there are human settlements nearby a considerable percentage fly into them. Having taken a blood-meal they lose the positive phototaxis to twilight so characteristic of empty females and the majority of them remain in the premises. Moreover, some of the insects which have taken a blood-meal outside fly at dawn into premises. However, if the indoor microclimate is unfavourable the majority leave on the following night and may remain in their natural outdoor shelters.* Some stay there from the very beginning. Thus, even in the case of the so-called endophilic species, such as *A. maculipennis*, the daytime population is distributed between natural shelters and indoor resting-places; the distribution depends on the microclimate of the resting-places and on the distribution of the host.

Gillies (1956a) mentions three groups of exophily:

(1) *Obligate exophily*, where there are no buildings. As an example he quotes the *A. culicifacies* population in uninhabited jungle in Ceylon.

(2) *Facultative exophily*, where there are favoured hosts outside buildings but many human habitations and premises for animals in the neighbourhood. An example of this is the population of *A. gambiae* in the southern part of

Tanganyika, where the cattle are kept in open compounds on the edge of the villages. After taking a blood-meal the mosquitos fly off either into their natural resting-places or into human dwellings. Ecological factors to a large extent determine the choice of resting-place.

(3) *Pre-determined exophily.* Gillies divides this form of exophily into two types. Type A—Senior-White's endophagy—is that in which feeding takes place inside premises occupied by human beings or animals but where the mosquitos fly off to their resting-place in nature before daybreak. *A. maculatus* in Malaya and *A. aquasalis* in western India are examples. In type B—Senior-White's exophagy—feeding takes place in the open air and the gorged females avoid buildings as resting-places. Examples are *A. caustani* and *A. pharoensis*. The author states that the degree of exophily must be assessed only on the basis of females with blood in their stomachs. The finding of empty females cannot be of importance in any instance, since they may either be newly emerged or have just oviposited.

The data now obtained on the distribution of females of various ages between different types of resting-place show that there are differences in the factors which normally condition the behaviour of the females in their choice of resting-place, not only between individuals of different species but also between individuals of the same species, but of different physiological ages. These differences require an accurate analysis by the methods used in studying insect behaviour; the whole question of the choice of day resting-places by mosquitos is also in need of a detailed investigation.

On the basis of data on the age composition of the population, we considered it possible to determine the ratio between age groups in the mosquito population and hence between the mortality rates among females at various ages. We considered that the work carried out in various parts of the habitat of a species in the USSR makes it possible to determine also the difference in the mortality rate among populations living in completely different climatic conditions. However, the occurrence of the deficit mentioned previously hinders the determination of the mortality rate, since firstly the size of the initial age group, i.e., the nulliparous group, is unknown, and secondly in the majority of cases the proportion of females which have completed one oviposition is also unknown. Data on the relative sizes of age groups among females which have oviposited three or more times indicate a gradual decrease in the numbers of each consecutive age group, and therefore make it possible to estimate their mortality rates. On the other hand, the oldest age groups are small in numbers and representatives of those groups are few in the dissected sample, so that differences in their numbers are statistically unreliable. Thus in general it is possible to estimate the mortality rate only for a few intermediate age groups, viz., from the third to the sixth.

The rough calculations based on the data on the age composition of females and carried out by Markovich, Prokopenko & Ivanova (1949), Detinova (1953d), Kozhevnikova (1953), and Almazova, Prokopenko, Shesterikova & Levitanskaya (1957) for *A. m. messeae* and by Ivanova (1956) and Almazova (1959) for *A. superpictus* do not give sufficiently reliable results, but they do show that among physiologically young females mortality is lower (roughly 50% in each gonotrophic cycle) than among physiologically old females (60%-75% and sometimes even higher). According to Shute's data (1936) (see Beklemishev, 1944) mortality among female *A. m. atroparvus* under laboratory conditions was identical in the first four age groups at 20%, and thereafter gradually increased with age, but by the thirteenth to the sixteenth gonotrophic cycle it had only reached 27%.

From these data it can be concluded that in natural conditions the chances of survival in each cycle are considerably less than in laboratory conditions, where the most favourable circumstances for the life of female *Anopheles* have been created.

Analysis of Data on the Age Composition of Female *Anopheles maculipennis* in the Moscow Oblast during the 1952 Season

Observations on the age composition of females were carried out in the Moscow area in 1952 during almost the whole season of the activity of *A. maculipennis*. Systematic dissections enabled one to study the changes in the age composition of the population from the time of flight from hibernating places until the complete disappearance of gonoactive summer females from the day shelters, and the flight of diapausing females to their winter quarters. The results of these dissections are shown in Table 1.

The beginning of flight from places of hibernation was noted in 1952 during the first days of April. At the end of April, when investigations began, the majority of overwintered females still consisted of nulliparous females and comparatively few had completed one or two ovipositions by that time. This combination of age groups indicates the protracted period of flight from places of hibernation, a fact which is known also from direct observation. During the first ten days of May the proportion of nulliparous females considerably decreased, and from 12 May onwards the finding of nulliparous females among those dissected ceased altogether, a fact which showed that flight from hibernating places had been completed. In the second ten-day period of May a considerable aging was noted among the females which had flown earlier and gradually there appeared insects of subsequent ages. By the end of the month some of the dissected females were found to be in the fourth to seventh gonotrophic cycle. The aging of the overwintered females continued in June. The last female of this group was found on 26 June in its twelfth gonotrophic cycle. In view of

TABLE 1. AGE COMPOSITION OF THE *ANOPHELES MACULIPENNIS* POPULATION IN 1952, MOSCOW OBLAST
(AS A PERCENTAGE OF THE TOTAL NUMBER DISSECTED)

Period of dissections	Number dissected	Percentage relationship of females of various ages														
		Nulliparous		Parous (number of gonotrophic cycles)												
		Gonoactive	Non-gonoactive	1	2	3	4	5	6	7	8	9	10	11	12	Very old
25-30 April	81	81.4	—	13.6	4.9	—	—	—	—	—	—	—	—	—	—	—
6-10 May	62	1.6	—	87.0	11.3	—	—	—	—	—	—	—	—	—	—	—
11-15 May	60	1.6	—	3.3	65.0	28.3	1.6	—	—	—	—	—	—	—	—	—
21-25 May	7	—	—	—	42.8	28.5	28.5	—	—	—	—	—	—	—	—	—
26-31 May	51	—	—	—	5.8	31.3	52.9	7.8	1.9	—	—	—	—	—	—	—
1- 5 June	95	7.3	—	—	2.1	5.2	22.1	41.0	18.9	3.1	—	—	—	—	—	—
6-10 June	170	53.5	—	7.6	—	—	4.7	15.8	11.7	6.4	—	—	—	—	—	—
11-15 June	270	62.9	—	27.5	—	0.4	1.1	4.8	3.3	—	—	—	—	—	—	—
16-20 June	285	23.0	—	57.0	13.7	0.3	—	—	—	1.4	3.1	1.4	—	—	—	—
21-25 June	394	19.0	—	31.5	39.1	8.5	0.3	—	—	—	0.3	0.5	0.5	0.3	0.3	—
26-30 June	283	7.4	—	26.8	38.8	19.1	6.3	1.1	—	—	—	—	—	—	—	—
1- 5 July	240	3.7	—	17.5	27.9	27.5	15.8	6.2	1.2	—	—	—	—	—	—	—
6-10 July	209	8.1	—	14.3	22.4	20.5	18.2	11.9	2.9	1.4	—	—	—	—	—	—
11-15 July	275	12.3	—	10.5	19.6	22.5	21.8	8.7	2.5	1.8	—	—	—	—	—	—
16-20 July	299	18.0	—	17.4	19.3	13.4	16.7	9.3	4.6	1.0	—	0.3	0.7	—	—	—
21-25 July	284	13.4	—	21.5	16.2	14.5	10.3	10.5	7.0	3.4	2.1	0.3	0.7	—	—	—
26-31 July	220	15.0	—	25.0	19.0	12.7	7.7	10.4	3.2	5.5	1.4	—	—	0.4	—	—
1- 5 August	230	5.6	—	34.7	24.7	13.9	7.4	7.8	3.0	0.9	0.9	0.4	—	—	—	—
6-10 August	186	1.6	—	15.6	24.7	17.1	15.6	12.3	9.7	1.6	1.1	0.5	—	—	—	0.9
11-15 August	260	—	0.8	8.4	21.5	25.0	22.9	11.1	4.6	5.3	0.4	0.7	—	—	—	—
16-20 August	220	—	0.45	2.8	15.9	25.9	22.2	15.0	7.7	5.9	2.7	0.4	—	—	—	—
21-25 August	130	—	2.3	0.7	4.7	16.9	29.3	26.1	10.0	7.8	1.4	0.7	—	—	—	—
26-31 August	193	—	3.1	2.5	6.2	11.9	24.9	28.0	12.4	6.7	2.0	1.6	0.6	—	—	0.46
1- 5 September	213	—	4.7	0.46	7.0	20.8	22.5	21.6	12.7	6.5	2.8	0.46	—	—	0.3	0.3
6-10 September	280	—	9.3	0.3	4.3	8.5	25.8	21.1	13.9	10.0	4.7	1.1	0.3	—	—	0.7
11-15 September	131	—	28.2	—	4.6	4.6	16.8	17.6	12.2	8.4	4.6	1.5	0.7	—	—	—
16-20 September	81	1.2	29.6	—	1.2	6.1	6.1	23.5	16.0	4.9	8.6	2.5	—	—	—	—
21-25 September	46	—	41.3	4.3	—	2.2	10.8	13.0	10.8	10.8	6.6	—	—	—	—	—
26-30 September	15	—	33.3	—	—	—	—	33.3	26.6	6.6	—	—	—	—	—	—

the fact that some females die in each gonotrophic cycle and that no new batches of nulliparous overwintered females appeared, the total number of overwintered insects gradually fell and latterly only few were found. The data obtained made it possible to estimate the actual calendar—and physiological—age of females which had gone into hibernation. Since in the Moscow Oblast the overwhelming majority of females go into hibernation at the end of August and throughout September, and are encountered again in the following spring up till the end of June, a small proportion of them must live for as much as nine or ten months. The gonoactive life of a certain proportion of overwintered females in the spring-summer period lasted for two or three months only, and in most cases it was considerably shorter. There were occasional females which during this period managed to complete 10 or 11 ovipositions. It is quite possible that in nature there were females of greater age, which we did not find because of their small numbers.

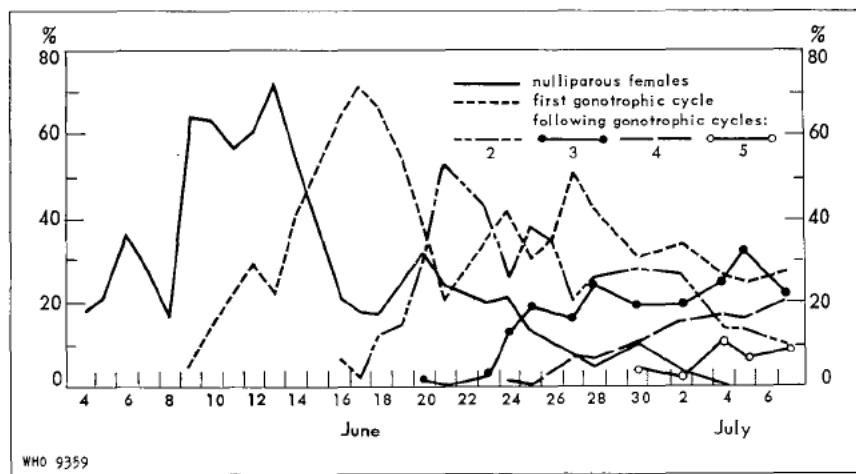
The beginning of flight of the first generation to the day resting-places was noted on 4 June. On that day the first summer nulliparous females were found with blood in the stomach in Sella's stage 2. Emergence of the first generation reached its peak between 10 and 15 June. During the next days of June a gradual aging of the young insects was noted. Daily dissection of females made it possible to study this aging process. As can be seen from Fig. 27, the first female in the first gonotrophic cycle was discovered on 4 June, in the second cycle on 9 June, in the third on 16 June, in the fourth on 20 June, in the fifth on 24 June, in the sixth on 30 June and in the seventh on 5 July. On these dates females were found in Sella's stage 2, which indicates the actual beginning of a new gonotrophic cycle. Fig. 27 illustrates the gradual increase in the proportion of females belonging to each successive age group and shows how a preponderance of females in one age group among those dissected is accompanied by a gradual increase in the proportion of those in the age group above it. The duration of each gonotrophic cycle differed and depended on the environmental temperature.

In the period between 4 June (when the first females of the first generation appeared in the day resting-places) and 26 June (when the last overwintered female was found) two well-defined groups of females differing in physiological age were discovered in the population: (1) physiologically young, pauciparous and nulliparous individuals of the first generation; and (2) multiparous, overwintered females in the fifth to the twelfth gonotrophic cycle.

As will be seen from Table 1, there was always a gap of several age classes between these two groups. The absence during this period of "middle-aged" females is explained by the fact that young females of the first generation had not yet managed to complete a large number of ovipositions and all the overwintered females which had survived until that time

had already become multiparous. Thus the absence of females in the middle age groups makes it possible to distinguish the overwintered group from all the summer mosquitos, which are considerably younger than the youngest overwintered insects. A similar picture was noted for the majority of the populations studied in other places. However, in some instances, particularly in the south of the USSR, a very protracted period of flight from hibernating places was noted, making it more difficult to divide the whole population in the spring-summer period into the two above-mentioned groups. In such cases it is easy to distinguish only the

FIG. 27. GRADUAL INCREASE OF AGE OF THE FIRST GENERATION OF FEMALE *ANOPHELES MACULIPENNIS* IN 1952 (MOSCOW OBLAST, KOLONETZ VILLAGE)



group of physiologically old, overwintered females and it would be helpful to use Perry's method (1912) as an additional guide.

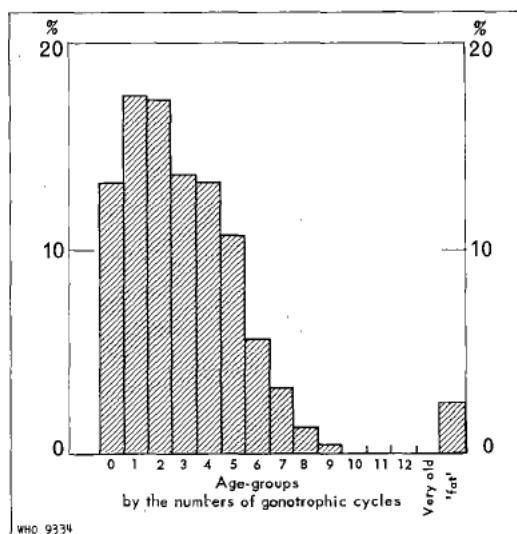
During the season mentioned, a considerable aging had taken place by the end of June 1952 among the summer females. Some were found in their sixth gonotrophic cycle.

During the period from 26 June to 10 August, when diapausing females began to be caught in the settlements, only females of the summer generation were found among those dissected. Since during this period the meteorological conditions were favourable for the life of the population, considerable aging was noted, and the proportion of multiparous females grew significantly. This composition of the population pointed to a high survival rate of females in successive age groups in the summer of 1952. The first female in its eighth gonotrophic cycle was found on 8 July, in the tenth on 18 July, and in the eleventh on 22 July. Very old females, in their tenth to twelfth gonotrophic cycle, were found almost daily, although in

very small numbers, but the numbers of females in their fifth to ninth cycle were consistently high.

From the second ten-day period in August, when the emergence of gono-active females had almost completely ceased, a reduction in the proportion of pauciparous, and an increase in the proportion of multiparous, females was noted. The oldest was a female in its thirteenth gonotrophic cycle, found on 10 September. Among the dissected some females were found whose ages it was impossible to determine. The ovaries had a structureless appearance and we believe that these individuals had completed a still greater number of ovipositions. By the end of the season the numbers of multiparous females had considerably increased.

FIG. 28. PROPORTION OF VARIOUS AGE GROUPS
OF A POPULATION OF *ANOPHELES MACULIPENNIS*
IN 1952 (MOSCOW OBLAST)



As will be seen from the figures given in Table 1, the proportion of nulliparous females during the whole season was smaller than the proportion of uniparous females. This indicates a deficit of nulliparous females in the day resting-places in the settlements, a fact which has already been discussed. Only on odd days in the season, when individuals of new generations emerged, was there a slight preponderance of the first age group over the second. The relative size of age classes among all those dissected during the season is shown in Fig. 28.

The figures given show clearly that among the mosquitos dissected a deficit was found, not only of nullipars, but also of females having completed one gonotrophic cycle. The total number of females in the

second gonotrophic cycle was the same as in the third. With no deficit in this group, the total number of first females should have been higher than that of second females, since mortality occurs at each gonotrophic cycle.

Repeated observations on the age composition carried out in the same place during the next season, in 1953, again confirmed the occurrence of the deficit of nulliparous females in the day resting-places. The capture of mosquitos from natural shelters in the zone of attraction of the settlement, and the examination of all the females captured, showed that among this group, nulliparous empty females predominated, and many of them were newly emerged. However, even among females at a particular stage of blood digestion, nulliparous females predominated. All these data lead to the conclusion that the deficit of nulliparous females is caused by the greater proportion of the physiologically young females remaining in nature.

In the first ten-day period of August 1952 the proportion of nulliparous gonoactive females among those dissected fell sharply, and in the second and third ten-day periods, no females of this group were encountered. However, the constant finding, throughout August, and even on some days in September, of females having completed one cycle indicates that gonoactive females continued to emerge. These data show that in the autumn the age-related characteristics of mosquito behaviour are preserved—the physiologically young females prefer the outdoor shelters.

When young gonoactive females ceased to fly in, nulliparous individuals gradually disappeared from the population and only the aging of earlier-emerged individuals took place. At the same time a gradual increase in the numbers of diapausing females occurred. The first diapausing female was found in the capture station in the settlement on 9 July 1952, but regular findings of such females began only on 11 August. Their numbers in the capture stations were always small, this being due to the character of these resting-places, which were very unfavourable, so that the females dispersed to some other resting-places which were not examined.

Main Features of Similarity and Difference in Age Composition Between *Anopheles maculipennis* Populations in Various Geographical and Climatic Conditions

The study of the age composition of female *A. maculipennis* carried out by the same methods in different parts of their habitat in the USSR ascertained the main similarities or differences in the age composition of populations living in various geographical and climatic zones.

The material collected was analysed separately for overwintered females and for females of the summer generations.

The comparative age composition of overwintered female *Anopheles maculipennis*

It was confirmed that everywhere a certain proportion of overwintered females not only survives until the emergence of the first generation but even lives sufficiently long afterwards to become as multiparous as the summer generations. The calendar—and physiological—age of overwintered females differed in various climatic conditions.

Since there is a considerable difference in the times of flight of females from hibernating places, it was impossible to compare their composition on the same days. Their age composition can only be compared for identical biological periods. According to the data collected by Shipitsina (1957) on the phenology of *A. maculipennis*, throughout the territory of the USSR the difference in the average time of flight from hibernation in the south and north was about three months. This difference means that at the same calendar period all the overwintered females in the south have died off, while in the north they are only beginning to fly from their hibernating places.

Where the study of the age composition of the population began long before the emergence of the summer generations, comparative over-all data were obtained on the relative size of age classes during the 30 days preceding the emergence of the first generation (which does not correspond to the whole period of activity of these females). In addition, in a number of populations, comparison was made of the age composition of the female population on the very day of flight of newly emerged insects of the first generation, and then in some cases the composition of the overwintered female population was compared for the last 15 days of their life in nature.

Comparative age composition of overwintered females during a period of 30 days before the first flight of individuals of the first generation

A comparison of the data for the 30 days preceding the emergence of individuals of the first generation showed a considerable difference in the relative sizes of the age classes in individual populations. The biggest difference was noted between populations living in the south and those living in the temperate central areas. A basic difference was noted in the proportion of nulliparous females. Thus, while in the populations in the forest zone the proportion of nulliparous females among all those dissected in a month averaged 0.8%-2.9%, in the southern populations it varied between 23.5% and 55.0%. This difference may be explained by the difference in the length of the period during which females fly from their hibernating places. While at the beginning of the stated period the number of females flying from winter quarters was still considerable, the majority

of nulliparous females belong to those which are counted during the 30 days and, in that case, the proportion of nulliparous females is high. The same was found in the southern populations. On the other hand, in areas where lower spring temperatures mean that a considerably longer interval elapses between the flight from hibernating places and the emergence of individuals of the first generation, the numbers flying in from winter quarters during the last 30 days are very small and therefore the total proportion of nulliparous females is also small. At the same time, in populations in the forest zone, a greater number of age classes and a considerably greater proportion of multiparous females were found. Thus, while in southern conditions females with four or five completed ovipositions were the oldest (although the proportion of such females was very low and did not exceed 4% of those dissected), under forest conditions the oldest were females that had completed seven to nine ovipositions, the total proportion of those which had completed four or more reaching 30%-40% of those dissected.

Analysis of the data on the age composition of overwintered females at the time of emergence of individuals of the first generation also showed considerable differences between females living in different parts of the habitat. Thus, for example, in a population from the Baltic area (Polikarpova, 1957) in 1953 and in one from Novosibirsk in 1952 (Mishukov, 1957), in addition to females which had already completed four and five ovipositions each, on the day of flight of newly emerged individuals, overwintered mosquitos were found at the end of the first and in the second gonotrophic cycles. Although the total numbers of such females were small, the fact that they were found indicates a very protracted period of flight from winter quarters. It is interesting that during observations on the composition of overwintered females in subsequent seasons no such physiologically young females were found, this being due to different weather conditions. In the overwhelming majority of populations, mainly in the forest belt (Chernysheva, 1955; Korzhenevskaya, 1957; Shevchenko, 1953, 1955; Almazova & Zotova (in preparation); Detinova (unpublished observations); Polikarpova, 1957; Kukharchuk, 1958; Pankova, 1959; Vlasenko, 1958, and many others), at the time of flight of individuals of the first generation there were already no nulliparous females among those which had overwintered, but there were some in their second gonotrophic cycle. In the 1952 season, in the Moscow Oblast, the youngest females in this period had oviposited three times. This shows that there had been a considerable interval between the end of flight from hibernating places and the emergence of individuals of the first generation.

The age of the oldest overwintered females varied in different populations and ranged from the third to the ninth oviposition. As has already been stated, the oldest females were found in the populations in the forest zone of the USSR.

Comparative age composition of overwintered females during the last 15 days they are found in nature

In order to compare the age composition of females during the period concerned, only a few populations were taken in which it was possible without error to separate, according to physiological age, overwintered females from the general mass of summer mosquitos existing by this time. The results, which are given in Table 2, show that the age composition of the females found differed in different populations. For example, under the conditions in the steppe zone (Stalingrad¹ Oblast, according to Kozhevnikova (1953)), where in the spring there are constant dry winds, the age composition showed a preponderance of young females. Here, even at the end of the existence of the overwintered females, a comparatively large number of females which had completed only one oviposition was encountered among those caught, and the oldest had completed only four ovipositions. This physiological youth indicates that the leaving of hibernating places was spread over a long period and that mortality among parous females was high.

The time-spread of flight from hibernating places is due mainly to the variety of such places. With the high mortality among overwintered females, this protracted flight period ensures gradual accumulation and development of the pre-imaginal stages and later large numbers of first generation mosquitos.

In populations of the forest zone the youngest were in their fourth or fifth gonotrophic cycle and the oldest in the twelfth. These data, like the previous ones, show that in the forest zone, with its lower temperatures and greater humidity, conditions are created for the gradual aging of females with a comparatively low mortality rate, as a result of which a larger number of age classes and a higher proportion of multiparous females are found among those dissected.

Comparative age composition of females of the summer generations

Determination of similarities and differences in the age composition of summer females was based on the data obtained by dissection throughout the whole season, from the time of flight of individuals of the first generation until the summer-emerged individuals have completely or almost completely died off in the autumn.

A comparison of the age composition of summer females for the whole season in various localities is possible because all the changes which take place in the composition of the population during the whole life-span of the summer females are taken into account. In this general analysis, the population composition is taken into account both at periods when

¹ Now Volgograd.

there is a mass emergence of all generations and at periods when there is very little flight of newly emerged females, as well as in the autumn, when the breeding of gonoactive females ceases altogether and only a gradual aging of already-emerged individuals is noted. As a result, a picture is obtained of the composition of the population for the whole season which is characteristic of a given locality in a given year. This makes it possible to compare the age composition of females from localities with different geographical and climatic conditions.

The data obtained on the relative sizes of age groups among the females examined are given in Table 3. As will be seen from the figures therein, the calendar dates of the beginning and end of the season of activity of summer females differed considerably in various populations. In all cases except one (Vitebsk Oblast, 1952), the life of a population was observed from the emergence of the first generation until the complete or almost complete dying-off of all the summer gonoactive females. In the Vitebsk Oblast alone, observations of age composition were completed on 1 August, when all the females caught in the capture stations in the settlement proved to be gonoactive. Despite the early completion of the work, the data for Vitebsk were included in the general table, since they are of considerable interest in so far as the relative size of age classes was clearly different from that in the majority of populations studied in other conditions. These differences proved so considerable that it can be confidently stated that the ratios between age groups found in this instance would not have changed during the season even if the results of dissection had been added for September, when the proportion of gonoactive females decreases sharply as the number of diapausing females increases.

Table 3 shows figures for populations in which observations were systematically carried out during the whole season and the total number of dissections was sufficient to obtain reliable results. An analysis of the data obtained showed that, despite differences in the climatic conditions, a number of common features could be noted in the majority of the populations studied. The existence of these features (described below) in populations living under completely different conditions indicated that they were characteristic of the *A. maculipennis* species as a whole.

- (1) There was a deficit of nulliparous females among those caught in the day resting-places in the settlement.
- (2) Among all the dissected females, there was a preponderance of females which had completed one gonotrophic cycle and were in their second gonotrophic cycle.
- (3) There was a considerable reduction in the numbers of females which had completed four or more ovipositions.
- (4) Females were capable of completing a considerable number of consecutive ovipositions.

TABLE 2. THE RELATIONSHIP BETWEEN AGE CLASSES AMONG SUMMER FEMALE GENERATION UNTIL THE COMPLETE OR ALMOST COMPLETE DYING-OFF

Place	Year	Author	Period of observation (5-day periods and months)	Number dissected
Stalingrad Oblast	1952	Bandin (1953)	June-August	2432
Moscow Oblast	1953	Almazova & Zотова (in preparation)	1 5-day period 4 " June " September	2243
Moldavia	1949	Detinova (1952a)	3 " May 1 " October	7673
Latvia	1953	Polikarpova (1957)	6 " May 4 " September	2833
Moscow Oblast	1952	Detinova (1953d)	1 " June 6 " September	5134
Leningrad Oblast	1952	Sukhomlinova (1956)	2 " June 2 " September	4162
Vitebsk Oblast	1951	Zorin (1957)	1 " June 2 " September	2890
Molodechensk Oblast	1954	Korzhenevskaya (1957)	1 " June 6 " October	3367
Stalingrad Oblast	1952	Kozhevnikova (1953)	4 " May 4 " September	1911
Vitebsk Oblast	1952	Chernysheva (1955)	2 " June 6 " August	1793

TABLE 3. THE RELATIONSHIP BETWEEN THE VARIOUS AGE CLASSES AMONG OVER-

Place	Year	Author	Date of emergence of the first generation	Period of dissections
Stalingrad Oblast	1952	Kozhevnikova (1953)	17 May	20 May-5 June
Georgian SSR	1953	Tairova (1956)	27 May	5-20 June
Vitebsk Oblast	1951	Zorin (1957)	5 June	6-19 June
Vitebsk Oblast	1952	Chernysheva (1955)	7 June	12-26 June
Moldavian SSR	1949	Detinova (1952a)	11 June	19 May-2 June
Moscow Oblast	1952	Detinova (1953d)	4 June	13-27 June

ANOPHELES MACULIPENNIS FROM THE MOMENT OF FLIGHT OF THE FIRST
OF THE GONOACTIVE FEMALES IN VARIOUS REGIONS OF THE USSR

Nulliparous	Percentage of number dissected belonging to age (gonotrophic cycle):												
	1	2	3	4	5	6	7	8	9	10	11	12	Very old
52.5	30.3	11.2	4.3	1.5	0.1	0.04	—	—	—	—	—	—	—
26.6	21.5	18.9	13.9	8.7	4.3	2.1	2.0	0.9	0.6	0.3	0.1	0.05	—
25.0	28.2	23.7	11.8	7.2	2.5	1.2	0.3	0.1	0.04	—	—	—	—
32.3	43.2	15.9	5.3	2.0	0.8	0.3	0.1	—	0.03	—	—	—	—
13.6	18.1	18.0	14.0	13.7	11.1	5.9	3.4	1.3	0.5	0.2	0.04	0.02	0.09
20.2	37.3	17.5	13.3	6.8	3.6	1.0	0.2	0.02	0.02	—	—	0.02	—
11.0	26.6	24.5	18.1	9.7	5.4	2.6	1.1	0.7	0.2	0.06	—	—	—
19.5	50.4	16.2	7.0	3.4	1.9	0.8	0.4	0.2	0.1	—	0.03	0.03	—
14.6	44.3	18.8	11.1	5.8	3.1	1.0	0.7	0.4	0.1	—	—	0.05	—
5.4	22.2	24.8	20.5	10.4	7.5	4.2	2.7	1.7	0.5	0.1	—	—	—

WINTERED FEMALES DURING THE LAST 15 DAYS OF THEIR PRESENCE IN NATURE

Total	Number dissected												
	Belonging to age (gonotrophic cycle):												
	0	1	2	3	4	5	6	7	8	9	10	11	12
36	—	20	10	4	2	—	—	—	—	—	—	—	—
106	—	—	3	36	30	27	10	—	—	—	—	—	—
139	—	—	16	97	23	3	—	—	—	—	—	—	—
360	—	—	5	36	71	73	99	41	35	—	—	—	—
17	—	—	—	8	3	1	2	1	2	—	—	—	—
34	—	—	—	—	1	1	6	6	10	6	3	1	—

Naturally, these characteristics varied in populations living under different conditions. Among the large number of populations in which the relative sizes of the age classes during the whole season of activity of the summer females were successfully established, it was only in very rare cases that no deficit of nulliparous females was noted among all those examined in the settlements. The absence of such a deficit may be noted, for example, in an *A. m. messeae* population living in the steppe zone (Bandin, 1953) and an *A. m. sacharovi* population living in the hot, dry climate of Central Kazakhstan (Kannegisser & Riazantseva, 1953). In these two populations the proportion of nulliparous was considerably greater than that of uniparous females. It is interesting that, during the following season in Kazakhstan in the same centre of population but with greater humidity, the relative sizes of the age classes changed considerably and nulliparous females were only slightly more numerous than those which had completed one gonotrophic cycle. The nulliparous females made up 38.3% of all those dissected and the uniparous 30.8%. This relationship also pointed to a deficit of nulliparous females in that season. A similar relationship was noted in some other populations—for example, in one of the settlements of the Moscow Oblast and in the Baltic area in the 1954 season. The change in the relative sizes of age classes in the same centres of population in different seasons leads to the conclusion that the inter-age relationship varies under the influence of external factors, which produce more favourable or less favourable conditions in the natural shelters. In all the other populations, a clearly marked deficit of nulliparous females was noted in the settlements. The greatest deficits were found in populations living in the mild climate of Byelorussia.

From the data supplied, it can be seen that in a number of populations, in addition to the deficit of nulliparous females, there was also a deficit of uniparous insects (Moscow, Novosibirsk, Vitebsk and other oblasts). In the same oblasts, but in different centres of population and in different years, the relative number of uniparous females only slightly exceeded the proportion of females having completed two cycles, which points to a deficit also in that group. Thus, this phenomenon was common among female *A. maculipennis* living mainly in the forest zone.

It is interesting that the deficit of nulliparous females is sharply marked among those examined in the first month of life of the summer generations, when the proportion of nulliparous gonoactive females is highest. Only in individual populations was there a preponderance of nulliparous over uniparous females. However, this preponderance was not so great as to invalidate the general deficit of nulliparous females in settlements. It was an exception to the rule caused by particular conditions of place and time.

Thus, on the basis of all the material examined, it can be concluded that the occurrence of a deficit of nullipars among the females dissected

at the beginning of their active season and during the season as a whole does not depend on the place where the population lives or on the number of generations emerging during one season or on the speed of aging of the females. It is a regular phenomenon for the *A. maculipennis* species throughout its habitat. The absence of such a deficit in the day resting-places in settlements among all summer females and among females examined at the beginning of their active season is rather an exception and is caused by specific external factors determining the behaviour of females in selecting their resting-places. A deficit of uniparous females was also characteristic of many populations.

The data obtained on the relative sizes of age classes among populations living in the most varied climatic conditions show that females of *A. maculipennis* are well adapted to environmental conditions throughout a considerable part of their habitat. This adaptation is shown in the fact that the females are capable of completing a large number of consecutive gonotrophic cycles. In the overwhelming majority of the studied populations a proportion of the females managed to complete between 9 and 12 ovipositions during their lifetime. The finding of these females at one of the stages of blood digestion and ovarian development shows the possibility of completing the cycle and ovipositing. The failure to find females in still higher age groups is an indication of their scarcity.

Despite the identical physiological age of the oldest females in different populations, the calendar age of these individuals differed, depending on the speed with which gonotrophic cycles were completed. Thus, in southern conditions (Stalingrad Oblast), the females managed to complete 12 gonotrophic cycles in 42 days, whereas in the forest zone of the Moscow Oblast the same number of cycles took 79 days. These data show that in different conditions females complete a different number of ovipositions during identical calendar periods. In view of the fact that deaths of females occur mainly at the beginning and at the end of a gonotrophic cycle, the faster the gonotrophic cycles are completed the greater is the calendar period mortality of the females.

Analysis of all the data obtained shows that for females of *A. maculipennis* of the summer generations and for overwintered females the conditions in the forest zone are most favourable. As a result of this the proportion of females surviving to physiological old age is much greater than in the more southerly populations and the total number of age classes is also considerably greater, since in these conditions females can complete a greater number of gonotrophic cycles.

Chapter 8

DETERMINATION OF THE
EPIDEMIOLOGICAL IMPORTANCE OF
POPULATIONS OF *ANOPHELES MACULIPENNIS*
BY THEIR AGE COMPOSITION

The epidemiological effectiveness of a vector population depends on many factors. Among the more important are the total numbers of mosquitos, their degree of activity, the degree of their contact with man, the proportion which survive to a dangerous age, their susceptibility to infection with plasmodia. Most of these factors have been thoroughly studied in connexion with the epidemiology of malaria, and need not be reviewed here. The importance of calculating the proportion of mosquitos surviving until the end of sporogony for the evaluation of the epidemiological importance of the *Anopheles* population was shown as early as 1910 by Ross. Not having any factual data on mosquito survival, Ross took the above factor as being constant. It has now been established that the proportion of mosquitos capable of surviving until the end of sporogony is not constant and differs not only among populations living in different climatic conditions, but even in one and the same population during the given season of potential malaria transmission. The data obtained lead to the conclusion that this least-studied factor is decisive in evaluating the epidemiological effectiveness of a vector population. The problem of longevity of malaria vectors in relation to the epidemiology of malaria has been ably presented by Macdonald (1957).

Each of the factors which determines to some degree the epidemiological significance of a population follows an annual trend, and the extent to which such factors coincide is extremely important; one example is the coincidence of large numbers of parasite carriers with large numbers of vectors and the ability of the latter to survive until the end of sporogony. It has already been shown that the age composition of the population is not constant throughout the season, but undergoes considerable changes. The age composition of the populations of different species found in one and the same locality also varies, at least in countries with well-defined seasons.

Obviously, the differences in the age composition of females determine the epidemiological effectiveness of the populations. Since each blood-meal affords another chance of the female's becoming infected the importance of each age group consequently increases in relation to the increase of its physiological age. However, because of the increasing mortality during each gonotrophic cycle, only a very small proportion of females survives to physiological old age. As Beklemishev (1944) points out, "the older a particular age class of the mosquito population, the higher its infectivity rate, but the smaller its numbers". Obviously then, the younger the physiological age at which females can become malaria vectors, the more often are they found in a population and the greater is their epidemiological effectiveness.

The length of the period permitting mosquito activity and the development of the parasites into sporozoites varies considerably in different climatic conditions. In the tropics, malaria is transmitted practically throughout the year. In temperate latitudes, it can only be transmitted during the warm season except for cases of infection inside houses. Thus, in the USSR the following differences were found in the length of the malaria transmission season: on the northern border of the area of distribution of the vector the transmission period is limited to one or one and a half months; in the central areas it lasts two to two and a half months, in the south it is as long as five to six months (Moshkovsky & Rashina, 1951; Prokopenko, 1960; Sergiev & Yakusheva, 1956). Naturally, the longer the period during which mosquitos can become infected and transmit malaria, the greater is the epidemiological effectiveness of their populations. In all countries with a cold or cool winter there is a longer or shorter interruption in malaria transmission. In these areas it is always necessary to determine the beginning, the middle and the end of the transmission season. The ability to determine correctly the duration of the period when infection of mosquitos and transmission of malaria are possible gives a clue to the correct timing of mosquito control measures and of chemotherapy. It also gives some idea of the time of possible appearance of the last primary cases of malaria in the autumn and the first cases in the spring.

The transmission season, or the season of mass malaria infection (Moshkovsky & Rashina, 1951), is that part of the year during which most cases of malaria infection occur. The period during which temperature conditions in a locality permit the development of malaria parasites to the sporozoite stage is called the "season of effective mosquito infectivity," while the period during which malaria is transmitted from mosquito to man is the malaria transmission season.

We have had no opportunity of establishing the relationship between the physiological age of females and their infection with sporozoites. In former years, when malaria infection was widespread in the USSR,

the age of the females could only be determined approximately from the size of their ampullae, and the results obtained were not very reliable. The data obtained by Avdeeva (1940) enable us only to state that among the females with larger ampullae, infected individuals were found more frequently, but it is impossible to establish at what physiological age the proportion of infected individuals increases. It would be desirable to carry out, wherever this is possible, a series of simultaneous investigations of the age composition and the infectivity of vectors in order to establish the actual epidemiological part played by each age class.

We estimate the beginning and the end of mosquito infectivity and of malaria transmission seasons, conditionally, by using actual data on the age composition of populations. Where the actual infectivity rate of females is not determined, and the only factors known are the possible duration of sporogony and the physiological age which females must reach by the end of the first sporogony, we refer to females which have reached that age as potentially dangerous. In other words, those *Anopheles* females whose age at a given time equals or exceeds the length of time necessary for the completion of sporogony are potentially dangerous during the epidemiological season. The duration of life of females is determined from the number of completed gonotrophic cycles and from the length of each cycle, calculated on the basis of the environmental temperature. This point will be discussed later.

To determine the age at which females are potentially dangerous we assume that they become infected during their first blood-meal. It will be shown that both the speed of sporogony and the time of completion of gonotrophic cycles change considerably in relation to various weather conditions, but not proportionately to each other, so that the age of the potentially dangerous females is not a constant quantity; it must be determined not only for each geographical zone but for every locality and every period of the season. An exception can probably be made in the case of the equatorial zone or the extreme south, where the temperature throughout the whole year may remain within the optimum range, and the duration of sporogony and the duration of gonotrophic cycles change very little. As already stated (see Chapter 7, page 96), under these conditions a gonotrophic cycle takes 48 hours. It has been established that in different parts of their habitat and at different times during the malaria transmission season, females reaching the same physiological age may not have the same epidemiological importance. A particular physiological age may be potentially dangerous in some cases and not in others. The younger the age at which females may become dangerous, the larger are their numbers in the population and the greater is the danger of malaria transmission by that population, other conditions being equal.

To establish the epidemiological effectiveness of malaria vectors, it is necessary to know:

- (1) the duration of the sporogony from each possible day of infection throughout the whole season;
- (2) the duration of the gonotrophic cycles, also throughout the whole season;
- (3) the age of the potentially dangerous females on each day during the season;
- (4) the proportion of potentially dangerous females among all those dissected daily;
- (5) the numbers of mosquitos in the day resting-places;
- (6) the degree of contact between the mosquitos and man.

Methods of Determining the Duration of Sporogony

It is well known that the whole process of sporogony covers the period from the taking of a blood-meal by the female from a malaria case to the appearance of sporozoites in its salivary glands. From that moment the females become epidemiologically dangerous, i.e., each subsequent blood-meal is infective. The duration of the process of sporogony, like that of all biological processes in insects, depends directly on environmental factors. As was first stated by Grassi (1901) and later confirmed by many research workers (Khodukin & Lisova, 1927; Nikolaev & Shub, 1934; Veshchezerov, 1928; Boyd, 1932; Nikolaev, 1935; Kligler & Mer, 1937; Stratman-Thomas, 1940; Remennikova, 1938, 1948; Moshkovsky, 1946; Lysenko & Levitanskaya, 1952; Pavlova, 1952, and others), the main factor determining the rate of this process is temperature.

Nikolaev (1935) established that the sporogony of *Plasmodium vivax* takes place at a temperature not lower than 16°C, and of *P. falciparum* at a temperature of not less than 18°C. It has also been found that there is a direct relationship between the environmental temperature and the speed with which sporogony proceeds in the mosquito.

From the point of view of malaria epidemiology, the determination of the duration of sporogony in the natural habitat of mosquitos is of great interest, since the changes in temperature have an effect on the development of plasmodia. Knowledge of the laws governing the course of sporogony at changing temperatures permits the determination of the commencement, duration and end of the epidemiological season. At the present time we have two methods for the estimation of the duration of sporogony under natural conditions: the Moshkovsky method (Moshkovsky, 1946; Moshkovsky & Rashina, 1951) and the Oganov-Rayevsky method (Oganov, 1947). Both methods use as a basis the experimental data obtained by Nikolaev in 1935.

The mean diurnal temperatures are used in the calculation and are taken as constant. All calculations of the duration of sporogony, digestion of the blood and maturation of the ovaries are based, not on the outdoor temperatures, but on the temperatures of the premises used as the main day resting-places of the mosquitos. It is therefore necessary in the calculations to make a correction for the difference between the outside air temperatures and the temperatures inside those premises. Such a correction for temperate climates was introduced by Oganov (1947). Naturally a correction factor valid for all climatic zones cannot be given. It can be used only for the given type of climate and premises for which it was determined.

Both the Moshkovsky and the Oganov-Rayevsky methods are based on calculation of the sum of heat necessary for completion of the cycle of sporogony.

Moshkovsky's method for determining the duration of sporogony

Moshkovsky (1946) used the Blunck hyperbola and the Bodenheimer formula for determining the relationship between the rate of the sporogonic process and the temperature, and demonstrated that the hyperbolic relationship between these factors agrees with the "sum of heat" theory, according to which a certain total of degree-hours or degree-days is required for the completion of the cycle. "The sum of heat is the total number of degree-days in the given period of time. The degree-day (or, to be more accurate, the degree-24 hours) is the number of degrees by which the mean temperature of the day concerned exceeds the lower threshold temperature for the development of the organism of the given species, i.e., the temperature below which its development cannot occur" (Moshkovsky & Rashina, 1951).

The sum of heat in degree-days for the maturation of the sporozoites in the mosquito's body is 105°C for *P. vivax*, 111°C for *P. falciparum* and 144°C for *P. malariae*.

To establish the possible duration of sporogony it is necessary to subtract from the mean diurnal temperature in each 24 hours the estimated lowest development temperature threshold, which is equivalent to 14.5°C for *P. vivax* and 16°C for other species of malaria parasites, and then in sequence to add the differences obtained in order to reach the total necessary for the development of the given species of parasite. An example of the calculation is given in Table 4.

The Oganov-Rayevsky method of determining the duration of sporogony

Oganov (1947), using a method suggested by Rayevsky (1942), calculated the proportion of the whole process of sporogony of *P. vivax* occurring during 24 hours at each temperature. His data for each tenth of a degree are shown in Table 5.

TABLE 4. THE MOSHKOVSKY TECHNIQUE OF CALCULATING
THE DURATION OF SPOROGONY*

Date	Mean diurnal temperature		Degree-days			
			<i>Plasmodium vivax</i>		<i>Plasmodium falciparum</i>	
	External air	Day resting-place	External air	Day resting-place	External air	Day resting-place
20 June	19.0	20.0	4.5	5.5	3.0	4.0
21 June	21.5	21.0	7.0	6.5	5.5	5.0
22 June	22.5	22.5	8.0	8.0	6.5	6.5
23 June	23.0	22.5	8.5	8.0	7.0	6.5
24 June	22.5	22.0	8.0	7.5	6.5	6.0
25 June	22.0	21.0	7.5	6.5	6.0	5.0
26 June	24.5	22.5	10.0	8.0	8.5	6.5
27 June	25.0	23.0	10.5	8.5	9.0	7.0
28 June	25.5	21.0	11.0	6.5	9.5	5.0
29 June	24.0	22.5	9.5	8.0	8.0	6.5
30 June	24.5	22.0	10.0	7.5	8.5	6.0
1 July	25.0	22.0	10.5	7.5	9.0	6.0
2 July	25.0	23.0	105.0	8.5	9.0	7.0
3 July	26.0	23.0		8.5	10.0	7.0
4 July	23.0	24.0		105.0	7.0	8.0
5 July	26.0	25.0			111.0	9.0
6 July	27.0	26.0				10.0
						110.0

* After Moshkovsky & Rashina (1951).

To determine the duration of sporogony in *A. maculipennis* at varying temperatures, the proportions of the duration of sporogony corresponding to the mean diurnal temperature for each day are added until they make up 100%. In practice, in these calculations all the days of the season are successively recorded from the moment when a temperature occurs at which sporogony is possible. For each day of the season the main diurnal temperature and the percentage of the total process of sporogony which can occur during one day at that temperature are entered. Then from each day of the season these percentages of sporogony duration are added until they reach 100%, and thus the duration of sporogony can be determined for all females which have taken a blood-meal on any day during a season.

TABLE 5. DURATION OF SPOROGONY OF *PLASMODIUM VIVAX*
IN THE *ANOPHELES* MOSQUITO AT VARIOUS TEMPERATURES *

Tem- pera-ture in °C	Duration of the whole process in days	Development per day as a percentage of the completed process	Temperature in °C	Duration of the whole process in days	Development per day as a percentage of the completed process
16	55	1.82	19.5	22	4.55
16.1	53	1.89	19.6	21.5	4.65
16.2	51	1.96	19.7	21	4.76
16.3	49	2.04	19.8	25	4.88
16.4	47	2.13	19.9	20	5
16.5	45	2.22	20	19	5.26
16.6	44	2.27	20.1-20.3	18.5	5.4
16.7	42.5	2.35	20.4-20.6	18	5.55
16.8	41	2.44	20.7-20.9	17.5	5.74
16.9	40	2.5	21	17	5.8
17	38.5	2.6	21.1-21.3	16.5	6.05
17.1	37	2.7	21.4-21.5	16	6.25
17.2	36	2.78	21.6-21.8	15.5	6.45
17.3	35	2.86	21.9	15	6.66
17.4	33	3.03			
17.5	32	3.12	22	15	6.66
17.6	31.5	3.17	22.1-22.2	14.5	6.9
17.7	31	3.22	22.3-22.4	14	7.14
17.8	30	3.33	22.5-22.8	13	7.7
17.9	29.5	3.39	22.9	12.5	8
18	29	3.45	23	12.5	8
18.1	28.5	3.51	23.1-23.2	12.5	8
18.2	28	3.57	23.3-23.6	12	8.33
18.3	27.5	3.64	23.7-23.9	14.5	8.7
18.4	26	3.85	24	11	9.09
18.5	26	3.85	24.1-24.4	11	9.09
18.6	25.5	3.92	24.5-24.9	10.5	9.52
18.7	25.5	3.92	25-25.5	10	10
18.8	25	4	25.6-25.9	9.5	10.52
18.9	25	4	26-26.4	9	11.11
19	24.5	4.08	26.5-26.9	8.5	11.8
19.1	24	4.16			
19.2	23.5	4.26	27-27.4	8	12.5
19.3	23	4.35	27.5-27.9	7.5	13.3
19.4	22.5	4.44	28	7	14.2

* Calculated by the method of Oganov-Rayevsky (Oganov, 1947).

To determine the possible date of appearance of infected mosquitos among overwintered females, it is recommended that the proportions of the process of sporogony for each day be added together, beginning with the day when mean diurnal temperatures above 16°C are consistently noted (during a long period with lower temperatures the sporozoites perish). The same calculation is made for mosquitos of the summer generations. The beginning of calculation should coincide with the date of establishment of favourable temperatures and with the actual discovery of the first summer females which have taken a blood-meal.

To determine the possible date of the last sporogony in a given season, the calculation begins with the last day during which the temperature was not lower than 16°C. In this case the percentages of sporogonic development are added in reverse, and also finish on the day when the total reaches 100%. This day is considered as the possible beginning of the last successful sporogony in the given season, while the day from which the calculation

TABLE 6. METHOD OF DETERMINING THE DURATION
OF SPOROGONY OF *PLASMODIUM VIVAX*

Date	Mean diurnal temperature in °C	Development of sporogony during one day as a percentage of the completed process	Period during which sporogony took place
15 July	23.0	8.0	
16 "	24.0	9.09	
17 "	25.5	10.00	
18 "	24.9	9.52	
19 "	23.5	8.33	
20 "	23.3	8.33	
21 "	22.9	8.0	
22 "	22.7	7.7	
23 "	21.0	5.8	
24 "	23.0	8.0	
25 "	24.5	9.52	
26 "	23.0	8.0	
		100.29	15-26 July
27 "	19.5	4.55	
28 "	19.7	4.76	
		101.6	16-28 July

began indicates the last day of the possible completion of sporogony during the season. The method of calculation is shown in Table 6.

From the example given in Table 6 it follows that sporogony beginning at the temperatures mentioned on 15 July could have finished on 26 July. In females which could have been infected on 16 July, the end of sporogony would have been on 28 July. In adding up the percentages of sporogonic development the round sum of 100 is rarely obtained, and the total sum nearest to that figure is therefore taken.

This method makes it possible to determine the duration of sporogony with sufficient reliability, since the calculations take into account all the changes in the speed of the process depending on temperature fluctuations.

The accuracy of the results obtained by these methods of calculation has been proved in the first place by the agreement between the actual dates of sporogony and the calculated dates for the same temperatures (Remennikova, 1938; Oganov, 1947; Dukhanina, 1944) and secondly by the agreement between the calculated end of the first cycle of sporogony in the season and the actual appearance of fresh cases of malaria (Almazova & Zotova (in press); Dukhanina (1956) and Yakusheva (unpublished observations).

Methods of Establishing the Duration of the Gonotrophic Cycle

The duration of the gonotrophic cycle depends on the sum of the duration of its three phases (Beklemishev, 1940): (1) the search for a host and the attack; (2) the digestion of the blood-meal and the maturation of the ovaries; and (3) the search for a suitable body of water and the oviposition.

Much research has been done on the duration of the second phase of the cycle, i.e., blood digestion and ovarian development. De Buck, Schoult & Swellengrebel (1932), Hecht (1933), Beklemishev, Vinogradskaya & Mitrofanova (1934), Shlenova (1938), West & Eligh (1952), Detinova (1953b) and others have shown the existence of a direct relationship between the duration of these processes and the environmental temperature and humidity. Until comparatively recently, the duration of the other two phases of the gonotrophic cycle could be estimated only from a small number of individual observations which were often of a contradictory character. The following sections present a brief outline of the methods used for the estimation of the duration of each of the three phases beginning with the second.

Method of determining the duration of the second phase of the gonotrophic cycle

Shlenova (1938) set up extensive experiments on the duration of blood digestion and ovarian development in *A. maculipennis*. The experiments were arranged in various controlled temperatures combined with relative humidities of 30-40%, 70-80% and 90-100%. In processing the material

$$\text{Shlenova used the Blunck hyperbolic equation expressed as } S = \frac{k}{C^0 - N},$$

where S is the duration of digestion; k is a constant; and N is the assumed lowest temperature limit at which digestion can take place. On the basis of factual data obtained on the speed of digestion at different humidities, Shlenova calculated the constant for the hyperbola and established the lower limits of temperature at which digestive processes were still possible at a given humidity.

At a relative humidity of 30- 40%, $N = 4.5^\circ\text{C}$; $k = 1570$.

At a relative humidity of 70- 80%, $N = 9.9^\circ\text{C}$; $k = 875$.

At a relative humidity of 90-100%, $N = 7.7^\circ\text{C}$; $k = 890$.

The sum of degree-hours at different humidities is composed of the differences between the actual temperature at each hour and the threshold temperature, which at a humidity of 30-40% is 4.5°C ; at 70-80%, 9.9°C ;

and at 90-100%, 7.7°C. The making up of this total, which it would be more correct to call the sum of effective temperatures, to 1570, 875 and 890 at the corresponding rates of humidity points to the completion of the second phase of the gonotrophic cycle.

On this basis, the hyperbolic equation at various humidities is as follows:

$$S = \frac{1570}{C^{\circ} - 4.5}; \quad S = \frac{875}{C^{\circ} - 9.9}; \quad S = \frac{890}{C^{\circ} - 7.7}$$

The data obtained by Shlenova on the duration of the second phase of the gonotrophic cycle in *A. maculipennis* at constant temperatures were used by me in an attempt to determine the length of this phase in mosquitos in natural conditions with constantly changing temperature. For all calculations I took the mean diurnal temperatures as constant and transformed Shlenova's constants for degree-hours into degree-days, after dividing them by 24.

As a result the equations took on the following appearance:

$$\begin{aligned} \text{At a relative humidity of 30-40\%}, \quad S &= \frac{65.4}{C^{\circ} - 4.5} \\ \text{At a relative humidity of 70-80\%}, \quad S &= \frac{36.5}{C^{\circ} - 9.9} \\ \text{At a relative humidity of 90-100\%}, \quad S &= \frac{37.1}{C^{\circ} - 7.7} \end{aligned}$$

To determine, in practice, the duration of the second phase of the gonotrophic cycle in mosquitos living in natural conditions it is essential to have data on the mean diurnal temperature and humidity. The difference between the mean diurnal temperature and the threshold temperature is calculated for each 24-hour period. These differences are added until the sum of the effective temperatures at 30-40% humidity is 65.4; at 70-80%, 36.5, and at 90-100%, 37.1. Thus, the making up of the sum of effective temperatures to obtain these figures indicates the completion of the second phase of the gonotrophic cycle. An example of this calculation is given below:

Date	Mean diurnal temperature (°C)	Threshold temperature (°C)	Difference between mean diurnal and threshold temperature
1 July	19.5	9.9	9.6
2 July	19.7	9.9	9.8
3 July	18.9	9.9	9.0
4 July	18.2	9.9	8.3
			36.7

The example shows that females which took a blood-meal on 1 July finished their blood digestion and ovarian development on 4 July. This method of calculation is similar to Moshkovsky's method for determining the duration of sporogony.

In practice, when the data are being processed the required total of degree-days does not always amount to the exact final figure. In these cases calculation must be stopped on the day which gives the total temperature nearest to that sought.

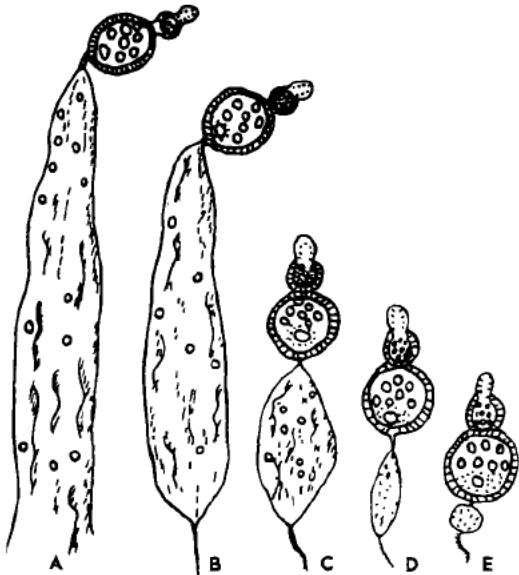
The possibility of using similar calculations for summer females was confirmed by observations on the dates of appearance of freshly fed females at each new physiological age in natural conditions and by calculation of the speed of the processes on the basis of the mean diurnal indoor temperatures at the time (Detinova, 1953d). Complete agreement between the actual dates and those calculated was noted on days when temperatures were closest to optimum. When summer temperatures were low, some disparity was noted between the dates.

However, these methods of calculation prove unsuitable for the spring portion of the season with its low temperatures. The actual age of overwintered females is usually higher than the calculated age. This can probably be explained by the considerable difference between the temperature of the small resting-places in which the females gather and the mean diurnal outdoor temperature. It should be noted that according to Shlenova (1940) the spring distribution of mosquitos in day resting-places differs from their summer distribution.

Method of determining the duration of the first phase of the gonotrophic cycle

When females were dissected for determination of their physiological age, I discovered that in different females in stages 1, 2 and 3 of Sella's classification, the terminal portions of the ovarioles showed a varying degree of follicular dilatation (Detinova, 1953c). Knowing that the contraction of the sac formed after the passage of the mature egg occurs gradually, I tried to use this difference to determine the time which had elapsed since oviposition. The condition of the terminal portions of the ovarioles and the time of dissection were noted. In addition the speed of contraction of these terminal sections was determined for wild females which had oviposited in the laboratory. The time of oviposition of each female and the time of her dissection were recorded. These data made it possible to keep under continuous observation the periods of contraction of the sacs and the times of completion of the process. If the condition of the ovarioles is examined in females caught at Sella's stages 1, 2 and 3 and if it is known in what condition they should be after a particular number of hours following oviposition, the approximate time between oviposition and dissection can be estimated.

FIG. 29. THE PROCESS OF CONTRACTION
OF OVARIOLES FROM WHICH A MATURE EGG HAS
BEEN PASSED



- A The terminal portion of an ovariole of the long-sac type such as are found in females which have just oviposited
- B Signs of contraction have appeared in the terminal portions of the ovariole
- C The terminal portions of the ovariole have noticeably contracted
- D Dilatations in the terminal portions of the ovariole formed after passage of the mature egg and of almost normal dimensions
- E Dilatations of normal dimensions

dissected in order to determine the rate of contraction of the ovarioles after oviposition. Observation of the condition of the ovarioles in these females showed that they usually retain their sac-like shape for 19-20 hours, after which the process of contraction speeds up considerably and normal-sized dilatations are found in the end portions of the ovarioles during the second 24-hour period.

The dissection of females given a blood-meal after oviposition showed that their ovarioles contracted at the same rate as those of empty females. It can thus be concluded that a blood-meal has no effect on the speed of this process.

Among all the empty females dissected, after capture in the day resting-places, 66% had the terminal portions of the ovarioles still in the sac form, indicating that oviposition had taken place within the past 24 hours. In the remaining 34% the dilatations were of normal or almost normal size, showing that the females had oviposited more than 24 hours before but for some reason had not taken a blood-meal.

In processing the collected material all the females were divided into three main groups with the following characteristics:

(1) the terminal portions of the ovarioles without any signs of contraction or with only the first signs of it (Fig. 29A, B);

(2) the terminal portions of the ovarioles noticeably contracted (Fig. 29C); and

(3) the dilatations in the end portion of the ovarioles of normal or almost normal dimensions (Fig. 29D, E).

In studying a Moscow Oblast population of *A. m. messeae*, the condition of the ovarioles was examined in 1669 females, of which 76 were in Sella's stage 1, 1245 in stage 2, and 348 in stage 3. Ninety-seven females which had oviposited in the laboratory were

In females dissected in Sella's stage 2 (i.e., freshly fed females), 52% showed sac-like ovarioles, 16% had noticeably contracted dilatations and 32% possessed dilatations which had contracted fully or almost fully. This last group was composed of females which remained empty during more than 24 hours after oviposition.

Of females dissected in Sella's stage 3, 23% had the ovarioles in the sac-like shape, which probably indicates that these females had taken a blood-meal shortly after oviposition.

Further observations on females in the south of the USSR (North Kirghizia) showed almost complete agreement with the results given for the Moscow Oblast as far as the condition of the terminal portions of the ovarioles in empty and freshly fed females was concerned. Among females in Sella's stage 3 dissected in the south, a large proportion had sac-like ovarioles and this can be explained by the difference in the speed of digestion of females at various temperatures. In the south the process of blood digestion (corresponding to the reaching of stage 3) progresses with considerably more speed and the ovarioles do not contract completely during that time.

The condition of the ovarioles was examined in 549 female *A. m. messeae*, of which 138 were dissected at a varying number of hours after oviposition in the laboratory, 229 were empty females caught in the day resting-places and 128 were in Sella's stage 2 and 54 in stage 3.

It is well known that the great majority of females oviposit either in the evening after sunset or in the morning during sunrise (Russell & Rao, 1942 a, b; Beklemishev, 1944). Having established that clear signs of contraction in the ovarioles are seen after 19-20 hours, it follows that among all the insects found in the morning with expanded ovarioles, those which had oviposited in the evening and those which had done so in the morning are equally represented.

The conclusion may be drawn, therefore, that about two-thirds of females take a new blood-meal either immediately after oviposition or seven to eight hours later. This last group comprises mosquitos which have oviposited in the evening and have taken a blood-meal in the early morning hours. The finding of females with considerably contracted terminal portions of the ovarioles shows that not all females that have oviposited in the morning take a blood-meal immediately; most of them do so only in the evening. The finding of females in which the dilatations in the ovarioles were of normal dimensions points to the fact that less than one-third of the females remain unfed for about 24 hours after oviposition. According to Gillies' observations (1953, 1954) female *A. gambiae* also take a blood-meal soon after oviposition.

Method of determining the duration of the third phase of the gonotrophic cycle

The available data on the duration of the third phase of the gonotrophic cycle, i.e., the time between completion of ovarian development and oviposition are very contradictory. Thus, Martini (1922a, b) indicated that anopheline females seek out suitable bodies of water and oviposit when only seven to ten hours have elapsed after the maturation of eggs. According to Sautet (1934) an *Anopheles* female after the completion of egg development flies from the feeding place to suitable bodies of water and begins to oviposit after one to three days.

The large amount of material collected (Beklemishev, 1944; Zviagintzev, 1946; Shmeleva, 1935; Rayevsky, 1940; Rakhmanova, 1940; Kiseleva, 1953) shows that females with mature eggs are often found in day resting-places with hosts nearby, although in comparatively small numbers.

These observations show that not all females that have completed ovarian development leave the resting-places immediately. In resting-places with no host nearby and in natural shelters the females in Sella's stage 7 are considerably more numerous. This also indicates that when they disperse from day resting-places not all females immediately fly away to a body of water to lay their eggs, but the available data are insufficient to judge the length of time before the beginning of oviposition. In laboratory conditions (Detinova (& Kopteva), 1936), females which have completed blood digestion and have alighted on water oviposit immediately.

The first attempt to solve the problem of the duration of the third phase of the gonotrophic cycle of *A. maculipennis* in natural conditions was made by Tairova (1957). The females caught in Sella's stage 6 were placed in a breeding-cage, and after they had passed into stage 7 were marked with dry methylene-blue powder and released into day resting-places. Subsequently, for the next seven days, all the insects in all premises in the settlement were caught and checked for the presence of the stain and at the same time for the determination of the stage of blood digestion. It was shown that during the first 24 hours after completion of ovarian development the majority of females not only leave the day resting-places but succeed in laying eggs, returning to the settlement and taking a blood-meal, i.e., starting a new gonotrophic cycle. However, in one of the settlements used for the experiment 27%, and in another 47%, of females remained for a further 24 hours in the day shelters after maturation of the eggs.

Similar observations were made by Kalmykov (1959) on *A. superpictus* in the Tadzhik SSR. Marked females with mature eggs released in the evening were caught the following morning in Sella's stage 2, i.e., when they had just taken a blood-meal after oviposition.

On the basis of all the material on the duration of the three phases of the gonotrophic cycle the following conclusions may be drawn:

(1) The duration of the second phase (digestion of the blood and the development of the ovaries) depends on the microclimatic conditions of the given environment. Not only can the duration of the process be observed, since the stages of blood digestion and ovarian development are clearly visible through the abdominal integument, but it can also be calculated.

(2) The first phase, from oviposition to a new blood-meal, takes, in most females, not more than seven to eight hours. In a considerably smaller portion of females it takes about 24 hours.

(3) The duration of the third phase, from maturation of eggs to oviposition, has been studied least of all but the data obtained by Tairova and Kalmykov show that in most females it does not exceed 24 hours.

(4) Since the majority of females take their blood-meal soon after oviposition, there is every reason to believe that usually the duration of the first and third phases taken together is 24 hours.

(5) The duration of each gonotrophic cycle for the majority of females depends on the duration of the second phase, which is determined every time, with the addition of the 24 hours necessary for the completion of the first and third phases. This assumption was made in all subsequent calculations. For a minority of the females, a longer period must be added, probably equal to 48 hours, but we have not introduced this figure into our calculations.

Method of Determining the Age of Potentially Dangerous Females

Female *Anopheles* are considered potentially dangerous when they have reached the physiological age at which a cycle of sporogony could already have taken place in their body if they had been infected during their first blood-meal.

To establish the physiological age of potentially dangerous females it is necessary to know two facts: (1) the duration of sporogony; and (2) the duration of each gonotrophic cycle during the same period. By comparing these figures we can find out how many gonotrophic cycles can be completed during a cycle of sporogony. In this way the initial age of potentially dangerous females is established. Naturally, all females which at the time are in physiologically older age groups are also included in the group of potentially dangerous females.

It is known that the duration of sporogony of the malaria parasite and of gonotrophic cycles of the *Anopheles* are not constant and are conditioned

by climatic factors. Beklemishev (1944) and Moshkovsky & Rashina (1951) studied the relationship in detail and found that it is variable; therefore, the initial physiological age of potentially dangerous females is not constant either. The younger the age of such females, the greater is the total number of potentially dangerous females and the more important the vectorial capacity of the local anopheline population.

To determine the age of potentially dangerous females for each day of the season from the time when temperatures occur at which sporogony is possible, the mean diurnal temperatures are recorded every day together with the proportion of the sporogonic cycle corresponding to those temperatures and the effective temperature for determining the duration of gonotrophic cycles. Thus the methods already described are used for the calculation of the duration of sporogony for each day of the season and of the duration of each gonotrophic cycle, 24 hours being added for the first and third phases. Then the number of gonotrophic cycles within a sporogonic cycle is determined. An example of the calculation is given in Table 7.

It follows from this example that during the sporogonic cycle at the given temperatures the females have completed four gonotrophic cycles and on this particular day their physiological age will be the initial potentially dangerous age.

The group of potentially dangerous females in autumn includes females which could have been infected before the beginning or at the beginning of the last cycle of sporogony, but the life of these females extends for a considerable time after the completion of the last cycle.

In further calculations, the total number of females, assessed for every day of their potentially dangerous age and their proportion among those dissected, is noted. In view of the fact that groups of females are dissected without any preliminary selection among those caught, we consider, as stated earlier, that the age composition of the dissected group corresponds approximately to the age composition of the whole population on a given day.

Hence the proportion of all females at a potentially dangerous age among those dissected on a particular day corresponds to their proportion among all the females which have flown into the settlement on that day.

Determination of Absolute Numbers of Female *Anopheles* of Potentially Dangerous Age

An evaluation of the epidemiological effectiveness of a vector population throughout the season in any particular locality requires the calculation, not of the proportion of females at a potentially dangerous age, but of the absolute numbers of such females flying into the locality. The absolute numbers of potentially dangerous females determine the epidemi-

TABLE 7. METHOD OF DETERMINING THE AGE
OF POTENTIALLY DANGEROUS FEMALES

Date	Mean diurnal temperature in °C	Proportion of sporogony taking place per day as a percentage of the completed process	Difference between the mean diurnal temperature and the threshold temperature ($= 9.9^{\circ}\text{C}$) during the completion of gonotrophic cycles	Notes on the corresponding gonotrophic cycles
4 June	22.1	6.06	12.2	
5 "	23.0	8.0	13.1	
6 "	23.6	8.33	13.7	
			39.0	Completion of phase 2
7 "	22.7	7.7	—	Time taken for phase 3 and phase 1
8 "	21.7	6.45	11.8	
9 "	19.8	4.88	9.9	
10 "	13.5	—	3.6	
11 "	15.1	—	5.2	
12 "	17.4	3.03	7.5	
			38.0	Completion of phase 2
13 "	18.1	3.5	—	Time taken for phase 3 and phase 1
14 "	22.7	7.7	12.8	
15 "	21.8	6.45	11.9	
16 "	22.1	6.9	12.2	
			36.9	Completion of phase 2
17 "	22.4	7.14	—	Time taken for phase 3 and phase 1
18 "	21.9	6.66	12.0	
19 "	21.8	6.45	11.9	
20 "	20.4	5.55	10.5	
21 "	20.4	5.55	10.5	
	100.35		44.9	Completion of phase 2
22 "	20.3	5.4	—	Time taken for phase 3 and phase 1

logical importance of vector populations. Where the proportion of dangerous females is high and the total population is low, its epidemiological effectiveness is considerably smaller than where the respective proportion is low but total numbers are high.

In view of the large numbers of mosquitos flying into settlements, because of the way they are distributed among various types of indoor

resting-place and on account of their flight to resting-places outside the settlement, the absolute size of the population cannot be determined. One usually estimates the changes in the size of the population by the number of mosquitos caught in indoor capture stations. It is assumed that the number of mosquitos collected reflects reliably the actual size of the population. The mean number of potentially dangerous females coming into the capture station is determined on the basis of the average numbers of mosquitos in those indoor resting-places and the proportion of potentially dangerous females among those dissected.

Methods of Determining the Degree of Contact Between *Anopheles* and Man

The degree of contact between mosquitos and man depends on a number of factors, of which the following are of the greatest importance: the relative size of the mosquito population (average number of mosquitos per man), the number of mosquitos at a dangerous age among the attackers, the total number of cattle in the settlement concerned, the way the cattle are kept, the disposition of dwellings in the settlement, the degree of accessibility of premises used by human beings and cattle for mosquitos flying in. Rayevsky (1940), Beklemishev (1944) and others demonstrated a direct relationship between the number of cattle in the settlements and the number of mosquitos attacking man. The greater the number of cattle the smaller is the proportion of mosquitos attacking man. The degree of contact of mosquitos with man is determined by the proportion drinking his blood.

The species to which the blood in the mosquito's stomach belongs is determined by two methods. The first is the precipitation method, usually used as modified by Rice & Barber (1935) (see also Demina & Zvyagintsev, 1936). By means of the precipitation reaction, where a set of sera is available, the proportion of mosquitos taking a meal from various animal species is determined. However, in assessing the epidemiological danger of a population it is essential to determine only that proportion of females with human blood in their stomachs. Only human blood sera need therefore be used.

The second method is that of agglutination. It was introduced into malariological practice by Vlasenko (1956). The method is easy of application under practical conditions and makes it possible to determine the proportion of mosquitos which have fed on man at the time when the females are being dissected. The blood from the abdomen of the dissected female is smeared on to an object glass and triturated in a small drop of saline, with the subsequent addition of serum of group IV human blood. If the stomach of the female contains the blood of any animal then this will quickly coagulate and form flakes. If human blood is present in the stomach of the female, no coagulation takes place.

**The Epidemiological Effectiveness of Populations of
Anopheles maculipennis
in Various Geographical and Climatic Conditions in the USSR**

The data obtained on the age composition of populations studied in various geographical and climatic zones allow us to estimate the epidemiological effectiveness of individual populations and the reasons for similarities and dissimilarities between them.

The epidemiological importance of overwintered *Anopheles maculipennis messeae* females

Until comparatively recently the epidemiological role of overwintered females was assessed by:

(1) the occurrence among infants (in winter or early spring) of fresh cases of malaria contracted before the females of the summer generations could possibly transmit the infection; and

(2) the finding of overwintered females with sporozoites in their salivary glands.

In view of the difficulty of finding infected mosquitos, even when the incidence of malaria is comparatively high, the chances of determining the proportion infected among overwintered females are not sufficiently great, and the absence of infected mosquitos among those dissected does not mean that the overwintered females have no epidemiological significance. The actual absence of any epidemiological danger from this group of females could be postulated if a very large number were dissected at a time when, according to calculations, females with sporozoites could already be found among them and when the actual probability of infection is very great. However, nobody has carried out special investigations on this point.

To determine the epidemiological effectiveness of overwintered females of various populations the following factors were used as a basis for analysis:

- (1) the date of possible completion of the first cycle of sporogony;
- (2) the date of the actual finding of the last overwintered females;
- (3) the total number of overwintered females surviving to the day of the possible completion of sporogony and after;
- (4) the actual survival of females to a potentially dangerous age and their absolute numbers in indoor resting-places during the whole period.

Analysis of all the available material showed that during the years of observation in the overwhelming majority of vector populations in completely different climatic conditions in the USSR (in a number of places in the forest zone, in Central Asia, in the south and in Siberia) overwintered females could not have any epidemiological importance. In all these cases the females perished before the possible completion of sporogony. The physiological age of females was high in a number of places (up to nine to eleven ovipositions); this indicates favourable climatic conditions for the vector itself, although for the malaria parasite they were already unfavourable.

In other centres of mosquito population, mainly in the forest zone, a proportion of the overwintered females survived until the end of possible spòrogony. Their importance was determined in each case by the total numbers of females surviving to a potentially dangerous age and by the length of time during which such females were encountered among those that attempted to feed.

In the majority of cases the epidemiological effectiveness of this portion of the population is limited, because of their very low numbers; this also explains the almost complete absence of contact between man and these dangerous mosquitos.

Only in extremely rare cases was it possible to consider that overwintered females could, for a short period of time, be of epidemiological importance. Thus, for example, in Moldavia, when conditions in the Spring of 1949 were very favourable, overwintered females became potentially dangerous when they had completed four to five gonotrophic cycles, and a comparatively large proportion of these females survived to that age by the end of May.

The epidemiological importance of female *Anopheles maculipennis messeae* of the summer generations

To compare the epidemiological importance of summer females the same populations were used in the main as those examined to determine similarities and differences in age composition (see Chapter 7). The data obtained cannot be considered to be exhaustive for the epidemiological assessment of populations of *A. m. messeae* throughout its distribution area. They make it possible to assess merely the importance of individual populations of the species from places in which its living conditions are not identical.

The age composition of the females in a number of cases makes it possible to assess more accurately the time of the possible beginning and end of the season of mosquito infection and of the possible infection of human beings with malaria, and to establish the duration of the whole malaria transmission season.

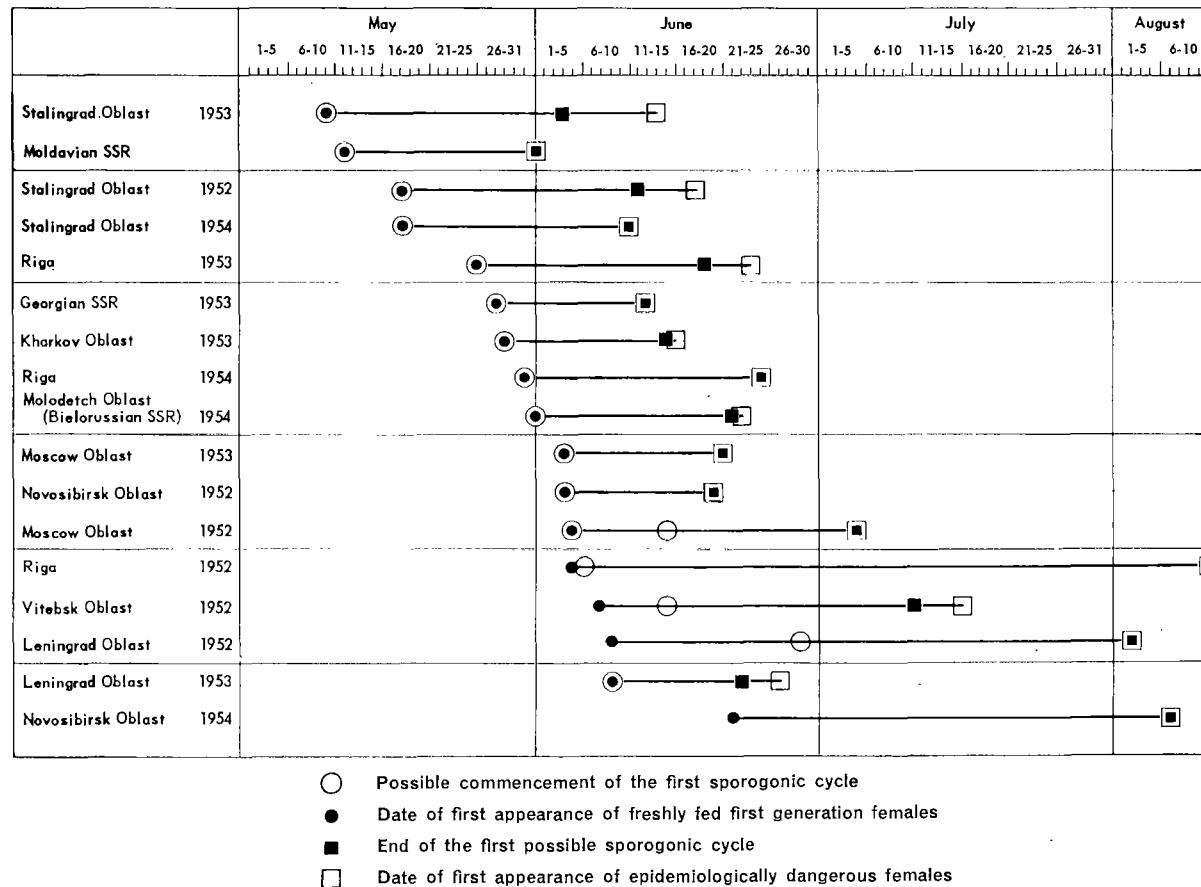
The beginning of the season of possible infection of mosquitos and the season of human infection with malaria

As already stated, the beginning of the mosquito infection season is taken to be the date on which mean diurnal temperatures reach 16°C and above, provided that females of the first generation are present in nature. An analysis of all the data on the periods of possible first infection of summer females among populations studied in various geographical and climatic conditions has shown that there is a time lag of one month between the two factors mentioned above (Fig. 30).

It is well known that the development of mosquitos and the completion of gonotrophic cycles are possible at temperatures below the threshold of sporogony (16°C) and therefore in a number of places there is a lack of agreement between the date of flight of individuals of the first generation and the possible date of the first sporogony. The data given in Table 2 show that in the overwhelming majority of the populations examined the temperatures at the first flight of summer females are favourable for the commencement of sporogony. In all these cases we assume that the beginning of the transmission season coincides with the flight of the first summer females. In rare instances (e.g., in the Leningrad Oblast in 1952, the Vitebsk Oblast in the same year and in some other regions) the temperatures favourable for sporogony occurred after seven to twenty days from the time of flight of the first generation. In such cases, even where the risk of infection is very high, it can only occur during the second blood-meal.

The beginning of the malaria transmission season occurred in the great majority of cases during the second half of June. The duration of the cycle of sporogony, which determines the interval between the season of mosquito infection and the season of human infection, differed very considerably not only in the various parts of the habitat of the species but also in a given locality in different years, this being due to weather conditions at the time. In a number of places, despite the early onset of the possible infection of mosquitos, the sporogony cycle took longer and therefore the females became dangerous at the same period as in other vector populations, where the sporogony had taken less time even though it began considerably later. Thus, for example, in the steppe zone, the beginning of the sporogony cycle in 1953-54 was on 16 May, but in fact females of a potentially dangerous age did not appear until 20-25 June. In the forest zone, the sporogony cycle in many places began on 4-8 June but potentially dangerous females were being discovered in the last third of June. Among all the populations examined the shortest first possible cycle of sporogony—16 days—was found in the Moscow Oblast in 1952. The longest was found in Riga in 1952 and lasted 66 days; it finished only on 10 August.

FIG. 30. COMMENCEMENT OF THE SEASON OF POSSIBLE INFECTION OF MOSQUITOS AND TRANSMISSION SEASON OF MALARIA
IN VARIOUS TOPOGRAPHICAL AND GEOGRAPHICAL CONDITIONS OF THE USSR



As will be seen from Table 2, in the overwhelming majority of populations, despite a considerable difference in the duration of the first possible cycle of sporogony, females managed to complete as many as four or five ovipositions. Only in rarer cases did this age correspond to that of females which had oviposited a greater number of times. In summer the difference in the initial age of potentially dangerous females among vector populations in various areas was considerably greater.

As will be seen from Fig. 30, it is not always possible to determine the beginning of the season for human infection from the dates of possible completion of sporogony. In a number of instances, e.g., in the Stalingrad Oblast in the 1952 and 1953 seasons, in Riga in 1953 and in other places, at the time of possible completion of sporogony the populations contained no potentially dangerous females. This may have been due to increased mortality of individuals as a result of unfavourable weather conditions. Thus a population takes on epidemiological importance from the time when individuals at a potentially dangerous age begin to appear among it, and when the total numbers in the population become high, thus ensuring contact between mosquitos and man.

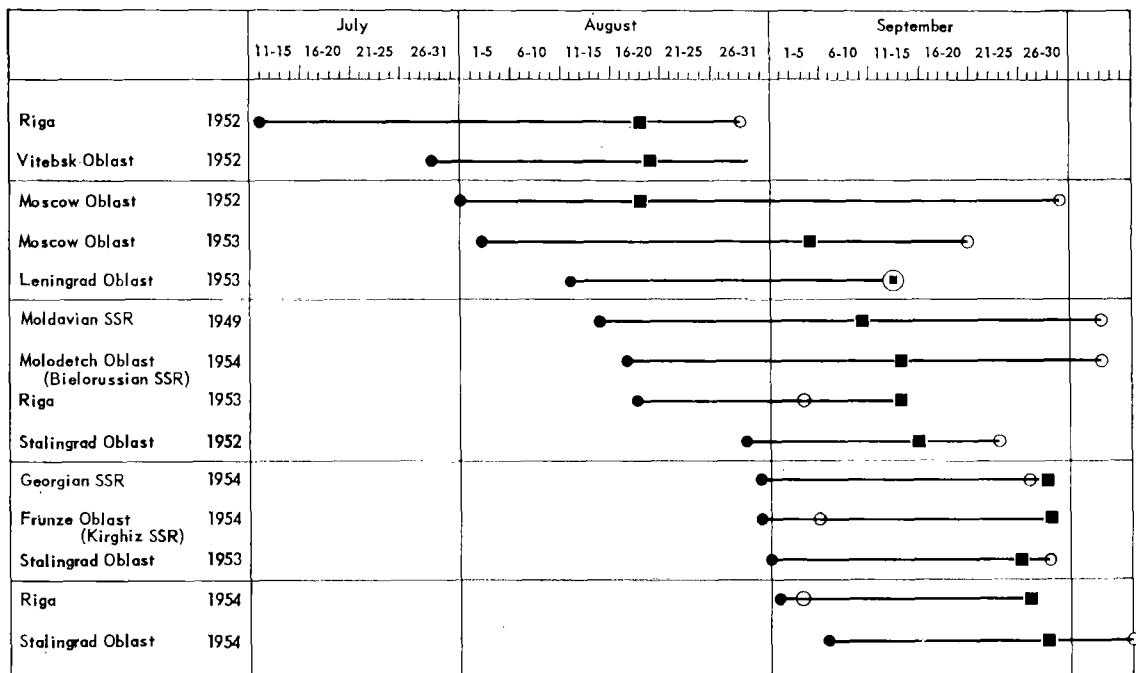
The end of the season of possible mosquito infection and of human infection with malaria

The different duration of the whole period during which malaria transmission was possible in the various climatic zones is shown in Fig. 31.

In the overwhelming majority of populations the calculated dates of completion of sporogony did not correspond to the actual finding of the last potentially dangerous females. In some populations females of a potentially dangerous age, in which sporogony could have been completed before the onset of lower temperatures, continued to be found during a number of days when new sporogony cycles could not have taken place. In other instances, on the contrary, all the potentially dangerous females perished before the date of the possible completion of sporogony in that year. Thus the data on age composition establish with adequate accuracy the actual duration of the malaria transmission season in varied climatic conditions. In the absence of these data, when only the temperatures at which sporogony is possible are taken into account, an assessment of the duration of the transmission season is often misleading. In some cases an unduly short, and in others an unduly long, period will be estimated by this method.

We discovered that the epidemiological effectiveness of individual populations differs not only among females living in different climatic conditions but also in the same locality in different years. This is due to the changes in weather conditions during the season of the potential malaria transmission.

FIG. 31. TIME OF POSSIBLE ENDING OF TRANSMISSION SEASON IN VARIOUS TOPOGRAPHICAL AND GEOGRAPHICAL CONDITIONS OF THE USSR



- Possible commencement of the last sporogonic cycle
- End of the last possible sporogonic cycle
- Date of finding of the last epidemiologically dangerous females

Comparison of the epidemiological dangerousness of an *Anopheles maculipennis* population in the Moscow Oblast in two consecutive seasons

Data obtained in the Moscow Oblast in 1952 will serve as a good example. In that year the first possible cycle of sporogony could have started (at the temperature observed in the indoor resting-places) on 14 June and could have finished on 4 July. At this time the whole vector population consisted only of summer females. The last cycle of sporogony could have lasted from 31 July to 18 August, despite the fact that during the whole of the first half of August temperatures in the indoor resting-places remained favourable for sporogony and mosquitos could have been infected. Nevertheless, the sporogonic cycle could not have been completed, because of the cold weather in the second half of the month. The duration of the possible sporogony during the season was not constant and fluctuated between 17 and 28 days. The initial age of potentially dangerous females in July and August was almost constant at four gonotrophic cycles and only on single days did it equal five cycles. From the last days of August onwards the physiological age of females gradually increased and by the end of the second decade of September it was equal to eight gonotrophic cycles.

Weather conditions during that period were favourable both for the vector and for the parasite so that not only was the proportion of physiologically old females high, but the proportion of potentially dangerous females among them was also high. Thus in July the proportion of potentially dangerous females among all those dissected on single days varied between 18% and 49%, in August between 17% and 66%, and in the first half of September it was about 20%. The females found in September could have been infected at the end of July. Thus the life-span of these females was considerably greater than the duration of sporogony.

In view of the fact that the influx of mosquitos into the settlement was not large and did not exceed 160 per capture station, although there was a high proportion of potentially dangerous females among those dissected, their absolute number at various periods during the season amounted only to 2-30 per capture station per day.

The spring-summer period of 1953 was characterized by higher temperatures, as a result of which the time of possible infection of the first generation females coincided with the beginning of their first blood-meal. The first cycle of sporogony could have begun on 3 June and finished on 20-22 June, i.e., a fortnight earlier than in the previous season. The last cycle of sporogony could have begun on 2 August and finished only on 4 September. The duration of the sporogonic cycle varied from 18 to 34 days.

The proportion of potentially dangerous females among those dissected during the whole season changed considerably and was in general lower

than in the previous season, this being explained by higher mortality. The age of potentially dangerous females during the whole summer period was the same as in the previous season and corresponded to four or five gonotrophic cycles. In the last days of June the proportion of potentially dangerous females among those dissected varied from 0.9% to 17.5%; in July, from 2.4% to 32.5%; and in August, from 26% to 40%—the highest level, which was maintained through September. Despite the fact that the last cycle of sporogony ended on 4 September, the season of malaria transmission lasted until the middle of September, when potentially dangerous females were still being found. Since the numbers of mosquitos in the capture stations were, as in the previous year, comparatively small, the absolute number of potentially dangerous females was relatively low, varying from 1 to 26 per indoor resting-place.

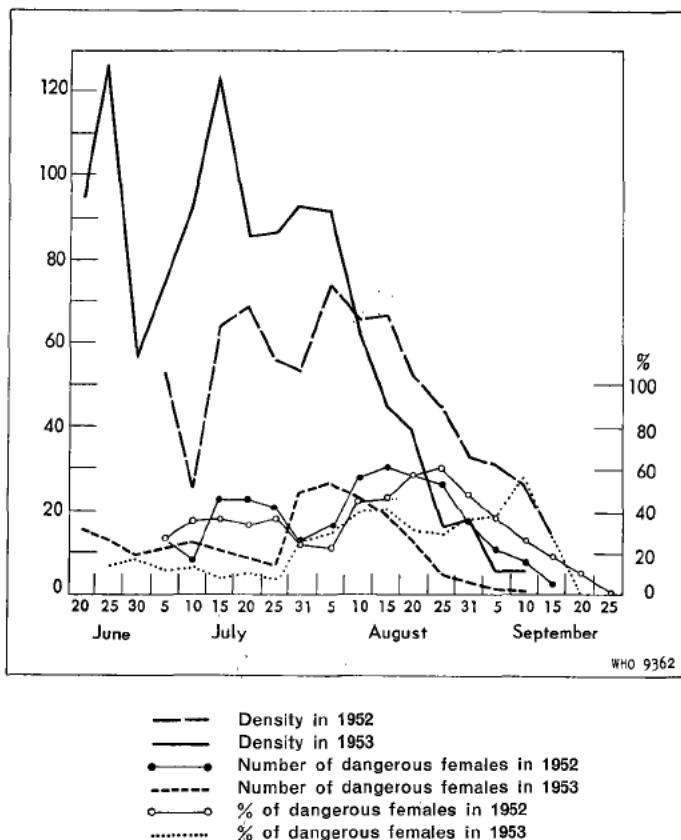
Taking into account the fact that during both seasons potentially dangerous females made up a large proportion of all biting females, it is obvious that had there been any human gametocyte carriers the epidemiological effectiveness of the vector population would have been quite high. Comparative data for the two seasons in the Moscow Oblast are given in Fig. 32, which shows that the number of potentially dangerous females in the indoor resting-places was higher in 1952, whereas the total number of females in those places was higher in 1953.

I am not able to give here a detailed analysis of the data used for evaluating the epidemiological effectiveness of all the populations studied on the basis of the age composition of the females. I shall limit myself to mentioning some general points relating to this problem and shall give a few examples.

First of all it must be said that the temperature has a many-sided effect on the possibility of malaria transmission. The temperature determines the speed of completion of sporogony during the whole malaria transmission season, the speed of completion of gonotrophic cycles and consequently the age of potentially dangerous females. It also influences the mortality among females and hence the size of the portion of the population capable of surviving to an epidemiologically important age.

If one considers the vector populations which live in different climatic conditions of the species habitat, the survival rate in each gonotrophic cycle will increase until the zone is reached with an optimum temperature, after which as temperatures increase the survival rate will considerably decrease. The effect of temperature on the duration of sporogony and on the number of gonotrophic cycles completed within one sporogonic cycle changes within the considered temperature limits in the same direction, i.e., the duration of sporogony decreases from lower temperatures to higher and the number of gonotrophic cycles completed during one sporogonic cycle also decreases. The epidemiological effectiveness of a vector population, other conditions being equal, will be highest in those

FIG. 32. RELATIVE EPIDEMIOLOGICAL EFFECTIVENESS
OF THE POPULATION OF VECTORS IN 1952-53 (MOSCOW OBLAST)



places where the total proportional mortality of females during the sporogonic cycle is lowest. This is true in cases when minimum mortality in each gonotrophic cycle coincides with the minimum number of gonotrophic cycles completed during one sporogonic cycle.

To illustrate this, we might consider briefly three vector populations in completely different climatic conditions:

(1) In Riga in 1952 the temperatures in spring and summer were low, so that the first possible cycle of sporogony in the bodies of summer females, according to Polikarpova's data (1957), could have been completed only by 10 August. To become dangerous the females would have had to complete no fewer than eight gonotrophic cycles during this time. In actual fact only a negligibly small proportion of the females had completed as many as seven cycles. The gradual aging of the females and the possibility of

their survival, although in very small numbers, to a considerable physiological age, shows that conditions for mosquito life were still favourable but these same conditions proved unfavourable for the causative agent of malaria.

Such years occur fairly often in these places, although they alternate with warmer seasons in which malaria transmission is possible. This was confirmed by the material obtained in the two subsequent seasons.

(2) In the Moscow Oblast both seasons (1952 and 1953) in which the population was studied were quite warm, and as a result on the great majority of days four gonotrophic cycles took place for one cycle of sporogony. Mortality of females at subsequent ages was comparatively low and a considerable proportion survived to a potentially dangerous age. The high proportion of females at a potentially dangerous age shows the existence of completely favourable conditions for the life of the vector and for the causative agent of the disease.

(3) In the Moldavian SSR in the 1949 season the temperatures in July and August were constantly high and ensured the swift completion of sporogony and of gonotrophic cycles. As a result the age of potentially dangerous females at this time corresponded to that of a female which had oviposited three times. Mortality among females at the existing temperatures, which were above the optimum for the life of *A. m. messeae*, caused a reduction in the total proportion of females at a potentially dangerous age, because fewer survived to physiological old age. The very high numbers of mosquitos, coupled with a comparatively small proportion of mosquitos surviving to a dangerous age, nevertheless gave great epidemiological importance to the population, compared with those living under other conditions. In general these conditions were completely favourable for the development of the sexual cycle of the causative agent of malaria but caused a higher mortality among *A. m. messeae*.

Thus, other things being equal, the vector population in the Moscow Oblast was of the greatest epidemiological importance in the years mentioned. This leads to the conclusion that in the southern limits of their habitat the existence of a moderately heat-loving species such as *A. maculipennis* cannot ensure the intensity of malaria transmission that might have been expected, even though the environmental temperature is favourable for the reproductive cycle of the causative agent.

Naturally, it would be wrong to conclude that in southern areas of the USSR the environment is less favourable for contracting malaria. The southern limits of the habitat of each species of *Anopheles* borders on the habitat of another vector species, of which the mortality at those temperatures is lower, and consequently the percentage of females surviving to a dangerous age is higher. In the USSR thermophilic species consist

mainly of *A. superpictus*, *A. pulcherrimus* and the subspecies of *A. maculipennis*: *A. m. sacharovi*, *A. m. subalpinus*, and partly *A. m. maculipennis*. Since the prevailing conditions, with high temperatures, are favourable for these thermophilic species the total numbers in the mosquito population are high and malaria can always easily be transmitted.

Chapter 9

THE AGE COMPOSITION OF FEMALE *ANOPHELES* AS A CRITERION IN ESTIMATING THE EFFECTIVENESS OF ANTIMOSQUITO MEASURES

Since the beginning of the 20th century a great deal of attention has been paid throughout the world to the control of malaria vectors. As data have been accumulating on the biology, ecology and physiology of *Anopheles* in all phases of their development, it has become necessary to plan mosquito control measures as correctly as possible, to establish their proper timing, and to find improved methods of entomological evaluation of the effectiveness of the applied methods.

There is no need to review all the stages of the extensive research work carried out in all parts of the world. I shall mention only the methods of applying contact insecticides such as DDT and BHC in malaria control and the entomological and epidemiological evaluation procedures of malaria vectors. The successes gained in malaria control were due mainly to the contact insecticides with a long residual action. DDT preparations were first used for malaria control in 1944, as mentioned by Trapido (1946) and Aitken (1946). In subsequent years this insecticide was widely used, not only for the control of malaria vectors, but also for the control of vectors of other diseases. During the early years of the use of DDT in malarial control, many research workers (Aitken, 1946; Ribbands, 1947; Hinman & Cutcomb, 1947; Davidson, 1947, 1953; Nabokov & Nikiforova, 1947, 1948; Vinogradskaya, Nabokov & Shmeleva, 1949; Rageau, Adam & Rivola, 1953; Pozdeev, 1954) showed that it is effective even after a single spraying.

It was also reported (Downs, Colorado & Gahan, 1948; Nikiforova, 1949; Chinaev, 1952; Trapido & Aitken, 1953; Prokopenko, 1960) that in some conditions a single spraying of the residual insecticide can be effective for one to three years. At the same time there were indications (Muirhead-Thomson, 1951) of the failure of DDT preparations to give good results in malaria control.

Failures in such cases may be due to the species composition of the local vector population. Where the vectors belong to exophilic species

which do not, or only rarely, fly into human habitations, the treatment of houses can never give good results in malaria control. In working out the tactical use of residual insecticides, account must always be taken of the species composition and ecological characteristics of the vectors. During the last few years many papers have been published demonstrating the phenomenon of resistance in mosquitos to preparations of the chlorinated hydrocarbon type. However, the lack of success in control of the vector by means of these preparations cannot always be ascribed to insect resistance. Often unsuccessful spraying is due to the species composition of the malaria vectors and their way of life. In the control of endophilic species, better results can be obtained when all internal surfaces of the dwellings are treated and when stabled cattle are also treated. In the control of exophilic mosquitos which fly into the settlement only to take a blood-meal, the best results can be obtained by treating the places where most of the mosquitos accumulate in natural shelters in the vicinity of the human settlement (Fastovskaya & Kalmykov, 1956) and by treating cattle, which may attract mosquitos both outside and inside the village. To assess the effectiveness of these measures it was necessary to work out methods which would enable a constant check to be carried out on the quality of the control measures. The best criterion for judging the success of these measures is the age composition of the female vectors. Extensive work on the control of *Anopheles*, *Phlebotomus* (Dolmatova, 1949a,b; Dolmatova & Okulov, 1950, 1951a,b; Dolmatova, Kuvitchinsky & Leibman, 1953) and the house-fly *Musca domestica* (Lineva, 1950) showed that it was possible to use the data on the age composition of female insects not only for the evaluation of a particular measure, but also for making all the necessary corrections where errors had been made in the spraying procedures. In all cases those surfaces should be treated which will ensure constant contact of the insect, taking into account the biological features of each species of *Anopheles*.

It was pointed out by Beklemishev (1947, 1957b) that even the most intensive use of DDT does not lead to the immediate eradication of the vector population. DDT treatment only increases by many times the number of females dying during each gonotrophic cycle. A numerical decrease of mainly older females is due to their increased chance of alighting on a treated surface in the course of each gonotrophic cycle. At the present time it is universally believed (Beklemishev, 1947; Sergiev, 1953; Gabaldon, 1953; Pampana, 1954; Garrett-Jones, 1959) that DDT can be used for the complete eradication of malaria without the need for eradicating the vector, but on condition that all females are eliminated before they reach an epidemiologically dangerous age.

At the present moment, several methods of using DDT and BHC preparations have been worked out for the control of malaria vectors (the complete coverage of all localities, barrier spraying, selective spraying

and the spraying of stabled cattle). The choice of the method depends in every case on the amount of malaria among the human population and on the number and distribution of the main breeding-places of the vector; in addition to that, the layout of the settlement, the nearness of other habitations and of untreated places, the species composition of the vector population, features of their biology and ecology are equally important. The methods of entomological assessment change according to the method of treatment.

The effectiveness of any malaria control measures in the USSR is always assessed by what is known as the three-stage system. The first stage is to assess the direct effect of the measure concerned on the vector, the second is to determine the effectiveness of the measure against the vector population as a whole, and the third stage is to assess measures on an epidemiological basis, i.e., on the basis of a reduction in the incidence of malaria.

In regard to assessing the effectiveness of malaria control measures using products of the DDT type, Beklemishev (1947) suggests a system consisting of the following steps:

- (1) Direct estimation of the effectiveness of the treatment: (*a*) checking of the absence of live mosquitos during the daytime in sprayed premises, and (*b*) checking that all premises have been sprayed.
- (2) Confirmatory entomological assessment (assessment of the general effectiveness of spraying): (*a*) measurement of the abundance of larvae in bodies of water, and (*b*) determination of the age composition of the adult population, if only on the basis of the percentage of nulliparous females.
- (3) Epidemiological assessment of effectiveness—counting the number of fresh cases of malaria in the community to be protected.

The checking of the effectiveness of control measures by means of this three-stage system makes it possible in good time to discover and eliminate any errors committed during the work, to make a correct estimate of the quality of the work and to decide whether the aim of the vector control campaign has been achieved and whether the necessary epidemiological effect has been attained.

Complete Spraying Coverage and Entomological Evaluation of its Results

Complete coverage of a settlement with contact insecticides is necessary wherever the incidence of malaria among the population is high and where important movements of the population are combined with the presence of malaria cases in the area, with high vector densities and with the influx

of mosquitos into all parts of the settlement. Complete coverage can also be recommended in small settlements surrounded by large *Anopheles* breeding-areas.

The main purpose of this method of spraying is to increase the mortality rate of the vector in each gonotrophic cycle to such an extent that the numbers of females surviving to the age when sporozoites can appear in their salivary glands fall to negligible proportions. In view of this, the main method of evaluating the effectiveness of complete coverage is to keep a record of the density of the mosquito population and to determine the age composition of the females caught in the settlement to be protected. In field trials the results are compared with the age composition and density of the vector population in a settlement where no measures against mosquitos have been taken.

Despite the tremendous mortality of mosquitos with the method of complete coverage, nobody has yet achieved an eradication of the whole vector population. This is due to the fact that a considerable portion of the physiologically young females remain in natural shelters and because even inside the settlements there exist various small and safe hiding-places in which the females can complete their ovarian development. Because of this the vector population is sustained at a certain, often very low, level. Even when mosquitos were apparently not to be found in settlements during a complete coverage, their presence has been proved by means of artificial traps (Gahan & Lindquist, 1945; Hinman & Cutcomb, 1947; Fletcher & Krause, 1948; Adam, 1956) or by means of "inlet and outlet" mosquito traps (Detinova, 1948; Vinogradskaya, Nabokov & Shmeleva, 1949; Nikiforova, 1949; Markovich, Prokopenko & Ivanova, 1949; Buyanova & Kekhcher, 1960; Prokopenko, Shmeleva & Timrot, 1960), or by their discovery in premises left unsprayed for control purposes in a sprayed area (Markovich, Prokopenko & Ivanova, 1949; Speranskaya, 1952; Buyanova & Kekhcher, 1960; Ivanova, Fastovskaya & Farber, 1960, and others), or finally by the discovery of mosquitos in resting-places in nature (Thomson, 1947; Georgopoulos, 1951; Farid, 1954; D'Alessandro, 1956, and many others) and the discovery of larvae in bodies of water near the sprayed settlements (Fletcher & Krause, 1948; Nabokov & Nikiforova, 1948; Rageau, Adam & Rivola, 1953).

Before evaluating the results of the complete coverage of settlements from the data on age composition of vectors, I wish to deal briefly with a number of entomological factors on the basis of which one can judge of the effect of the insecticide and study the behaviour of mosquitos in conditions where they cannot avoid contact with a toxic surface. As was first pointed out by Kennedy (1947), mosquitos which fly into treated premises and come into contact with a toxic surface do not as a rule receive an immediately lethal dose of the poison. When *Anopheles* receive a sublethal dose they become strongly excited and try to fly to a light-coloured

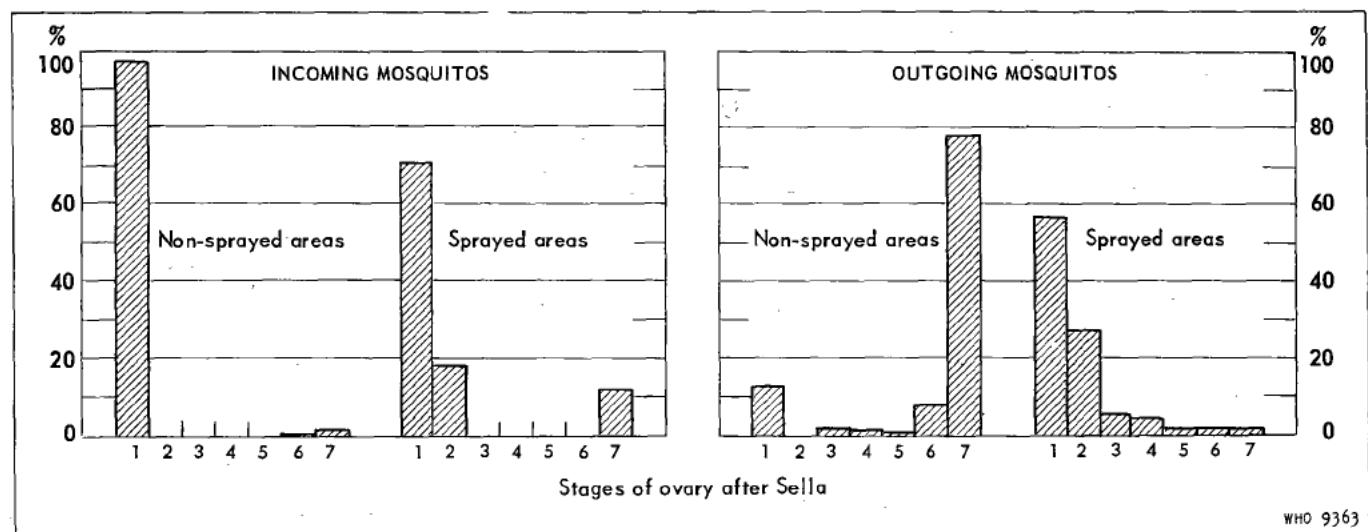
surface. During the hours when mosquitos are active, such surfaces are presented by open doors and windows. The affected mosquitos which fly out of the premises meet with various fates. If they fly into treated premises and settle again on a toxic surface, thus receiving an additional dose of poison, they cannot, in all probability, avoid death. If by chance they find a resting-place which has not been treated, then some of the females, after going through a period of paresis, remain alive and complete their gonotrophic cycle. Kennedy's data have been confirmed by a number of research workers in the USSR (Detinova, 1948; Nikiforova, 1949; Vinogradskaya, Nabokov & Shmeleva, 1949) for *Anopheles* populations studied in various geographical conditions.

Considerable difference has been noted in the composition of females according to stages of blood digestion in settlements treated with DDT and in untreated settlements. It is known (Vinogradskaya & Shmeleva, 1934; Shmeleva, 1935; Markovich, 1939; Shlenova, 1940) that in untreated settlements the majority of females flying away from their resting-places already contain mature eggs; freshly blood-fed females are very rare among them. On the other hand, among those flying into the houses there is always a preponderance of empty females in search of a blood-meal.

In settlements with complete coverage most of the mosquitos flying out are those that have just taken a blood-meal and also empty females. This is clearly due to the action of the DDT on the mosquitos. If an empty female flying into a treated room comes into contact with a toxic surface before it takes a blood-meal then, as was stated before, it is irritated and flies out, without having a blood-meal. Females which, on flying into treated premises, attack their host, have a blood-meal and only after that settle on a treated surface, fly out when freshly fed because of the irritant action of the toxicant. The stage of the gonotrophic cycle of mosquitos flying into inlet mosquito traps differs in untreated settlements. In this case there is a considerable proportion of freshly fed females, consisting mainly of females that have already been in treated rooms and are seeking new resting-places, as a result of the irritation (actual observations have shown that many of them show signs of poisoning). The flight of some mosquitos in treated settlements to new resting-places was observed by Ribbands (1947). He showed that in settlements where only some of the premises had been treated, dead mosquitos were also found in untreated premises, which indicates that they must have flown there after having been poisoned. Comparative data on the gonotrophic-stage composition of mosquitos in traps in treated (Detinova, 1948) and untreated (Shmeleva, 1935) settlements are given in Fig. 33.

It follows that the composition of samples of females leaving or entering treated premises can be used in evaluating the toxicity of the treated surfaces. The presence of empty and freshly fed females among those flying

FIG. 33. COMPOSITION OF INCOMING AND OUTGOING MOSQUITOS IN AN UNSPRAYED VILLAGE *
AND SPRAYED VILLAGE **



* After Shmeleva (1935).
** After Detinova (1948).

out shows that the treated surfaces have a strong toxic action. On the other hand, the finding of females in Sella's stages 6 and 7 points to the absence of, or only slight, toxic effect of the treated surfaces, or else to the existence of some indoor resting-places safe for mosquitos. Where mosquito traps are not used, the evaluation may be based on the stages of blood digestion among the collected females. As was pointed out by Buyanova & Kekhcher (1960), Kekhcher & Markovich (1960) and Prokopenko, Shmeleva & Timrot (1960), the finding in the early morning hours of freshly fed females in treated premises does not always mean that the insecticide has no toxic action. The females could have been those that had only just flown in and had not yet been poisoned. On the other hand, the finding on the same walls of females in the intermediate stages of blood digestion points to the probability of their having completed the whole cycle of ovarian development, either in the indoor resting-place where they were found, or somewhere else in the settlement. These conclusions must be drawn particularly if resting females are discovered during the daytime, when the flight between houses practically ceases. The finding of mosquitos for a second time on treated surfaces shows that the spraying must be repeated (Prokopenko, Shmeleva & Timrot, 1960; Buyanova & Kekhcher, 1960).

The assessment of mosquito density is one of the important indicators of the effectiveness of complete spraying. There is, until now, no standard method of calculating the numbers of mosquitos in a treated settlement. The difficulty lies in the fact that when the toxicant acts strongly the mosquitos cannot remain on the treated surfaces, and it is impossible to establish the actual numbers of females flying into the settlement by examining the treated indoor resting-places. Several research workers (Markovich, Prokopenko & Ivanova, 1949; Speranskaya, 1952, and many others) have established the numbers of *Anopheles* in treated settlements from the numbers flying into untreated indoor capture stations in which all mosquitos were systematically collected.

Analysis of the data led Markovich, Prokopenko & Ivanova (1949) to the conclusion that the untreated capture stations gave an exaggerated idea of the numbers of mosquitos in the settlement, since in these places mosquitos crowd together under the irritant influence of the DDT from treated premises. Moreover, normal females may also enter these capture stations direct after flying in from their natural shelters. Beklemishev (1949) showed that it is impossible to equate the number of mosquitos in a few untreated premises with the total number of mosquitos in a settlement, since a considerable proportion flies away from the treated premises under the effect of the toxicant and hides in various inaccessible shelters. Relatively reliable data on the numbers of mosquitos in a locality can be obtained by means of mosquito traps set in varied types of indoor resting-place situated in different parts of the settlement. However, Beklemishev

(1949, 1957a) pointed out that even these methods give an exaggerated idea of the numbers of mosquitos in a treated settlement, particularly because of the increased movements of the females irritated by the insecticide.

One of the criteria for evaluating the effect of complete coverage on the vector population in a locality is the number of anopheline larvae and the radius of distribution of such larvae in bodies of water around the settlement. However, this method is applicable only in the case of an isolated locality in the vicinity of a large treated area.

Undoubtedly the best criterion for judging the effectiveness of malaria control measures is the age composition of the females. In comparing the age composition of *Anopheles* in sectors with a complete DDT or BHC coverage with those sectors that were left untreated, one notes considerable changes in the age composition as a result of spraying. While in untreated settlements the vector population is in its natural state and mosquitos die only from natural causes, in treated settlements every female at any gonotrophic cycle is exposed to the toxicant. Under these conditions the chance of females surviving to physiological old age is extremely small, since it is most improbable that females which have completed a large number of ovipositions would not have, at least once, come into contact with a toxic surface. Thus the complete coverage of a settlement with DDT or BHC reduces the age of the whole mosquito population.

The effectiveness of the spraying is evaluated in the following manner. If all the dissected females caught in a settlement under a complete coverage are physiologically young and nulliparous, then the mosquitos are emerging from the surrounding breeding-places. Having flown into the settlement, these young females die after contact with the toxic surface. The presence, among the dissected females, of any that have completed one or two gonotrophic cycles indicates either that a proportion of the females remained in natural shelters or that some premises have been treated with insufficient completeness or care. However, these physiologically young females are not dangerous under temperate conditions, since the process of sporogony could not yet have been completed even if they had been infected during their first blood-meal. For epidemiological assessment of the age groups of female malaria vectors it is necessary in every individual instance to establish the age of the potentially dangerous females. In any case the finding of females which have completed three or more gonotrophic cycles — i.e., females which, if all conditions were favourable, would already form part of the group of dangerous females—in conditions where settlements have been given complete coverage indicates serious shortcomings in malaria control. This may be due to the missing of a proportion of dwellings during spraying or to the poor quality of the insecticide. In such cases, it is necessary to discover and respray the premises having walls

and ceilings non-toxic for mosquitos. Sometimes the presence of old females is explained by their influx from untreated settlements.

If the biology of the local vector is well known, the possibility of such flights can always be estimated from the topography of the district and from the situation of the locality in regard to the breeding-places. Naturally, if cases of malaria are also noted in untreated settlements from which mosquitos are incoming then appropriate measures must be taken to spray them.

Thus, in each individual case the age of the potentially dangerous females during the season can be determined so that the effectiveness of the control measures can be judged accordingly, and all the shortcomings corrected. Where large-scale measures are instituted for the complete coverage of an inhabited locality and when there is a shortage of well-trained entomologists capable of using the method of determining the physiological age of females, an approximate assessment of these measures can be made from the ratio of parous to nulliparous females, determined by the conditions of the tracheal system of the ovaries. Females of endophilic *Anopheles* species are more likely in each gonotrophic cycle to come in contact with a treated surface; therefore, if the spraying has been properly carried out the proportion of parous females in the population should be considerably lower, and conversely the proportion of physiologically young nulliparous females considerably higher than in an unaffected vector population. In other words, the higher the mortality of mosquitos under the influence of contact insecticides the higher is the proportion of nulliparous females in the population. If there are shortcomings in the control work then the proportion of parous females will always increase.

Some factual data on the evaluation of the effectiveness of complete DDT coverage are of interest.

In 1947 the Institute of Malaria of the USSR Ministry of Health carried out a complete coverage of 15 adjoining settlements in a specific area. The total sprayable surface of the treated premises was over 420 000 square metres (Nabokov & Nikiforova, 1948). Larvicidal measures were not carried out. It fell to my lot, as a participant in this expedition, to make an entomological evaluation of the effectiveness of the insecticide measures carried out. Four settlements were under constant observation:

(1) A settlement where all premises were given a complete coverage. This locality was completely isolated from the untreated zone.

(2) A settlement where all premises were given the complete coverage but where the locality itself was situated at the edge of the treated zone. At a distance of half a kilometre from its most outlying houses there was an untreated settlement. Because of this and of the existence of bodies of water between the two localities mosquitos could easily fly from the untreated into the treated zone.

(3) The untreated settlement mentioned in (2). The entomological data collected in it made it possible to establish the effect on the vector population of the nearby treated zone.

(4) An untreated settlement completely isolated from the treated zone, where there could be no effect of DDT on the mosquito population.

The numbers of mosquitos resting in the sprayed settlements were counted by means of mosquito traps and in the untreated settlements by catching all mosquitos entering indoor capture stations. Because of their small total numbers all mosquitos caught in the traps were dissected but only a proportion of those from the unsprayed sectors were dissected. There were considerable differences between the numbers of mosquitos in the two sectors.

Careful daily inspection of all premises in the sprayed settlement isolated from the untreated zone showed an almost complete absence of female *Anopheles*. Only a few days during the season were we able to catch one or two females in the whole locality. In the second treated settlement the mosquito densities were extremely low and the average daily number caught in each dwelling varied from 0.3 to 1.7. In the untreated settlement near the treated locality the numbers were intermediate between the sprayed and unsprayed sectors and varied at different times during the season from 4 to 21 per capture station. In the untreated settlement isolated from the treated zone the average numbers were considerably higher and varied from 8 to 183, although on some days in July still higher figures were recorded.

The total number of dissected females in each group of settlements was small, amounting to 434 in the treated sector and 925 in the untreated. Despite this, considerable differences in age composition were discovered. Thus in the first isolated and treated settlement the proportion of nulliparous females was highest and amounted to 79%. Among the small remaining portion some females were found which had completed from one to four gonotrophic cycles. Since it was difficult for mosquitos to have flown into this settlement from the outside, the finding of parous females can only be explained by the presence of some limited unsprayed shelters in which a small proportion of *Anopheles* had been able to find refuge. In the second treated sector, bordering on an untreated zone, the proportion of nulliparous females was also high, amounting to 73%. Among the parous females the 17% of uniparous females predominated. The numbers of females at subsequent ages were proportionately less and the oldest one had completed five ovipositions. In the untreated sectors the proportion of nulliparous females was considerably lower, being 52.6% in the non-isolated settlement and 56.2% in the isolated settlement. The oldest females in the former settlement were in their seventh, and in the latter in their eighth, gonotrophic cycle. It is very probable that if a larger

number of females had been dissected, even older individuals would have been found.

These data indicate that the measures taken were highly effective. The sharp reduction in numbers decreased by many times the contact of mosquitos with man and among those surviving only a negligibly small number had reached a potentially dangerous age.

In 1949 the Institute of Malaria carried out in southern areas of the USSR another extensive field trial in malaria eradication by DDT spraying. The conditions of this trial were difficult on account of unlimited breeding-foci of *Anopheles* in very broken country. Curative and prophylactic drugs were widely used at the same time. The check sector where no malaria control measures were carried out was completely isolated from the effect of DDT and its character and topography were the same as in most of the treated settlements. All recording of numbers and age composition of the vector population was carried out according to a standardized method.

This field trial showed that the numbers of mosquitos in treated settlements were thousands of times lower than in the untreated sectors. Thus, in June the average daily numbers of females per dwelling varied in treated settlements from 0.03 to 1.6; the relevant figure in the non-treated sector was equal to 2500. A similar or sometimes even greater difference was noted during all the subsequent months of summer and autumn.

The age composition in treated sectors differed completely from that in the untreated sectors. In all the treated settlements the proportion of nulliparous females averaged during the whole period between 60% and 66%, whereas in the untreated sector the respective proportion was 29%. This difference shows that DDT had a pronounced effect on the females and caused a high mortality which was repeated in every gonotrophic cycle. Despite the very high mortality there were individual females which succeeded in completing four to seven gonotrophic cycles. The proportion of such females was, however, negligible, and varied in different settlements between 0.1% and 0.02% of all mosquitos dissected during the summer and autumn. The presence of parous and nulliparous females in the treated settlements can be explained by the fact that a proportion of the vector population found a safe refuge outside and inside the settlements. Such refuges may be varied: new dwellings built after spraying, vegetation, etc.

Among females collected from the untreated settlements there was a considerably larger proportion of old age groups, together with a considerably greater total number of all age classes, indicating a greater survival rate among the females. It might be pointed out, for example, that the proportion of females which had completed three ovipositions varied in the treated sectors between 0.3% and 0.9%, whereas the relevant figure in the untreated sectors was more than 11%. A similar difference was noted

among females that had completed four ovipositions. In the treated areas their proportion varied from 0.05% to 0.1%, whereas in the untreated areas it was 5.5%.

An equally significant difference was noted at subsequent ages. The large number of mosquitos dissected (from 3000 to 6000 in treated sectors and 9000 in the untreated sectors) leads to the conclusion that the relative proportions of age classes correctly reflected the actual relationships in each population.

The combined data of mosquito numbers and of the proportion of potentially dangerous females among those dissected showed a tremendous difference in the epidemiological importance of the population in settlements under DDT spraying in comparison with the unsprayed settlements. Thus, while in the treated settlements the average daily number of potentially dangerous females in resting places was 0.1, in the untreated sectors there were from 250 to 350 or 400 such females per resting-place. Thus all the data quoted indicated the very high effectiveness of the measures carried out.

During the following years many research workers studied the age composition of the vector population in order to judge the effectiveness of malaria control measures. This work proved in the first place the high value of DDT or BHC preparations in antimalaria spraying by the method of total coverage; secondly it proved that it was possible, by means of data on the age composition of females, to assess with high accuracy the quality of the measures carried out and the duration of the insecticidal effect obtained (Beklemishev, 1960; Markovich (& Bandin), 1960; Markovich & Yaguzhinskaya, 1960, and others).

Barrier Spraying with DDT and BHC and Entomological Evaluation of its Results

In the conditions found in large cities and other centres of population, where the main breeding-places are often concentrated in one part of the area (even when the incidence of malaria among the population is high), barrier spraying with DDT or BHC can be used with success, instead of the complete coverage of all dwellings. If there are any additional breeding-places of *Anopheles* inside the protected area it is necessary to carry out simultaneous antilarval measures. The application of barrier spraying, as stated by Beklemishev (1949), is based on the observation that mosquitos flying into a settlement usually stay for some time in buildings situated nearer the breeding-places, and therefore only a small part of the vector population penetrates deep into the settled area. The depth and amount of barrier spraying should be determined in accordance with entomological indices such as the assessment of numbers of mosquitos in dwellings

situated at varying distances from the breeding-places. Excellent results may be obtained by the correct planning of barrier spraying, since here, as with complete coverage, the chances are repeated that in every gonotrophic cycle most of the females will come into contact with the treated surface. This explains why the entomological evaluation of the effectiveness of barrier spraying is the same as for the technique of complete coverage.

Among dissected females found in resting-places situated within the treated zone (i.e., the barrier) there should be no multiparous individuals. Their presence shows that the barrier is not wide enough to prevent the flight of mosquitos into the settlement.

The effectiveness of the method has been tested by a number of workers (Tishchenko, Kucher & Andrievsky, 1951; Zapluysvechka, 1952; Kozhevnikova, 1952; Segay (& Suchkova), 1952; Medvedeva, 1953; Moldavskaya et al., 1953; Reingard, Goritskaya & Zabudko-Reingard, 1953; Grinberg & Derkautsan, 1960a,b). Most workers state that barrier spraying is effective, by causing a general reduction in the numbers of mosquitos in settlements and an obvious change in their age composition. Among a small number of females dissected from a barrier zone, only nulliparous and uniparous females were found. Inside the barrier zone in addition to nulliparous and uniparous females, single individuals were found which had completed a varying number of ovipositions. The oldest was a female which had oviposited five times. The presence of such females can be explained only by the flight of a certain number of mosquitos into the settlement over the barrier. In only one case (Reingard et al., 1953) did the barrier spraying fail to have an effect and this the authors explain by topographic peculiarities of the area. The village in which the experiment was carried out was on a slope down to a river and the mosquitos were able to penetrate into the streets which were situated higher, missing altogether the barrier streets, which were nearer to the river.

It follows that in carrying out the barrier spraying it is necessary to take into account all the features of local topography and to decide in each case on the width and depth of the barrier from the numbers and distribution of mosquitos in the settlement.

Selective Spraying of Premises with DDT or BHC and Entomological Evaluation of its Results

In recent years, when the incidence of malaria in the USSR has sharply fallen, complete and barrier spraying have yielded place increasingly often to selective spraying. This method was first proposed and put into practice by Sarikian (1950), Sergiev & Sarikian (1953) and Sarikian & Zhukova (1959), who also assessed it epidemiologically. The method is used when

the local number of actual or former malaria cases is very small. Spraying is carried out in and around the houses of malaria patients and symptomless parasite carriers; former cases of malaria or those discovered by surveillance in the current year or in the previous year are also included, since among the latter parasite carriers might also be present (Sergiev & Yakusheva, 1956). The method is based on some aspects of the bionomics of the vector species.

It is known that freshly fed mosquitos cannot accomplish lengthy flights because of the considerable increase in their weight, and the majority of them settle not far from the place where they took their blood-meal. These places are usually the walls and ceilings of the premises in which the malaria patient lives and also other neighbouring buildings. Thus, selective spraying achieves the elimination of the epidemiologically most dangerous females which have taken a blood-meal from malaria patients.

To avoid the possible escape of mosquitos which have been exposed to sublethal doses a higher dosage of insecticide (Sergiev & Sarikian, 1953) or else a quicker-acting insecticide such as BHC must be used for this method of focal spraying.

The entomological assessment applicable in this instance is the counting of mosquitos in the treated premises and the investigation of the composition of collected females according to the stages of blood digestion. These data make it possible to establish the duration of the action of the insecticide. The constant finding of females on treated surfaces will indicate that the toxicant has ceased to act and that a repeated spraying is necessary. Since there is no marked reduction in the numbers of mosquitos when the spraying covers only a small total area, and since only a negligible proportion of the mosquito population is killed by the insecticide, naturally no noticeable changes in the age composition of the females can be expected.

Treatment of Stabled Cattle with Contact Insecticides and Entomological Evaluation of its Results

It has long been known that domestic animals spending the night in stables attract not less than 80%-90% of all the empty female *Anopheles* flying in. Ross pointed out (1910) that the chances of people becoming infected with malaria decreases as the number of cattle increases, since a considerable proportion of the infective bites are inflicted on the animals, which are not susceptible to malaria. The same effect has been indicated by Falleroni (1927), Rayevsky & Beylin (1940), Rayevsky (1942), Beklemishev & Sergiev (1942), Beklemishev (1944, 1949), and others.

The spraying of domestic animals with various insecticides as a permanent or temporary method of control of pests and ectoparasites (insects

and ticks) has long been practised, and generally gives good results. The spraying of cattle with DDT as an additional measure of control of malaria vectors was carried out for the first time in the USSR by Nabokov & Nikiforova (1948).

The authors came to the conclusion that wherever premises were sprayed at the same time as the local cattle better results were obtained. The same conclusions were reached by Neghme, Gutiérrez & Alée (1955), who carried out observations in Chile. Bandin (1953, 1957a,b, 1958, 1960) worked out in detail and gave scientific reasons for the method of treating cattle with contact insecticides in malaria control. He established the need for repeated spraying and assessed the importance of obtaining data on the numbers and age composition of mosquito populations in settlements where the cattle were sprayed.

Bandin showed that this method increased the chances of killing female *Anopheles* even more than in the method of complete coverage with DDT. The high mortality is explained by the greater contact of the females with the treated surface. While on treated walls the contact of the insect with the poison is mainly through the tarsal claws and labellae, while in feeding on a treated animal almost the whole body of the *Anopheles* touches the insecticide. Moreover, while females in sprayed premises can be irritated by the toxicant, fly away to find themselves some safe resting-place and thus avoid death, when cattle are treated, despite the excitatory effect of the poison, mosquitos in search of a blood-meal settle repeatedly on the body of the animal, thus increasing their chances of death. Another advantage of this method is that when cattle are treated the mosquitos are poisoned independently of the place where they have taken their blood-meal, in the open air far from settlements. Thus, there are increased chances of killing not only females flying into the settlements but also physiologically young, nulliparous females, which to a large extent remain out of doors and feed mainly on pasturing cattle. A serious shortcoming of this method is the need for repeated spraying. The duration of action of the insecticide on the coat of the animal depends on whether it rains or not, on the strength of the wind, the height of the grass and many other factors.

Any dwellings can be used as capture stations in the experimental sector; collected mosquitos are counted and the age composition of females is established. The effectiveness of spraying is estimated, in the same way as in the case of complete coverage, from the age composition of the females.

This method of control cannot lead to complete elimination of the vector population for the following reasons.

- (1) Some of the female mosquitos feed on man and also on animals such as pigs which constantly wash off the insecticide in mud or puddles, and on wild animals, birds and other hosts.

(2) Mosquitos may fly in from neighbouring settlements where control measures have not been carried out.

(3) The insecticide is not constantly present on the coat of the sprayed animals.

Similar conclusions were reached by Burnett (1954), who investigated the effectiveness of DDT spraying of animals to control *Glossina morsitans* and *G. swynnertoni*.

The method of spraying stabled cattle with residual insecticides is suitable wherever the malaria vectors belong to exophilic species or in the case of anthropophilic vectors having exophilic tendencies. An example of this is *A. m. maculipennis* in the warm and damp climate of many regions of the Caucasus. In addition, the spraying of cattle is indicated in isolated mountain settlements, as also in places where there is an important silkworm industry, and where the silkworm rearing sheds may serve as the main resting-places for *Anopheles* (Bandin, 1957a; Lysenko et al., 1957).

In organizing malaria control measures by spraying cattle it must be remembered, as Bandin (1957a) points out, that the success of this method depends on there being a sufficient number of cattle in the locality.

Observations carried out over a number of years have led the author to the conclusion that in settlements where, for example, there are 1.4 head of cattle per farmstead, between 94.5% and 99.3% of all mosquitos caught contain animal blood. The author recommends that in places where there are no other contra-indications against cattle spraying it should only be carried out if there is at least one cow or horse per farmstead. In these cases the attractant role of the cattle will be great and will cause high mortality among the female *Anopheles*, as a result of which they will not be able to survive to an epidemiologically dangerous age and thus malaria transmission will be interrupted.

According to Bandin, who carried out his observations on *A. maculipennis* populations in the steppe zone of the European part of the USSR and in Siberia, and to Dudkina (1954), working in the Ukraine, and in agreement with Lysenko et al. (1957), who observed *A. superpictus* in Central Asia, the numbers of mosquitos in settlements where cattle were sprayed was constantly much lower than in unsprayed localities. Thus, in Central Asia in an experimental sector there was an average of 2-15 females per capture station per day; in the non-sprayed sector there were several hundred. Bandin notes that after each spraying of cattle there was a sharp reduction in the number of resting mosquitos in capture stations. After two to four days, as the insecticide gradually disappeared from the coats of animals, the mosquito density rose again.

The systematic dissection of females permitted the determination of the ratio between various age groups of the vector population and (by comparison of the data from experimental and control sectors) made it possible to judge the effectiveness of the measures carried out. Bandin (1957a, b) found that among 2427 females dissected in an experimentally treated sector in the steppe zone, nulliparous females made up 82.78%, uniparous 14.9%, biparous 1.3%, and triparous 0.04%. No females of older age groups were discovered. The above ratio indicates a high degree of effectiveness of the measures carried out; in fact, the proportion of nulliparous females was even higher than in settlements which were given the complete treatment. A sharp reduction of the proportion of females in each subsequent age group also indicates a high mortality rate.

Of the 2432 females dissected in the untreated sector, the proportion of nulliparous (52.5%) was considerably smaller and the reduction in the proportion of each subsequent age group was much more gradual. The oldest females had completed six gonotrophic cycles. The best indicator of the effectiveness of this method is the proportion of females living to a potentially dangerous age. In the experimentally treated sector not a single female of such an age was found, whereas in the unsprayed sector they averaged about 5% throughout the season.

According to the data obtained by Lysenko et al. (1956), in the unsprayed sector females which had oviposited four or more times made up, on an average, about 35% of the total vector population, whereas in the sector where cattle had been sprayed no such females were found except in the last ten days of August, when they constituted 1% of all dissected.

Thus, it is possible to conclude that the spraying of a sufficient number of cattle is a highly effective method of controlling malaria vectors.

From 1953 onwards, however, articles have appeared in the literature indicating that if cattle are sprayed with DDT or BHC products these insecticides may penetrate into the organism of the animals and have a bad effect not only on their general condition but also on the health of human beings who drink milk from sprayed cows (Shillinger, 1953; Serebryannaya, 1954; Pavlov, 1957). In view of this fact, it must be considered that where endemic malaria is weak cattle should not be treated, and some more acceptable method must be used in each particular case. However, in very serious foci of malaria, provided that the main vectors are exophilic species, it must be remembered that the serious consequences of the disease may many times outweigh the damage resulting from treating cattle with these insecticides. In such cases it could be considered permissible to use cattle spraying as a method of malaria control. At all events, the method of treating cattle with contact insecticides must be justified, and therefore efforts must be made to bring into wide use contact insecticides which do not prove toxic for warm-blooded animals but are highly effective against blood-sucking arthropods.

Entomological Assessment of the Effectiveness of Larval Control Methods

During the past years the effectiveness of larval control methods has been judged directly by the comparison of numbers of larvae found in the treated zone with those in breeding-places in non-sprayed zones. In addition, the number of adult mosquitos in settlements where control measures had been carried out was compared with the number in untreated settlements. At the present time, these methods can be supplemented with data on the age composition of female *Anopheles*.

The presence, at a given time, of females in their second gonotrophic cycle shows that breeding, previously possible, has now ceased. On the other hand, the presence of nulliparous females among those dissected after spraying has taken place indicates that mosquitos are still breeding and consequently that some shortcomings in the larvical work must be found and corrected. This may be due to defective or late applications of measures or to the presence of untreated breeding-places near the locality. The relationship of parous to nulliparous females is established by examining the tracheal system of the ovary, which permits a rapid investigation.

It follows that while in imagicidal control an increase in the proportion of nulliparous females confirms the effectiveness of the measures taken, in the case of larvical measures, on the contrary, an increase in the proportion of nullipars indicates some errors in the application of control measures.

* * *

Assessment of the Effectiveness of Eradication Measures in the Control of *Musca domestica* and *Phlebotomus*

From the data on the age composition of populations of *Musca domestica*, Lineva (1950) assessed the effectiveness of various control methods and established that the age composition of the fly population also changes when places where flies congregate or breed are treated with DDT. Thus the age composition of the fly population may indicate the success or failure of antifly measures.

In the control of adult flies, complete elimination of the population is usually not achieved, because breeding continues and not all adults are killed. The explanation of the reasons for failure to eliminate flies is found by examining the age composition. A high proportion of parous females among those dissected indicates that treatment of dwellings has not been adequate. If the proportion of nulliparous females is high, this indicates continuing breeding and hence the ineffectiveness of control measures applied to larvae. For evaluation of antifly measures the num-

bers of flies are taken into account and their age composition is compared with similar observations in sectors where fly control measures have not been carried out.

From 1948 onwards, and for a number of years a campaign has been successfully waged in the Crimea against *Phlebotomus papatasi*, the vector of sandfly fever. This campaign was carried out by means of residual contact insecticides under the leadership of Dolmatova (1949a,b). Sandfly fever has now been successfully eradicated in the cities of the Crimea in which it was formerly rife (Dolmatova & Okulov, 1950, 1951a,b; Dolmatova, Kuvitchinsky & Leibman, 1953; Dolmatova, 1955).

Dolmatova stated that the proportion of female *Phlebotomus* in gonotrophic cycles later than the first one decreases with the increase of treated areas. Obviously the greater the treated area the smaller is the opportunity for females to find a resting-place in an untreated shelter, the smaller the proportion of females in their second gonotrophic cycle or later, the smaller the vector population and the less its epidemiological importance.

Dolmatova stated that in sandfly control good results can be obtained if the species population composition is taken into account and if some allowance is made for the ecological peculiarities of each species. When endophilic species are present the inner surfaces of dwellings are treated. In the case of exophilic species the outer surfaces are treated—particularly the eaves, under which *Phlebotomus* concentrates.

All these examples show that the study of the age composition of insect vectors of communicable diseases is a valuable and accurate method, making it possible to follow up the changes brought about by control measures, and to assess reliably the effectiveness of such measures. If correctly assessed, the data on age composition and on the numbers of the vector population will be in full agreement with epidemiological observations; as numbers decrease and the age composition of the population changes, so will decrease the incidence of the disease transmitted by these blood-sucking insects.

ACKNOWLEDGEMENTS

As stated in the preface, the substance of this monograph was presented in the form of lectures at a seminar held at the London School of Hygiene and Tropical Medicine in April 1959. It is my pleasant duty to express my gratitude to Dr L. J. Bruce-Chwatt, Chief, Research and Technical Intelligence, Division of Malaria Eradication, WHO; to Professor G. G. Macdonald, Director of the Ross Institute of Tropical Hygiene, London; to Dr D. S. Bertram, Professor of Entomology, London School of Hygiene and Tropical Medicine; and to all the staff of the Ross Institute for their constant and varied assistance in the work of which this monograph is the embodiment.

REFERENCES

REFERENCES *

- Adam, J. P. (1956) Note faunistique et biologique sur les anophèles de la région de Jaoundé et la transmission du paludisme en zone forestière du Sud Cameroun. *Bull. Soc. Path. exot.*, **49**, 210
- Adler, S. & Theodor, O. (1935) Investigations on Mediterranean Kala Azar; further observations on Mediterranean sandflies. *Proc. roy. Soc. B*, **116**, 505
- Aitken, T. (1946) A study of winter DDT house-spraying and its concomitant effect on Anopheles and malaria in an endemic area. *J. nat. Malar. Soc.*, **5**, 169
- Alessandro, G. d' (1956) Aspect de l'anophélisme extradomestique en rapport avec la lutte insecticide en Sicile. *Rev. path. gén.*, **56**, 186
- Almazova, V. V. (1935) [Determination of the age of the mosquito from its oviduct]. *Med. Parazit. (Mosk.)*, **4**, 345
- Almazova, V. V. (1959) [Material on the ecology of *Anopheles superpictus* in the Pyandzha Valley (Western Pamirs)], Stalinabad [Collected works on malaria and helminthiasis], No. 2, p. 89
- Almazova, V. V., Prokopenko, L. I., Shesterikova, A. A. & Levitanskaya, P. B. (1957) [Age composition and epidemiological importance of populations of *A. maculipennis* in the Altai area in 1953 and 1954]. *Med. Parazit. (Mosk.)*, **26**, 61
- Amosova, I. S. (1956) [Fauna and the biology of sandflies (*Culicoides*, fam. *Heleidae*) in coniferous and broad-leaf forests of the southern coastal areas], Leningrad (Thesis, Zoological Institute of the USSR Academy of Medical Sciences)
- Avdeeva, T. Y. (1940) [Presence of plasmodium in the body of *Anopheles* and its changes during the growth]. *Vop. Fiziol. Ekol. Malar. Komara*, **1**, 153
- Bandin, A. I. (1953) [Influence of DDT spraying of domestic cattle on the population of *Anopheles* in a settlement]. *Med. Parazit. (Mosk.)*, **22**, 20
- Bandin, A. I. (1957a) [Advantages of spraying domestic cattle with contact insecticides in comparison with house-spraying in anti-anopheles campaigns]. *Med. Parazit. (Mosk.)*, **26**, 208
- Bandin, A. I. (1957b) [The importance of spraying farm animals with contact insecticides in malaria prophylaxis], Moscow (Thesis, USSR Academy of Medical Sciences)
- Bandin, A. I. (1958) [A reliable method of insect control]. *Med. Sestra*, **8**, 41
- Bandin, A. I. (1960) [Practical advice on the spraying of farm animals with contact insecticides]. *Med. Parazit. (Mosk.)*, **29**, 82
- Bar-Zeev, M. (1957) The effect of density on the larvae of a mosquito and its influence on fecundity. *Bull. Res. Coun. Israel*, **6**, 220
- Beklemishev, W. N. (1940) [Gonotrophic rhythm as a basic principle of the biology of *Anopheles*]. *Vop. Fiziol. Ekol. Malar. Komara*, **1**, 3

* Titles enclosed in square brackets are translations from Russian.

- Beklemishev, W. N. (1944) [Ecology of the malaria mosquito], Moscow, Medgiz
- Beklemishev, W. N. (1947) [Some perspectives of the use of DDT against arthropod vectors of disease]. *Med. Parazit. (Mosk.)*, **16**, 3
- Beklemishev, W. N. (1949) [Some results of 1948 on the use of DDT against mosquitos and sandflies]. *Med. Parazit. (Mosk.)*, **18**, 23
- Beklemishev, W. N. (1957a) [Assessment of the density of vector populations in a malaria focus in relation to the evaluation of effects of imagicidal programmes]. In: Beklemishev, W. N. & Shipitsina, N. V., ed. [Seasonal phenomena in the life of malaria mosquitos in the USSR], Moscow, Medgiz, p. 462
- Beklemishev, W. N. (1957b) [Population biology as one of the theoretical bases of anti-mosquito campaigns]. In: [Proceedings of a joint scientific session of the USSR Academy of Medical Sciences and the Ministry of Health of the Uzbek SSR, Tashkent, 1954], p. 92
- Beklemishev, W. N. (1957c) [Some general problems of the biology of blood-sucking Diptera]. *Med. Parazit. (Mosk.)*, **26**, 562
- Beklemishev, W. N. (1960) [Some general questions connected with the use of DDT against malaria mosquitos]. In: [Experience in the use of residual contact insecticides in malaria control in the southern regions of the USSR]. *Tr. Inst. med. Parazit. trop. Med.*, p. 365
- Beklemishev, W. N., Bryukhanova, A. A. & Shipitsina, N. V. (1931) [The basis for the epidemiology and prophylaxis of malaria in Magnitogorsk (according to data for 1930)], Magnitogorsk, Department of Health
- Beklemishev, W. N. & Detinova, T. S. (1940) [The physiological cycle of Malpighian tubes in female *A. superpictus* Grassi]. *Vop. Fiziol. Ekol. Malar. Komara*, **1**, 65
- Beklemishev, W. N., Detinova, T. S. & Polovodova, V. P. (1959) Determination of physiological age in anophelines and of age distribution in anopheline populations in the USSR. *Bull. Wld Hlth Org.*, **21**, 223
- Beklemishev, W. N. & Sergiev, P. G. (1942) [Antimalarial rationalization of rural economy and of inhabited rural areas]. *Med. Parazit. (Mosk.)*, **11**, 3
- Beklemishev, W. N. & Shipitsina, N. K., ed. (1957) [Seasonal phenomena in the life of malaria mosquitos in the USSR], Moscow, Medgiz
- Beklemishev, W. N., Vinogradskaya, O. N. & Mitrofanova, Y. G. (1934) [On the gonotrophic cycle of *Anopheles*]. *Med. Parazit. (Mosk.)*, **3**, 460
- Belskaya, M. K. (1952) [On the problem of outdoor resting-places of *A. maculipennis*]. *Med. Parazit. (Mosk.)*, **21**, 454
- Beltiukova, K. N. (1953) [Materials on the study of Simuliidae (Diptera) of the pre-Ural area], Perm (Thesis, Molotov State University)
- Bertram, D. S. & Samarawickrema, W. A. (1958) Age determination for individual Mansonoides mosquitoes. *Nature (Lond.)*, **182**, 444
- Boyd, M. F. (1932) Studies on *Plasmodium vivax*. II. The influence of temperature on the duration of the extrinsic incubation period. *J. Hyg. (Lond.)*, **16**, 851
- Brandt, A. (1876) [Comparative investigation on the ovarioles and eggs of insects]. *Izvest. imp. Obšč. Iubil. estest. Antropol. Etnograf.*, **23**, 1
- Brandt, A. (1878) *Über das Ei und seine Bildungsstätte*, Leipzig

- Buck, A. de, Schoutte, E. & Swellengrebel, N. H. (1932) Further investigations on the racial differentiation of *A. maculipennis* in the Netherlands and its bearing on malaria. *Riv. Malar.*, **11**, 137
- Burnett, G. F. (1954) The effect of poison bait cattle on populations of *Glossina morsitans* Westw. and *G. swynnertoni* Aust. *Bull. ent. Res.*, **45**, 411
- Buyanova, O. F. (1959) [Material on the ecology of *Anopheles superpictus* in the middle Bakhs valley], Stalinabad [Collected works on malaria and helminthiasis], No. 2, p. 101
- Buyanova, O. F. & Kekhcher, O. M. (1960) [Epidemiological and entomological observations in targets sprayed late: observations in the village of Kirnatsenya]. In: [Experience in the use of residual contact insecticides in malaria control in the southern regions of the USSR]. *Tr. Inst. med. Parazit. trop. Med.*, p. 223
- Chagin, K. P. (1943) [Hibernation of malaria mosquitos in the Ivanov Oblast]. *Med. Parazit. (Mosk.)*, **10**, 440
- Chauvin, R. (1949) *Physiologie de l'insecte*, Paris
- Chernysheva, V. A. (1955) [Survival of *Anopheles* until the epidemiologically dangerous age]. In: [Collected works of the Byelorussian Institute of Epidemiology, Microbiology and Hygiene], p. 143
- Chinaev, P. P. (1952) [Experimental use of DDT in mosquito control in a rice-field area]. *Med. Parazit. (Mosk.)*, **21**, 534
- Christophers, S. R. (1911) The development of the egg follicle in anophelines. *Paludism*, **2**, 73
- Christophers, S. R. (1923) The structure and development of the female genital organs and hypopygium of the mosquito. *Indian J. med. Res.*, **10**, 698
- Clements, A. N. (1956) Hormonal control of ovary development in mosquitoes. *J. exp. Biol.*, **33**, 211
- Colless, D. H. (1958) Recognition of individual nulliparous and parous mosquitoes. *Trans. roy. Soc. trop. Med. Hyg.*, **52**, 187
- Cunliffe, N. (1921) Some observations on the biology and structure of *Ornithodoros moubata* Murray. *Parasitology*, **13**, 327
- Dasgupta, B. & Ray, H. N. (1956) Observations on the "corpus luteum" and the "NR bodies" in the female gonads of the bed bug. *Proc. Zool. Soc. India*, **9**, 55
- Davidson, G. (1947) Field trials with gammexane as a means of malaria control by adult mosquito destruction in Sierra Leone. *Ann. trop. Med. Parasit.*, **41**, 193
- Davidson, G. (1953) Experiments on the effect of residual insecticides in houses against *Anopheles gambiae* and *A. funestus*. *Bull. ent. Res.*, **44**, 231
- Davidson, G. (1954) Estimation of the survival rate of anopheline mosquitos in nature. *Nature (Lond.)*, **174**, 792
- Davies, L. (1955) Behaviour of young and old females of the black-fly *Simulium ornatum* Mg. *Nature (Lond.)*, **176**, 979
- Davies, L. (1957) A study of the age of females of *Simulium ornatum* Mg. (Diptera) attracted to cattle. *Bull. ent. Res.*, **48**, 535
- Davis, N. C. (1928) Notes on the development of ovarian follicles in Argentine *Anopheles*. *Amer. J. Hyg.*, **8**, 467

- Day, M. F. (1951) Studies on the digestion of wool by insects. III. A comparison between the tracheation of the midgut of *Tineola* larvae and that of other tissues. *Aust. J. Sci. Res. Ser. B*, **4**, 64
- Déduit, J. (1957) Etudes sur la ponte par autogénèse des culicides. II. Données numériques sur l'acte de ponte chez la femelle fécondée de *Culex pipiens autogenicus* Roubaud. *C. R. Soc. Biol. (Paris)*, **151**, 974
- Demina, N. A. & Zvyagintsev, S. N. (1936) [Results of the precipitation test with *Anopheles maculipennis* stomachs according to material collected by an expedition in the Saratov suburban zone]. *Med. Parazit. (Mosk.)*, **5**, 3
- Denisova, Z. M. (1940) [Functional changes in the Malpighian tubes of *A. maculipennis messeae* in relation to its gonotrophic cycle]. *Vop. Fiziol. Ekol. Malar. Komara*, **1**, 86
- Denisova, Z. M. (1946) [Autogenous development of the ovaries in an Alma-Ata *Anopheles bifurcatus* population]. *Med. Parazit. (Mosk.)*, **15**, 40
- Derbenieva-Ukhova, V. P. (1935) [Influence of the food of females on the development of ovaries in *Musca domestica*]. *Med. Parazit. (Mosk.)*, **4**, 394
- Derbenieva-Ukhova, V. P. (1942) [Development of ovaries and feeding of adult manure flies]. *Med. Parazit. (Mosk.)*, **11**, 85
- Derbenieva-Ukhova, V. P. (1952) [*Flies and their epidemiological importance*], Moscow, Medgiz
- Detinova, T. S. (1936) [Seasonal changes of fertility in female *A. maculipennis messeae*]. *Med. Parazit. (Mosk.)*, **5**, 566
- Detinova, T. S. (1940) [*Experimental study of imaginal diapause in Anopheles maculipennis Mg.*], Moscow (Thesis, V. I. Lenin State Pedagogical Institute)
- Detinova, T. S. (1942) [On the biology of the genus *Aedes*]. *Med. Parazit. (Mosk.)*, **11**, 44
- Detinova, T. S. (1944) [Relationships of the size of females of *A. maculipennis atroparvus* and the stage of development of ovaries at the emergence]. *Med. Parazit. (Mosk.)*, **13**, 52
- Detinova, T. S. (1945a) [Action of inner secretory glands on the ripening of sexual products and the diapause of adults of malaria mosquitos]. *Zool. Ž.*, **24**, 291
- Detinova, T. S. (1945b) [Determination of the physiological age of female *Anopheles* from the changes of the tracheal system of the ovaries]. *Med. Parazit. (Mosk.)*, **14**, 45
- Detinova, T. S. (1946) [Numerical relationship of parous and nulliparous females of *A. maculipennis messeae* near Moscow in 1946]. *Med. Parazit. (Mosk.)*, **15**, 52
- Detinova, T. S. (1948) [Numerical prevalence and population composition of *A. maculipennis messeae* in a rural area sprayed with DDT]. *Med. Parazit. (Mosk.)*, **17**, 19
- Detinova, T. S. (1949) [Physiological changes of ovaries in females of *A. maculipennis*]. *Med. Parazit. (Mosk.)*, **18**, 410
- Detinova, T. S. (1952a) [Age composition and epidemiological importance of *A. maculipennis* in untreated areas]. *Med. Parazit. (Mosk.)*, **21**, 339
- Detinova, T. S. (1952b) [Age composition of *Anopheles* as a criterion of effectiveness of antimosquito measures]. *Nov. Med.*, **27**, 57

- Detinova, T. S. (1953a) [Changes in the ovarioles of *A. maculipennis* in conditions when the previous egg had been retained]. *Med. Parazit. (Mosk.)*, **22**, 279
- Detinova, T. S. (1953b) [Influence of the development of ovaries on the speed of digestion of blood by female *A. maculipennis*]. *Med. Parazit. (Mosk.)*, **22**, 337
- Detinova, T. S. (1953c) [On the duration of one gonotrophic cycle in *A. maculipennis* (from one oviposition to the blood-meal)]. *Med. Parazit. (Mosk.)*, **22**, 446
- Detinova, T. S. (1953d) [Age composition and epidemiological importance of the population of *A. maculipennis* in the Moscow Oblast]. *Med. Parazit. (Mosk.)*, **22**, 486
- Detinova, T. S. (1955a) [Age changes in the ovaries of *Hippobosca capensis*]. *Doklady Akad. Nauk S.S.R.*, No. 5, p. 937
- Detinova, T. S. (1955b) [Fertility of *A. maculipennis*]. *Med. Parazit. (Mosk.)*, **24**, 6
- Detinova, T. S. (1957) [Physiological age of arthropod vectors of diseases in relation to the assessment of effectiveness of control measures]. *Med. Parazit. (Mosk.)*, **26**, 673
- Detinova, T. S. (1960) [The epidemiological importance of *Anopheles maculipennis* in an undisturbed population in the village of Chobruchu]. In: [Experience in the use of residual contact insecticides in malaria control in the southern regions of the USSR]. *Tr. Inst. med. Parazit. trop. Med.*, p. 72
- Detinova, T. S. & Beltiukova, K. N. (1958) [On the repeated gonotrophic cycle of Simuliidae in Krasnoyarsk area]. *Med. Parazit. (Mosk.)*, **27**, 686
- Detinova, T. S. & Butenko, O. M. (1955) [Autogenous maturation of ovaries in female *Anopheles hyrcanus*, in Northern Kirghizia]. *Med. Parazit. (Mosk.)*, **24**, 445
- Detinova, T. S. (assisted by Kopteva, V. P.) (1936) [Some elements of behaviour of *A. maculipennis messeae* in relation to oviposition]. *Med. Parazit. (Mosk.)*, **5**, 25
- Detinova, T. S. (assisted by Lozgacheva, V. A.) (1953) [Mechanism of the gonotrophic harmony in *A. maculipennis*]. *Zool. Ž.*, **32**, 1178
- Dolmatova, A. V. (1942) [The life-cycle of *Phlebotomus papatasii*]. *Med. Parazit. (Mosk.)*, **11**, 52
- Dolmatova, A. V. (1946) [The autogenous development of eggs in *Phlebotomus papatasii*]. *Med. Parazit. (Mosk.)*, **15**, 58
- Dolmatova, A. V. (1949a) [Numerical prevalence and population of sandflies in a DDT-sprayed part of an urban area]. *Med. Parazit. (Mosk.)*, **18**, 143
- Dolmatova, A. V. (1949b) [Observations on the biology of sandflies in Feodosia]. *Med. Parazit. (Mosk.)*, **18**, 507
- Dolmatova, A. V. (1955) [Methods of permanent sanitation of sandfly fever foci in the Crimea]. *Med. Parazit. (Mosk.)*, **24**, 48
- Dolmatova, A. V., Kuvitchinsky, B. S. & Leibman, A. L. (1953) [*Phlebotomus* of the southern coast of the Crimea and their control]. *Med. Parazit. (Mosk.)*, **22**, 455
- Dolmatova, A. V. & Okulov, V. A. (1950) [Experience of two years of sandfly control by DDT in Feodosia (Crimea)]. *Med. Parazit. (Mosk.)*, **19**, 499
- Dolmatova, A. V. & Okulov, V. A. (1951a) [Sandflies and pappataci fever in Feodosia]. *Med. Parazit. (Mosk.)*, **20**, 160

- Dolmatova, A. V. & Okulov, V. A. (1951b) [Experience of three years of sandfly control]. *Med. Parazit. (Mosk.)*, **20**, 445
- Downs, W. G., Colorado, I. R. & Gahan, J. B. (1948) Residual effectiveness of DDT in the third season after application. *Amer. J. trop. Med.*, **28**, 741
- Dudkina, M. S. (1954) [The effect of insecticidal treatment of cattle on numbers and age composition of mosquitos]. In: [Summaries of papers read at the Third Zoological Conference of the Kiev State University], Kiev, State University Publishing House, Part IV, p. 114
- Duke, B. O. L., Creawe, W. & Beesley, W. N. (1956) The relationship between the size of the blood-meal taken in by *Chrysops silacea* and the development of the microfilariae of loa-loa taken in with the blood-meal. *Ann. trop. Med. Parasit.*, **50**, 283
- Dukhanina, N. N. (1944) [Epidemiological observations on *falciparum* malaria in the North and determination of its zones of distribution], Moscow (Thesis)
- Dukhanina, N. N. (1956) [Long-incubation tertian malaria: its distribution in the USSR and its epidemiological characteristics], Moscow (Thesis, USSR Academy of Medical Sciences)
- Dunn, Z. H. (1923) Observations on the oviposition of the house-fly *Musca domestica* L. in Panama. *Bull. ent. Res.*, **13**, 301
- Enikolopov, S. K. (1950) [Autogenous multiplication of *A. hyrcanus*]. *Med. Parazit. (Mosk.)*, **19**, 544
- Falleroni, D. (1926) Note sulla biologia dell'*Anopheles maculipennis*. *Riv. Malar.*, **5**, 353
- Falleroni, D. (1927) Discussione sulla zooprofilassi e sulla biologia degli anopheli italiani. *Riv. Malar.*, **6**, 4
- Farid, M. A. (1954) Ineffectiveness of DDT residual spraying in stopping malaria transmission in the Jordan Valley. *Bull. Wld Hlth Org.*, **11**, 765
- Fastovskaya, E. I. & Kalmykov, E. S. (1956) [Epidemiological effectiveness of total zonal coverage with DDT], Stalinabad [Collected works on malaria and helminthiasis], No. 1, p. 40
- Fletcher, O. K. & Krause, J. B. (1948) Mass destruction of adult anophelines by DDT as a suggested malaria control measure. *Amer. J. trop. Med.*, **28**, 323
- Formozov, A. N. (1946) [Snow cover in the life of the mammals and birds of the USSR]. Moscow, Publishing House of the Moscow Society of Naturalists, Zoological Section, Issue No. 5, p. 1
- Gabaldon, A. (1953) Possible effect of residual insecticides on the interruption of malaria transmission. *Riv. Malar.*, **32**, 155
- Gahan, J. & Lindquist, A. (1945) DDT residual sprays applied in buildings to control *Anopheles quadrimaculatus*. *J. econ. Ent.*, **38**, 223
- Garrett-Jones, C. (1959) *The place of entomology in malaria eradication* (Unpublished working document WHO/Mal/231)
- Georgopoulos, G. (1951) *A. sacharovi* (*A. elutus*) resistance to DDT. *Arch. Hyg. (Athinai)*, **2**, 246
- Giles, G. M. (1900) *A handbook of the gnats or mosquitos*, London, Bale & Danielsson
- Gillett, J. D. (1955a) Behaviour differences in two strains of *Aedes aegypti*. *Nature (Lond.)*, **176**, 124

- Gillett, J. D. (1955b) Role of the blood meal in *Aedes aegypti*. *Rep. E. Afr. Virus Res. Inst.*, p. 24
- Gillett, J. D. (1956a) Genetic differences affecting egg-laying in the mosquito *Aedes (Stegomyia) aegypti* (Linnaeus). *Ann. trop. Med. Parasit.*, **50**, 362
- Gillett, J. D. (1956b) Initiation and promotion of ovarian development in the mosquito *Aedes aegypti*. *Ann. trop. Ped. Parasit.*, **50**, 375
- Gillett, J. D. (1957a) Age analysis in the biting-cycle of the mosquito *Taeniorhynchus (Mansonioides) africanus* Theo. based on the presence of parasitomites. *Ann. trop. Med. Parasit.*, **51**, 151
- Gillett, J. D. (1957b) Variation in the time of release of the ovarian development hormone in *Aedes aegypti*. *Nature (Lond.)*, **180**, 656
- Gillett, J. D. (1958) Induced ovarian development in decapitated mosquitos by transfusion of haemolymph. *J. exp. Biol.*, **35**, 685
- Gillies, M. T. (1953) The duration of the gono-cycle in *Anopheles gambiae* and *Anopheles funestus*, with a note on the efficiency of hand-catching. *E. Afr. med. J.*, **30**, 129
- Gillies, M. T. (1954) Studies of house leaving and outside resting of *Anopheles gambiae* Giles and *A. funestus* Giles in East Africa. *Bull. ent. Res.*, **45**, 361, 375
- Gillies, M. T. (1956a) The problem of exophily in *Anopheles gambiae*. *Bull. Wld Hlth Org.*, **15**, 437
- Gillies, M. T. (1956b) A new character for the recognition of nulliparous females of *Anopheles gambiae*. *Bull. Wld Hlth Org.*, **15**, 451
- Gillies, M. T. (1958) A review of some recent Russian publications on the technique of age determination in *Anopheles*. *Trop. Dis. Bull.*, **55**, 713
- Glukhova, V. M. (1956) [Fauna and ecology of Culicoides of the Karelo-Finnish SSR], Leningrad (Thesis)
- Glukhova, V. M. (1958) [Gonotrophic cycle of Culicoides in the Karelian ASSR]. *Parasit. Sbornik zool. Inst. Akad. Nauk S.S.R.*, **18**, 239
- Gordon, R. M., Hicks, E. P., Davey, T. H. & Watson, M. (1932) A study of the house-haunting Culicidae occurring in Freetown, Sierra Leone. *Ann. trop. Med. Parasit.*, **26**, 273
- Grassi, B. (1901) *Die Malariastudien eines Zoologen*, Jena, Fischer
- Grinberg, A. I. & Derkautsan, M. Z. (1960a) [Experience in protective barrier spraying of the city of Kishinev with DDT products in 1948]. In: [Experience in the use of residual contact insecticides in malaria control in the southern regions of the USSR]. *Tr. Inst. med. Parazit. trop. Med.*, p. 263
- Grinberg, A. I. & Derkautsan, M. Z. (1960b) [Experience in protective barrier spraying of the city of Kishinev with DDT products in 1949]. In: [Experience in the use of residual contact insecticides in malaria control in the southern regions of the USSR]. *Tr. Inst. med. Parazit. trop. Med.*, p. 270
- Grinfeld, E. K. (1955) [Nectar and pollen feeding of Diptera and their role in plant pollination]. *Vestn. Leningr. Univ.*, **10**, 15
- Hecht, O. (1933) Experimentelle Beiträge zur Biologie der Stechmücken. III. Blutverdauung und Eireifung bei *A. maculipennis* am Ende der Überwinterung. *Z. angew. Ent.*, **20**, 126

- Hinman, E. & Cutcomb, J. (1947) Block residual spraying of premises with DDT for control of malaria. *Ann. J. trop. Med.*, **27**, 443
- Hocking, B. & Pickering, L. R. (1954) Observations on the bionomics of some northern species of Simuliidae (Diptera). *Canad. J. Zool.*, **32**, 99
- Holstein, M. H. (1954) *Biology of Anopheles gambiae: research in French West Africa*. Geneva (World Health Organization: Monograph Series, No. 9)
- Hosoi, T. (1954a) Egg production in *Culex pipiens pallens* Coquillett. III. Growth and degeneration of ovarian follicles. *Jap. J. med. Sci. Biol.*, **7**, 111
- Hosoi, T. (1954b) Egg production in *Culex pipiens pallens* Coquillett. IV. Influence of breeding conditions on wing length, body weight and follicle production. *Jap. J. med. Sci. Biol.*, **7**, 129
- Hovanitz, W. (1947) Physiological factors which influence the infection of *Aëdes aegypti* with *Plasmodium gallinaceum*. *Amer. J. Hyg.*, **45**, 67
- Huff, C. G. (1929) Ovulation requirements of *Culex pipiens* Linn. *Biol. Bull. Wood's Hole*, **56**, 347
- Imms, A. D. (1908) On the larval and pupal stages of *A. maculipennis*. *Parasitology*, **1**, 114
- Ivanov, P. P. & Meshcherskaya, K. A. (1935) [Physiological characteristics of mature and immature ovaries of insects and their cyclical changes]. *Arh. Biol. Nauk*, **37**, 786
- Ivanova, L. V. (1956) [Some data on the biology of untreated population of *A. superpictus*], Stalinabad [Collected works on malaria and helminthiases], No. 1, p. 46
- Ivanova, L. V., Fastovskaya, E. I. & Farber, N. A. (1960) [Observations in the village of Leontyevo]. In: [Experience in the use of residual contact insecticides in malaria control in the southern regions of the USSR]. *Tr. Inst. med. Parazit. trop. Med.*, p. 203
- Jackson, C. N. H. (1946) An artificially isolated generation of tsetse flies (Diptera). *Bull. ent. Res.*, **37**, 291
- Kalita, S. R. (1937) [Subspecies of *A. maculipennis* of the southern part of the Azov-Black Sea area]. *Med. Parazit. (Mosk.)*, **6**, 710
- Kalmykov, E. S. (1959) [The season during which *Anopheles superpictus* can be effectively infected with the causative agent of malaria], Stalinabad [Collected works on malaria and helminthiases], No. 2, p. 163
- Kalmykov, E. S. & Lysenko, A. Y. (1959) [Material for the rationalization of methods of using DDT for malaria control in areas of distribution of *Anopheles superpictus*], Stalinabad [Collected works on malaria and helminthiases], No. 2, p. 33
- Kannegisser, N. N. & Riazantseva, T. H. (1953) [Survival of female *A. maculipennis sacharovi* to the epidemiologically dangerous age]. *Med. Parazit. (Mosk.)*, **22**, 21
- Kazantsev, B. P. (1959) [Observations of the ecology of *Anopheles superpictus* in the Adyr area of central Tadzhikistan], Stalinabad [Collected works on malaria and helminthiases], No. 2, p. 125
- Kekhcher, O. M. & Markovich, N. Y. (1960) [Observations in Bendery and its neighbourhood and in the village of Khadzhimus]. In: [Experience in the use of residual contact insecticides in malaria control in the southern regions of the USSR]. *Tr. Inst. med. Parazit. trop. Med.*, p. 163

- Kennedy, J. S. (1947) The excitant and repellent effect on mosquitos of sub-lethal contacts with DDT residual films. I. The persistence and toxicity of deposits from kerosene solutions on wall-board. *Bull. ent. Res.*, **37**, 593
- Khelevin, N. V. (1938) [Hibernating places in nature of malaria mosquitos in the Ivanov Oblast]. *Med. Parazit. (Mosk.)*, **7**, 144
- Khelevin, N. V. (1941) [Characteristics of the hibernation of malaria mosquitos in the Ivanov Oblast]. *Med. Parazit. (Mosk.)*, **10**, 440
- Khodukin, N. I. & Lisova, A. I. (1927) [The possibility of winter infections with malaria]. *Med. mysl. Uzbek.*, **6-7**, 71
- Kholodkovski, N. A. (1912) [*A course of theoretical and applied entomology*], 3rd ed.
- Kiseleva, I. A. (1953) [Observations on the biology of *A. maculipennis sacharovi* in the Tashan Oblast of the Turkmen SSR]. *Med. Parazit. (Mosk.)*, **22**, 514
- Kiseleva, I. A. (1955) [*Biology and epidemiological importance of A. maculipennis sacharovi in the Tashan Oblast of the Turkmen SSR*], Stalinabad (Thesis)
- Kligler, J. Y. & Mer, G. G. (1937) Studies on the effect of various factors on the infection of *Anopheles elutus* with different species of *Plasmodium*. *Ann. trop. Med. Parasit.*, **31**, 71
- Korzhenevskaya, J. V. (1957) [Survival of *Anopheles* to the epidemiological age in the Molodechensk Oblast of the Byelorussian SSR]. *Med. Parazit. (Mosk.)*, **26**, Supplement to No. 1, p. 15
- Kozhevnikov, G. A. (1903) [Observations on the biology of *Anopheles* collected by the Voronezh malaria expedition], Moscow [*Collected works on the study of malaria in Russia*], p. 69
- Kozhevnikov, G. A. (1905) [Observations on the natural history of the honey bee. On the polymorphism of the honey bee and other insects]. *Izv. imp. Obšč. Ijubit. estest. Antropol. Etnograf.*, **99**, 2
- Kozhevnikova, S. M. (1952) [The barrier method of spraying in Stalingrad]. *Med. Parazit. (Mosk.)*, **21**, 246
- Kozhevnikova, S. M. (1953) [Survival of *Anopheles* to the epidemiologically dangerous age in the Stalingrad area]. *Med. Parazit. (Mosk.)*, **22**, 500
- Kraan, H. (1956) Enkele opmerkelijke vondsten van Culiciden (Dip.). *Entomol. Ber.*, **16**, 236
- Krivosheina, N. P. (1957) [On the autogenous maturation of ovaries in female *Culicoides*]. *Med. Parazit. (Mosk.)*, **26**, Supplement to No. 1, p. 53
- Kudo, K. (1924) A biologic and taxonomic study of the microsporidia. *Illinois biol. Monogr.*, **9**, 268
- Kudo, K. (1929) [Studies on microsporidia parasitic in mosquitos. VII. Notes on microsporidia of some Indian mosquitos]. *Arh. Protistenk.*, **67**, 1
- Kudo, K. (1930) [Studies on microsporidia parasitic in mosquitos. VIII. *Nosema aëdes*, new species parasitic in larvae of *Aëdes argenteus (aegypti)* of Puerto Rico]. *Arh. Protistenk.*, **69**, 23
- Kukharchuk, L. P. (1958) [The age composition of *Anopheles maculipennis messeae* Meig. in the Kulindinsk Steppe and their survival to an epidemiologically dangerous age]. In: [Questions of theoretical and clinical medicine]. *Tr. Novosibirsk. med. Inst.*, **30**, 19

- Kulagin, N. (1901) Der Bau der weiblichen Geschlechtsorgane bei *Culex* und *Anopheles*. *Z. wiss. Zool.*, **69**, 578
- Kunitskaya, N. T. (1960) [Study of the reproductive organs of female fleas and determination of their physiological age]. *Med. Parazit. (Mosk.)*, **29**, 688
- Kuzina, O. S. (1942) [On the gonotrophic relationships in horse-flies (*Stomoxys calcitrans* and *Haematobia stimulans*)]. *Med. Parazit. (Mosk.)*, **11**, 70.
- Kuzina, O. S. (1950a) [Comparative parasitological and ecological observations on horse-flies (*Stomoxys calcitrans*, *Haematobia stimulans* and *Hyperosia irritans*)]. In: [Ectoparasites: fauna, biology and practical importance], Moscow, Publishing House of the Moscow Society of Naturalists, Issue No. 2, p. 139
- Kuzina, O. S. (1950b) [Some special features in maturation of the eggs of the biting housefly (*Stomoxys calcitrans L.*)]. *Med. Parazit. (Mosk.)*, **19**, 246
- Lachmajorova, J. (1950) Biologia *Anopheles maculipennis atroparvus* v. Thiel na wybrzeżu (1949, 1950). *Przegl. epidem.*, **4**, 1
- Lécaillon, A. (1900) Recherches sur la structure et le développement postembryonnaire de l'ovaire des insectes. 1. *Culex pipiens L.* *Bull. Soc. Ent. France*, No. 4, p. 96
- Lee, M. O. (1927) A note on the mechanism of respiration in the Orthoptera. *J. exp. Zool.*, **49**, 319
- Le Faucheur, M. (1956) Description du système drachéen de la larve de *Vermileo degeneri* Macquart (Diptera rhadionide). *Bull. Soc. zool. France*, **81**, 353
- Lewis, D. J. (1956) Biting times of parous and nulliparous *Simulium damnosum*. *Nature (Lond.)*, **178**, 98
- Lewis, D. J. (1957a) Aspects of the structure, biology and study of *Simulium damnosum*. *Ann. trop. Med. Parasit.*, **51**, 340
- Lewis, D. J. (1957b) A method of recognizing individual nulliparous and parous mosquitoes. *Trans. roy. Soc. trop. Med. Hyg.*, **51**, 561
- Lewis, D. J. (1958a) Observations on *Simulium damnosum* Theobald at Lokoja in Northern Nigeria. *Ann. trop. Med. Parasit.*, **52**, 216
- Lewis, D. J. (1958b) The recognition of nulliparous and parous *Anopheles gambiae* by examining the ovarioles. *Trans. roy. Soc. trop. Med. Hyg.*, **52**, 456
- Lineva, V. A. (1950) [Physiological age and seasonal trend of prevalence of *Musca domestica*], Moscow (Thesis)
- Lineva, V. A. (1953a) [Methods of assessment of the physiological age in females of *Musca domestica*]. *Med. Parazit. (Mosk.)*, **22**, 69
- Lineva, V. A. (1953b) [Physiological age of females of *Musca domestica*]. *Ent. Obozrenie*, **33**, 161
- Lineva, V. A. (1955) [Changes in the oogenesis of *Musca domestica* under the action of DDT]. *Zool. Ž.*, **34**, 1320
- Litvinova, L. N. (1941) [Relationship of fat larvae with fat young females of *A. maculipennis*]. *Zool. Ž.*, **20**, 562
- Litvinova, L. N. (1949) [Physiological changes in *A. maculipennis* during the pre-hibernation period and importance of these changes in the epidemiology of malaria]. *Uč. Zapiski Molotov. pedagog. Inst. (Perm)*, **12**, 101
- Love, G. J. (1954) Variations on hatching of ova in successive lots obtained from colonized *Anopheles quadrimaculatus* Say. *J. econ. Ent.*, **47**, 178

- Luppova, E. P. (1940) [Overwintering places of mosquitos in southern Kirghizia]. *Sovetsk. Zdravookhr. Kirgiz.*, **5**, 94
- Lysenko, A. Y., Lavrenko, I. M., Iliashenko, L. Y. & Korovai, A. F. (1957) [Antimalaria DDT spraying of domestic animals in the mountainous areas of Tadzhikistan]. *Med. Parazit. (Mosk.)*, **26**, 212
- Lysenko, A. Y. & Levitanskaya, P. B. (1952) [The effect of variable temperatures on sporogony in *Plasmodium relictum* and *Plasmodium vivax*]. *Med. Parazit. (Mosk.)*, **21**, 345
- Macdonald, G. (1957) *The epidemiology and control of malaria*, London, Oxford University Press
- Markovich, N. Y. (1938) [Maturation of the eggs of *Anopheles bifurcatus* without adult feeding]. *Med. Parazit. (Mosk.)*, **7**, 897
- Markovich, N. Y. (1939) [The biology of *Anopheles maculipennis* in the north]. *Med. Parazit. (Mosk.)*, **8**, 89
- Markovich, N. Y. (1941) [New data on the biology of *A. bifurcatus* in Northern Caucasus]. *Med. Parazit. (Mosk.)*, **10**, 24
- Markovich, N. Y. (1951) [Fertility of *A. bifurcatus* and its changes under the influence of environmental conditions]. *Med. Parazit. (Mosk.)*, **20**, 50
- Markovich, N. Y. (assisted by Bandin, A. I.) (1960) [The age composition of an undisturbed population of *Anopheles maculipennis sacharovi* in the Kura-Araks Depression according to observations in 1951]. In: [Experience in the use of residual contact insecticides in malaria control in the southern regions of the USSR]. *Tr. Inst. med. Parazit. trop. Med.*, p. 288
- Markovich, N. Y., Prokopenko, L. I. & Ivanova, L. V. (1949) [Experimental trial of DDT against malaria in the Moldavian SSR]. *Med. Parazit. (Mosk.)*, **18**, 28
- Markovich, N. Y. & Remennikova, V. M. (1939) [Observations of the early-spring activities of *Anopheles maculipennis* in the north]. *Med. Parazit. (Mosk.)*, **8**, 318
- Markovich, N. Y. & Yaguzhinskaya, L. V. (1960) [The effectiveness of complete coverage with residual contact insecticides in the control of *Anopheles maculipennis sacharovi* in Mugan (the Kherson group of settlements in the Ali-Bairamly Rayon of the Azerbaijanzhanian SSR)]. In: [Experience in the use of residual contact insecticides in malaria control in the southern regions of the USSR]. *Tr. Inst. med. Parazit. trop. Med.*, p. 298
- Martini, E. (1922a) Über den Einfluss der Wasserzusammensetzung auf die Keimlänge bei den Mückenlarven. *Arch. Schiffs- u. Tropenhyg.*, **26**, 82
- Martini, E. (1922b) Kritische Bemerkungen zur Theorie der misanthropen oder zoophilen *Anopheles*. *Arch. Schiffs- u. Tropenhyg.*, **26**, 257
- Martini, E. (1933) The hypopygia of certain *Anopheles* (Diptera Culicidae). *Proc. ent. Soc. Wash.*, **35**, 61
- Martini, E. (1952) *Lehrbuch der medizinischen Entomologie*, Jena, Fischer
- Mechnikov, I. I. (1866) Embryologische Studien an Insekten. *Z. wiss. Zool.*, **16**, 389
- Mechnikov, I. I. (1867) [Investigations on dipterous insects. (Letter to Behr)]. *Zapiski Akad. Nauk*, **10**, 78

- Mednikova, M. U. (1952) [Glands of internal secretion (corpora allata and corpora cardiaca) in mosquitos]. *Zool. Ž.*, **31**, 676
- Medvedeva, A. H. (1953) [Change from larvicidal methods to DDT spraying in the Stalingrad Oblast]. *Med. Parazit. (Mosk.)*, **22**, 133
- Mellanby, K. (1939) Fertilization and egg production in the bed-bug, *Cimex lectularius L.* *Parasitology*, **31**, 193
- Melnikova, Z. V. (1943) [Observations on *A. bifurcatus* in the Kara-Kaln region in the pre-spraying period]. *Med. Parazit. (Mosk.)*, **12**, 56
- Mer, G. G. (1931) Notes on the bionomics of *Anopheles elutus* (Diptera, Culicidae). *Bull. ent. Res.*, **22**, 137
- Mer, G. G. (1932) The determination of the age of Anopheles by differences in the size of the common oviduct. *Bull. ent. Res.*, **23**, 563
- Mer, G. G. (1936) Experimental study on the development of the ovary in *A. elutus*, Edw. (Dipt. Culic.) *Bull. ent. Res.*, **27**, 351
- Minayev, G. I. & Kravchenko, R. P. (1936) [The overwintering of *Anopheles maculipennis* in the southern rayons of the Krasnoyarsk Krai]. *Med. Parazit. (Mosk.)*, **5**, 211
- Mishukov, A. I. (1957) [Age composition and epidemiological importance of *A. maculipennis* near Novosibirsk in 1952]. *Med. Parazit. (Mosk.)*, **26**, Supplement to No. 1, p. 23
- Missiroli, A. (1925) I tubuli del Malpighi nell'*A. claviger* ibernante. *Ann. Igiene*, **35**, 113
- Missiroli, A. (1927) I tubuli del Malpighi nell'*A. claviger*. *Riv. Malar.*, **6**, 1
- Missiroli, A. (1929) Sui microsporidi parassiti dell'*A. maculipennis*. *Riv. Mal.*, **8**, 393
- Mitrofanova, Y. G. (1946) [Ecology of adult *A. hyrcanus* and *A. pulcherrimus* in the valley of Murgaba]. *Vop. Fiziol. Ekol. Malar. Komara*, **2**, 193
- Moldavskaya, V. D., Tishchenko, O. D., Ustinov, A. A., Moshchenskaya, F. A., Zalkind, H. B., Mikhailov, A. A. & Tsukanov, A. A. (1953) [Elimination of malaria in urban areas in the south of USSR]. *Med. Parazit. (Mosk.)*, **22**, 232
- Möllring, F. K. (1956) Autogene und anautogene Eibildung bei Culex, zugleichem Beitrag zur Frage der Unterscheidung autogener un anautogener Weibchen an Hand von Eiröhrenzahl und Flügellänge. *Z. Tropenmed. Parasit.*, **7**, 15
- Monchadsky, A. S. (1956) [Attack of mosquitos on man in the coastal area of the Volga Delta]. *Parazit. Sbornik zool. Inst. Akad. Nauk S.S.R.*, **16**, 89
- Moshkovsky, S. D. (1946) [The dependence upon temperature of the speed of development of malaria plasmodia in the mosquito]. *Med. Parazit. (Mosk.)*, **15**, 19
- Moshkovsky, S. D. (1950) [*The main laws governing the epidemiology of malaria*], Moscow
- Moshkovsky, S. D. & Rashina, M. G. (1951) [*Epidemiology and medical parasitology for entomologists*], Moscow
- Mouchet, Y. & Gariou, U. (1957) Exophilie et exophagie d'*Anopheles gambiae* Giles 1902 dans le Sud Cameroun. *Bull. Soc. path. exot.*, **50**, 446
- Muirhead-Thomson, R. C. (1951) *Mosquito behaviour in relation to malaria transmission and control in the tropics*, London, Arnold

- Müller, J. (1825) Über die Entwicklung der Eier im Eierstock bei den Gespenstheuschrecken und eine neuentdeckte Verbindung des Rückengefäßes mit den Eierstöcken bei den Insekten. *Nova Acta Acad. Leop. Caroz.*, **12**, 555
- Nabokov, V. A. & Nikiforova, A. V. (1947) [The use of DDT in malaria campaigns]. *Med. Parazit. (Mosk.)*, **16**, 10
- Nabokov, V. A. & Nikiforova, A. V. (1948) [Wide use of DDT in antimalaria campaigns]. *Med. Parazit. (Mosk.)*, **17**, 7
- Naumov, K. G. (1940) [Subspecies of *Anopheles maculipennis* in the Chu Valley]. *Sovetsk. Zdravookhr. Kirgiz.*, **5**, 84
- Naumov, N. P. (1955) [*Animal ecology*], Moscow, Publishing House "Sovetskaya Nauka"
- Neghme, A., Gutiérrez, J. & Alée, R. (1955) Attempt to eradicate *Anopheles* in the malaria zone of Chile. *Amer. J. trop. Med. Hyg.*, **4**, 1114
- Netski, G. I. (1957) [Results of phenological observations of *Anopheles maculipennis* in Omsk in the ten years 1940-1949]. In: Beklemishev W. N. & Shipitsina, N. K., ed. [*Seasonal phenomena in the life of malaria mosquitos in the USSR*], Moscow, Medgiz, p. 175
- Nicholson, A. J. (1921) The development of the ovary and ovarian eggs of the mosquito *A. maculipennis* Meig. *Quart. J. micr. Sci.*, **65**, 395
- Nikiforova, A. V. (1949) [Second year of widespread use of DDT in Orekhovo-Zuev peat-producing areas]. *Med. Parazit. (Mosk.)*, **18**, 52
- Nikolaev, B. P. (1935) [The influence of temperature on the development of the malaria parasite in the mosquito]. *Tr. Paster. Inst. Epidem. Bakt. (Leningr.)*, **2**, 108
- Nikolaev, B. P. & Shub, G. M. (1934) [Experimental infection of *Anopheles maculipennis* with malaria plasmodia in Leningrad]. In: [*Questions of social pathology and clinical practice in the Leningrad Oblast*], Leningrad, Leningrad Oblast Health Department, p. 202
- Novak, V. Y. A. (1959) *Insektenhormone*, Prague
- Oganov, L. I. (1947) [*Malaria and its seasonal changes in the Moscow Oblast*], Moscow (Thesis)
- Olsufiev, N. G. (1938) [*Tabanidae of the USSR*], Moscow (Thesis)
- Olsufiev, N. G. (1940) [Double character of feeding and sexual cycle in female Tabanidae]. *Zool. Ž.*, **19**, 445
- Pampana, E. J. (1954) Changing strategy in malaria control. *Bull. Wld Hlth Org.*, **11**, 513
- Pankova, A. P. (1959) [The age composition and epidemiological importance of overwintered and summer generations of *Anopheles* in various types of country in the south of the districts round the Ob River in the Novosibirsk Oblast]. In: [*Questions of theoretical and clinical medicine*]. *Tr. Novosibirsk. med. Inst.*, **33**, 24
- Pavlov, S. D. (1957) [The excretion of DDT in cow's milk after treatment of the animal's hide with various DDT formulations]. *Tr. vsesojuz. nauč.-issledovatel. Inst. Vet. Sanit. Ektoparazit.*, **11**, 260
- Pavlova, E. A. (1952) [The effect of temperature on the development of *Plasmodium gallinaceum* in *Aëdes aegypti* mosquitos]. *Med. Parazit. (Mosk.)*, **21**, 351
- Perfiliev, P. P. (1930) [Some data on the comparative anatomy of malaria mosquitos]. *Parazit. Sbornik zool. Inst. Akad. Nauk S.S.R.*, **1**, 75

- Perry, R. L. (1912) Malaria in the Jeypore Hill Tract and adjoining coastland. *Paludism.*, **5**, 32
- Plyater-Plokhotskaya, V. N. (1961) *Med. Parazit. (Mosk.)* (In press)
- Pokrovsky, S. V. (1935) [Experimental attempt at obtaining hibernating ovipositions of *A. maculipennis* in the laboratory]. *Vestn. Mikrobiol. Epidem. Parazit.*, **14**, 299
- Pokrovsky, S. V. (1946) [Observations on the population of *A. maculipennis* in the Nelidov region of Kalinin Oblast]. *Vop. Fiziol. Ekol. Malar. Komara*, **2**, 116
- Polikarpova, L. I. (1957) [Survival of *Anopheles* to the epidemiologically dangerous stage in Riga]. *Med. Parazit. (Mosk.)*, **26**, Supplement to No. 1, p. 27
- Polovodova, V. P. (1941) [Age changes in ovaries of *Anopheles* and methods of determination of age composition in mosquito populations]. *Med. Parazit. (Mosk.)*, **10**, 387
- Polovodova, V. P. (1947) [*Age changes in the female reproductive system of Anopheles and age composition of mosquito populations*], Rostov-on-Don (Thesis)
- Polovodova, V. P. (1949) [Determination of the physiological age of female *Anopheles*]. *Med. Parazit. (Mosk.)*, **18**, 352
- Polovodova, V. P. (1953) [Nervous system of the genitalia and rectum of malaria mosquitos]. *Zool. Ž.*, **32**, 635
- Pozdeev, T. S. (1954) [The effectiveness of BHC at various times of spraying of the day resting-places of the malaria mosquito]. *Med. Parazit. (Mosk.)*, **23**, 54
- Pozdeev, T. S. (1957) [Times of flight of *A. maculipennis* after hibernation in various microclimatic conditions of the Udmurtskaya ASSR]. In: Beklemishev, W. N. & Shipitsina, N. V., ed [Seasonal phenomena in the life of malaria mosquitos in the USSR], Moscow, Medgiz
- Pravikov, G. N. (1934) [Epidemiology of malaria in the Tashan area of Turkmenia]. *Sovetsk. Zdravoohr. Turkmen.*, **2-3**, 12
- Prokofieva, K. K. (1957) [Gonotrophic cycle of blood-feeding sandflies of Eastern Siberia]. *Zool. Ž.*, **36**, 1831
- Prokopenko, L. I. (1960) [The epidemiological effectiveness of contact insecticides used in combination with curative and prophylactic measures in the area of the Kura-Araks Depression]. In: [Experience in the use of residual contact insecticides in malaria control in the southern regions of the USSR]. *Tr. Inst. med. Parazit. trop. Med.*, p. 326
- Prokopenko, L. I., Shmeleva, Y. D. & Timrot, S. D. (1960) [Epidemiological and entomological observations on targets given early treatment. Observations in the villages of Kitskan and Kopanka]. In: [Experience in the use of residual contact insecticides in malaria control in the southern regions of the USSR]. *Tr. Inst. med. Parazit. trop. Med.*, p. 120
- Rageau, J., Adam, J. P. & Rivola, E. (1953) Etude préliminaire sur la biologie d'*Anopheles gambiae* Giles 1902, dans les régions forestières du Sud Cameroun. *Ann. Parasit. hum. comp.*, **28**, 425
- Rakhmanova, P. I. (1940) [On the types of gonotrophic cycle of *Anopheles* in natural conditions]. *Vop. Fiziol. Ekol. Malar. Komara*, **1**, 96
- Rayevsky, G. E. (1940) [Microclimate of hibernating places and behaviour of hibernating females of *A. maculipennis messeae*]. *Vop. Fiziol. Ekol. Malar. Komara*, **1**, 138

- Rayevsky, G. E. (1942) [Methods of epidemiological assessment of entomological observations]. *Med. Parazit. (Mosk.)*, **11**, 46
- Rayevsky, G. E. & Beylin, L. O. (1940) [Materials on the basic use of zooprophylaxis in malaria]. *Vop. Fiziol. Ekol. Malar. Komara*, **1**, 173
- Reingard, L. V., Goritskaya, V. V. & Zabudko-Reingard, T. N. (1953) [Causes of low effectiveness of barrier-spraying methods using BHC in some areas]. *Med. Parazit. (Mosk.)*, **22**, 237
- Remennikova, V. M. (1938) [Maturation of *P. vivax* in *A. maculipennis* in conditions of the northern zone of the European part of the USSR]. *Med. Parazit. (Mosk.)*, **7**, 530
- Remennikova, V. M. (1948) [The effect of various factors on the incidence of infection of malaria mosquitos]. *Med. Parazit. (Mosk.)*, **18**, 440
- Ribbands, C. R. (1947) The use of residual films of DDT and gammexane in malaria control. *Bull. ent. Res.*, **37**, 567
- Rice, J. B. & Barber, M. A. (1935) Malarial studies in Greece; modification of the Uhlenhuth-Weidanz precipitin test for determining the source of blood-meals in mosquitoes and other insects. *J. Lab. clin. Med.*, **20**, 876
- Richards, G. & Korde, F. H. (1950) Studies on arthropod cuticle. *Ann. ent. Soc. America*, **43**, 49
- Ross, R. (1910) *The prevention of malaria*, London, Murray
- Roubaud, E. (1929) Cycle autogène d'attente et générations hivernales suractives inaparentes chez le moustique commun, *Culex pipiens* L. *C. R. Acad. Sci. (Paris)*, **188**, 735
- Roubaud, E. (1934) Observations sur la fécondité des anophélinés. *Bull. Soc. Path. exot.*, **27**, 853
- Roubaud, E. & Mezger, J. (1934) La nourriture larvaire n'influe pas le développement de l'autogénèse chez les races de *Culex pipiens* spécifiquement anautogènes. *Ann. Parasit. hum. comp.*, **12**, 340
- Roy, D. N. (1931) On the ovulation of *A. stephensi*. *Indian J. med. Res.*, **19**, 629
- Roy, D. N. (1936) On the role of blood in ovulation in *Aedes aegypti*. *Bull. ent. Res.*, **27**, 423
- Rubtzov, I. A. (1936) Biology and ecology of black flies (Simuliidae) in Eastern Siberia. *Parazit. Sbornik zool. Inst. Akad. Nauk S.S.R.*, **6**, 169
- Rubtzov, I. A. (1955) [Changes in the activity of blood-sucking gnats in relation to the gonotrophic cycle]. *Tr. zool. Inst. Akad. Nauk S.S.R.*, **11**, 353
- Rubtzov, I. A. (1956) [*Fauna of the USSR. Insecta, Diptera*], Moscow, Publishing House of the USSR Academy of Sciences, Vol. 6
- Russell, P. F. & Rao, T. R. (1942a) On the swarming, mating and ovipositing behaviour of *A. culicifacies*. *Amer. J. trop. Med.*, **22**, 417
- Russell, P. F. & Rao, T. R. (1942b) Observation on the longevity of *A. culicifacies imagines*. *Amer. J. trop. Med.*, **22**, 517
- Saliternik, P. D. (1955) The specific biological characteristics of *Anopheles (Myzomyia) sergenti* (Theo.) and their correlation with malaria control in Israel. *Bull. ent. Res.*, **46**, 445

- Sarikian, S. Y. (1950) [The epidemiological effectiveness of spraying rural premises with DDT products in the vicinity of a large reservoir]. *Med. Parazit. (Mosk.)*, **19**, 202
- Sarikian, S. Y. & Polezhaev, V. G. (1941) [Epidemiological importance of mosquito control in dwellings]. *Med. Parazit. (Mosk.)*, **10**, 418
- Sarikian, S. Y. & Zhukova, T. A. (1959) [The organization of malaria control in a region with a low incidence of malaria (the Mozhaisk Rayon of Moscow Oblast)]. *Tr. Inst. med. Parazit. trop. Med.*, p. 215
- Saunders, D. S. (1960) Ovaries of *Glossina morsitans*. *Nature (Lond.)*, **185**, 121
- Sautet, J. (1934) Contribution à l'étude de l'*Anopheles maculipennis* en Corse. Gîtes d'été et d'hiver dans leurs rapports avec le paludisme. *Ann. Parasit. hum. comp.*, **12**, 8
- Sautet, J. (1937) Contribution à l'étude de l'exophilie de l'*Anopheles maculipennis labranchiae* et de l'*Anopheles sacharovi* dans ses rapports avec la transmission du paludisme. *Bull. Soc. Path. exot.*, **30**, 387
- Segay, E. M. (assisted by Suchkova, O. I.) (1952) [Epidemiological effectiveness of the use of DDT against malaria in Kursk]. *Med. Parazit. (Mosk.)*, **21**, 241
- Sella, M. (1920a) Relazione della campagna antianofelica di Fiumicino (1919) con speciale riguardo alla biologia degli Anofeli ed agli Anofeli infetti. *Ann. Igiene*, **30**, Supplemento 85
- Sella, M. (1920b) The antimalaria campaign at Fiumicino (Rome) with epidemiological and biological notes. *Int. J. publ. Hlth*, **1**, 316
- Serebryannaya, S. G. (1954) [The possibility of using milk from cattle treated with pentachlorine preparations (DDT)]. *Vračeb. Delo*, **8**, 717
- Sergiev, P. G. (1934) [The epidemiological importance of mosquito eradication indoors]. *Med. Parazit. (Mosk.)*, **3**, 315
- Sergiev, P. G. (1953) [Scientific basis of malaria eradication]. *Vestn. Akad. med. Nauk*, **4**, 9
- Sergiev, P. G. & Sarikian, S. Y. (1953) [Epidemiological effectiveness of focal DDT spraying in malaria programmes]. *Med. Parazit. (Mosk.)*, **22**, 224
- Sergiev, P. G. & Yakusheva, A. I. (1956) [*Malaria and its control in the USSR*], Moscow, Medgiz
- Shevchenko, A. K. (1953) [Survival of *A. maculipennis* to epidemiologically dangerous age in Kharkov Oblast]. *Med. Parazit. (Mosk.)*, **22**, 495
- Shevchenko, A. K. (1955) [*Malaria mosquitos in the Kharkov Oblast and their control using DDT and BHC*], Kharkov (Thesis)
- Shillinger, Y. I. (1953) [The passage of dichlorodiphenyltrichloroethane (DDT) into the milk of cows treated with DDT for the purposes of warble-fly control]. *Vop. pitan.*, **12**, 68
- Shipitsina, N. K. (1957) [Seasonal periodicity in the life of *A. maculipennis* and its importance in malaria control in the USSR]. In: Beklemishev, W. N. & Shipitsina, N. V., ed. [*Seasonal phenomena in the life of malaria mosquitos in the USSR*], Moscow, Medgiz, p. 485
- Shlenova, M. F. (1933) [Autumn changes in the physiological condition of female *Anopheles maculipennis* in Karatal (Semirechiye, Kazakhstan)]. *Med. Parazit. (Mosk.)*, **2**, 389

- Shlenova, M. F. (1938) [The speed of blood digestion in female *A. maculipennis messeae* at stable effective temperatures]. *Med. Parazit. (Mosk.)*, **7**, 716
- Shlenova, M. F. (1940) [Some practical results from experience of antimalarial measures in the peat industry of Ozeretsk]. *Med. Parazit. (Mosk.)*, **9**, 609
- Shlenova, M. F. (1941) [Sites of attacks of malaria mosquitos on the inhabitants of Kuibyshev]. *Med. Parazit. (Mosk.)*, **10**, 501
- Shmeleva, Y. D. (1935) [Biological observations on *A. maculipennis messeae* in peat bogs of the Moscow Oblast]. *Med. Parazit. (Mosk.)*, **4**, 369
- Shoshina, M. A. (1951) [Determination of a repeated gonotrophic cycle in sandflies]. *Doklady Akad. Nauk S.S.R.*, **28**, 181
- Shteinberg, D. M. (1932) [Cyclical method of the study of the reproductive system of the meadow butterfly]. *Sbornik VIZR'a (Leningr.)*, No. 4, 81
- Shute, P. G. (1936) A study of laboratory bred *A. maculipennis atroparvus* with special reference to egg laying. *Ann. trop. Med. Parasit.*, **30**, 11
- Shvanovich, B. N. (1949) [*A course of general entomology*], Moscow, Publishing House "Sovetskaya Nauka"
- Speranskaya, V. N. (1952) [Numerical prevalence of *A. maculipennis* in inhabited areas sprayed with DDT and BHC]. *Tr. Vo. med. Akad.*, **36**, 167
- Spielman, A. (1957) The inheritance of autogeny in the *Culex pipiens* complex of mosquitoes. *Amer. J. Hyg.*, **65**, 404
- Squire, F. A. (1950) Age grouping of tsetse flies as an aid in the study of their bionomics. *Nature (Lond.)*, **165**, 307
- Squire, F. A. (1951) Observations on mating scars in *Glossina palpalis* (R.D.). *Bull. ent. Res.*, **42**, 601
- Stratman-Thomas, W. K. (1940) The influence of temperature on *Plasmodium vivax*. *Amer. J. trop. Med.*, **20**, 703
- Sukhomlinova, O. I. (1956) [Seasonal biology of *A. maculipennis* in the Leningrad Oblast]. *Zool. Ž.*, **35**, 3
- Swellengrebel, N. H. (1929) La dissociation des fonctions sexuelles et nutritives (dissociation gonotrophique) d'*Anopheles maculipennis* comme cause du paludisme dans les Pays-Bas et ses rapports avec "l'infection domiciliaire". *Ann. Inst. Pasteur*, **43**, 1370
- Tairova, A. I. (1956) [Survival of *A. maculipennis* to epidemiologically dangerous age in the Stalinin region]. *Bjull. Minist. Zdravoohr. Gruz. S.S.R.*, **2**, 55
- Tairova, A. I. (1957) [Determination of the period between the flight of *A. maculipennis* from indoor resting-places after blood-digestion and the following blood-meal]. *Med. Parazit. (Mosk.)*, **26**, Supplement to No. 1, p. 32
- Tate, P. & Vincent, M. (1936) The biology of autogenous and anautogenous races of *Culex pipiens* L. (Diptera i Culicidae). *Parasitology*, **28**, 115
- Thomson, R. (1947) The effect of house spraying with pyrethrum and with DDT on *Anopheles gambiae* and *Anopheles melas* in West Africa. *Bull. ent. Res.*, **37**, 448
- Timrot, S. D. (1941) [Some observations on the natural hibernating places and the autumn distribution of mosquitos]. *Med. Parazit. (Mosk.)*, **10**, 455
- Tishchenko, O. D., Kucher, S. S. & Andrievsky, K. Y. (1951) [Effectiveness of barrier spraying of dwellings with DDT]. *Med. Parazit. (Mosk.)*, **20**, 458

- Trapido, H. (1946) The residual spraying of dwellings with DDT in the control of malaria transmission in Panama with special reference to *A. albimanus*. *Amer. J. trop. Med.*, **26**, 383
- Trapido, H. & Aitken, T. (1953) Study of a residual population of *Anopheles l. labranchiae* Falléroni in the Geremeas valley, Sardinia. *Amer. J. trop. Med. Hyg.*, **2**, 658
- Twinn, C. R., Hocking, B., McDuffie, W. C. & Cross, H. F. (1948) A preliminary account of the biting flies at Churchill, Manitoba. *Canad. J. Res., D*, **26**, 334
- Ustinov, A. A. (1941) [Seasonal changes of sizes of *A. maculipennis* in Abkhazia]. *Med. Parazit. (Mosk.)*, **10**, 457
- Vainshtein, N. B. (1938) [Seasonal changes of fertility in *A. maculipennis messeae* in Astrakhan]. *Med. Parazit. (Mosk.)*, **7**, 208
- Vainshtein, N. B. (1940) [Seasonal fertility of *A. maculipennis messeae*]. *Med. Parazit. (Mosk.)*, **9**, 301
- Verhein, A. (1921) Die Eibildung der Musciden. *Zool. Jahrb. Anat.*, **42**, 149
- Vermeil, C. (1953) De la reproduction par autogénèse chez *Aëdes detritus*. *Bull. Soc. Path. exot.*, **46**, 971
- Veshchezerov, N. I. (1928) [Material on artificial infection of *Anopheles* with malaria plasmodia]. *Tr. Severno-Kavkazsk. A.S.S.R. nauč.-issledovatel. Inst.*, **50**, 62
- Vinogradskaya, O. N. (1948) [Physiological condition of *A. maculipennis messeae* in the Moscow Oblast in autumn]. *Med. Parazit. (Mosk.)*, **17**, 166
- Vinogradskaya, O. N. (1960) [*The morphology and physiology of the respiratory apparatus and the water balance in adult blood-sucking mosquitos in relation to conditions in the habitat*], Moscow (Thesis, USSR Academy of Medical Sciences)
- Vinogradskaya, O. N., Nabokov, V. A. & Shmeleva, Y. D. (1949) [Observations on *A. maculipennis* in DDT-sprayed rooms]. *Med. Parazit. (Mosk.)*, **18**, 44
- Vinogradskaya, O. N. & Shmeleva, Y. D. (1934) [Turnover of *A. maculipennis* in its feeding-places]. *Med. Parazit. (Mosk.)*, **3**, 480
- Vlasenko, N. M. (1956) [*Ecological basis for the epidemiology and prophylaxis of malaria in the Barabinsk Depression*], Moscow (Thesis, USSR Academy of Medical Sciences)
- Vlasenko, N. M. (1958) [Age composition of *Anopheles maculipennis messeae* in the Barabinsk Depression and its survival to an epidemiologically dangerous age]. [Questions of theoretical and clinical medicine]. *Tr. Novosibirsk. med. Inst.*, **30**, 10
- Wanson, M. (1950) Contribution à l'étude de l'onchocercose africaine humaine (problèmes de prophylaxie à Léopoldville). *Ann. Soc. belge Méd. trop.*, **30**, 667
- Webb, Y. E. (1945) On the respiratory mechanism of *Melophagus ovinus* L. *Proc. Zool. Soc.*, **115**, 218
- Weidling, R. (1928) Die Beeinflussung von Eiröhrenzahl und Grösse einiger Dipteren durch Hunger im Larvenstadium mit einigen Beobachtungen über die Chaetotaxis der Hungertiere und über den Einfluss verschiedener physikalischer und chemischer Einwirkungen auf den Entwicklungsgang dieser Tiere. *Z. angew. Ent.*, **16**, 68
- West, A. S. & Eligh, G. S. (1952) The rate of digestion of blood in mosquitos. Precipitin test studies. *Canad. J. Zool.*, **30**, 267
- Wigglesworth, V. B. (1930a) A theory of tracheal respiration in insects. *Proc. roy. Soc. B*, **106**, 229

- Wigglesworth, V. B. (1930b) Some notes on the physiology of insects related to human disease. *Trans. roy. Soc. trop. Med. Hyg.*, **23**, 6
- Wigglesworth, V. B. (1938) The absorption from the tracheal system of mosquito larvae at hatching and moulting. *J. exp. Biol.*, **25**, 2
- Wigglesworth, V. B. (1939) Source of the moulting hormone in *Rhodnius* (Hemiptera). *Nature (Lond.)*, **144**, 753
- Wigglesworth, V. B. (1940) The determination of characters at metamorphosis in *Rhodnius prolixus*. *J. exp. Biol.*, **17**, 201
- Wigglesworth, V. B. (1947) The corpus allatum and the control of metamorphosis in insects. *Nature (Lond.)*, **159**, 872
- Wigglesworth, V. B. (1950a) A new method for injecting the tracheae and tracheoles of insects. *Quart. J. micr. Sci.*, **91**, 2
- Wigglesworth, V. B. (1950b) *The principles of insect physiology*, 4th ed., London, Methuen
- Wigglesworth, V. B. (1959) The role of the epidermal cells in the migration of tracheoles in *Rhodnius prolixus*. *Quart. J. micr. Sci.*, **100**, 315
- Woke, P. A. (1937) Comparative effects of the blood of different species of vertebrates on egg-production of *Aedes aegypti*, L. *Amer. J. trop. Med.*, **17**, 729
- Yaguzhinskaya, L. V. (1945) [Malaria vectors in the northern part of the Urgut region in 1943]. *Med. Parazit. (Mosk.)*, **14**, 47
- Zalutskaya, L. I. (1959) [Comparative data on the biology of *Anopheles minimus* and *Anopheles vagus* in the vicinity of the city of Tai-Nguen in the Democratic Republic of Viet-Nam]. *Med. Parazit. (Mosk.)*, **28**, 548
- Zaplyushevka, A. I. (1952) [Barrier spraying of premises with DDT and BHC in the control of malaria mosquitos]. *Med. Parazit. (Mosk.)*, **21**, 547 (Abstract)
- Zolotarev, E. K. (1936) [The Asian locust in the southern left-bank rayons of Kuibyshev Krai]. *Zool. Ž.*, **15**, 731
- Zorin, V. M. (1957) [Epidemiological assessment of various age-groups of the population of *A. maculipennis* in the Vitebsk Oblast]. *Med. Parazit. (Mosk.)*, **26**, Supplement to No. 1, p. 13
- Zviangintzev, S. N. (1946) [Biology of *A. maculipennis* in the Little Kabarda]. *Vop. Fiziol. Ekol. Malar. Komara*, **2**, 165



A N N E X

Annex

THE OVARY AND OVARIOLES OF MOSQUITOS

D. S. BERTRAM

*Professor of Entomology
London School of Hygiene and Tropical Medicine
London, England*

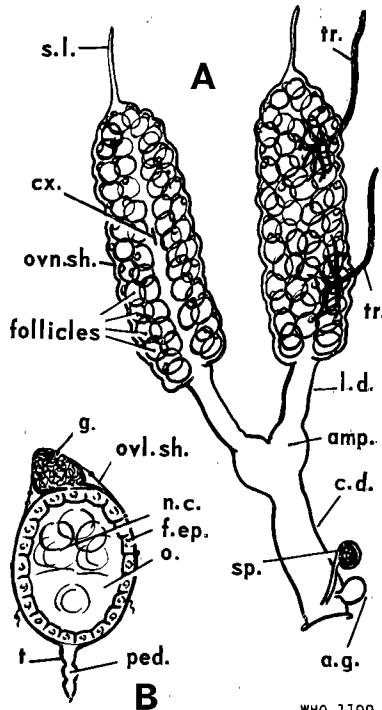
The glossary and illustrations which accompany this note were prepared for the London Course in Advanced Entomological Techniques. They were designed to illustrate the relationship of structures within the mosquito ovary—a subject particularly pertinent to the course—and to offer some clarification of differences and obscurities in interpretations and terminology of these structures. For this purpose, in addition to consulting the particular references cited in the text below, and other literature,¹ certain points of anatomy were checked in fresh dissections and in osmium-fixed preparations of ovaries of *Anopheles labranchiae atroparvus*, *Anopheles gambiae*, *Aëdes aegypti*, and *Mansonia uniformis*.

The present note, based on the glossary, includes modifications of the original lecture, in the light of some discussion of terminology during the course, and is supplemented by a brief account of the morphology of the reproductive system of female mosquitos and of oogenesis, or egg development. In the text and figures only the preferred term for a structure is consistently employed. Occasionally in the text note is made in parentheses of an alternative term, either because of its familiarity from considerable usage or for other reasons. In the glossary, the preferred term is followed in parentheses by one or several alternatives which appear in the literature. It is hoped that the present note may contribute to some stability in terminology. It is apparent that in mosquitos the morphology and function of certain structures in the reproductive organs are as yet

¹ For example: Bertram, D. S. & Samarawickrema, W. A. (1958) *Nature (Lond.)*, 182, 444; Bonhag, P. F. (1958) *Ann. Rev. Ent.*, 3, 137; Colless, D. H. (1958) *Trans. roy. Soc. trop. Med. Hyg.*, 52, 187; Puri, I. M. (1942) *A practical course for students of malariology*, 3rd ed., Calcutta, Government Press (*Hist. Bull.*, Vol. 3); Snodgrass, R. E. (1935) *The principles of insect morphology*. New York, McGraw-Hill; Wigglesworth, V. B. (1949) In: Boyd, M. F., *Malaria*, Philadelphia, Saunders; Wigglesworth, V. B. (1950) *The principles of insect physiology*, 4th ed., London, Methuen.

less certainly understood or established than current and probably future interest in these organs requires. But some recent progress towards a solution to such deficiencies is already apparent.

FIG. 1. A: REPRODUCTIVE SYSTEM OF FEMALE MOSQUITO; B: OVARIOLE OF A NULLIPAROUS FEMALE SHOWING STAGE I FOLLICLE, AS ISOLATED BY DISSECTION*



* A trace of ovariole sheath may be seen protruding laterally from follicle. Pedicel may not be complete.

General Morphology (Fig. 1A)

The reproductive organs of the non-gravid female mosquito include two fusiform ovaries, situated somewhat dorsally in the abdomen at about the level of the fifth segment, together with associated ducts. A lateral duct connects each ovary to a common oviduct, which communicates with the exterior at the genital opening situated ventrally between abdominal segments 8 and 9. Each lateral oviduct is expanded to form an ampulla at its junction with the opposite oviduct from the other ovary and with the common oviduct (gynaecophoric canal). Opening into the common oviduct on its dorsal surface near the genital opening is a small accessory (mucous, spermathecal or cement) gland, and in *Anopheles* the long, narrow duct leading to a single spermatheca. There are two or three spermathecae in culicine mosquitos. A spermatheca is a small, chitinized, spherical structure conspicuous for its brown to black colour and perforate appearance. It serves to store spermatozoa after insemination until eggs are ready for fertilization.

The Ovary (Fig. 1A and 2)

The limiting membrane forming the outer surface of an ovary is thin, elastic and characterized by small nuclei dispersed in an anastomosing system of fine muscle strands. Known as the ovarian sheath it forms an elong-

gate-ovoid chamber which, anteriorly, narrows to form the so-called suspensory ligament. Packed tightly together within this chamber are about 75 tapering tubes, the ovarioles, the sources of the eggs of a mosquito. Running centrally within the ovary is a tube, the calyx, its lumen being continuous with that of the lateral duct. The ovarioles are arranged more or less radially round the calyx throughout its length, attached to it by their broader, posterior, ends and with their tapered anterior ends disposed outwards and forwards so that they lie close under the ovarian sheath. The extreme anterior end of an ovariole in mosquitos is scarcely prolonged into a terminal filament. The extremities of the ovariole are termed, as above, anterior and posterior, since this designation defines their relative positions clearly and avoids the ambiguity which can arise if the terms distal and proximal are employed.

A rich tracheal supply to the ovarioles is provided by two conspicuous tracheae entering the ovary from the abdominal cavity, at positions some way from each end of the ovary. Dividing a number of times just before passing through the ovarian sheath, they branch more profusely again within the ovary and penetrate down between the ovarioles to form there tightly coiled leashes, or skeins, of fine tracheae and tracheoles (in a nulliparous ovary).

The Ovariole (Fig. 1B and 2A)

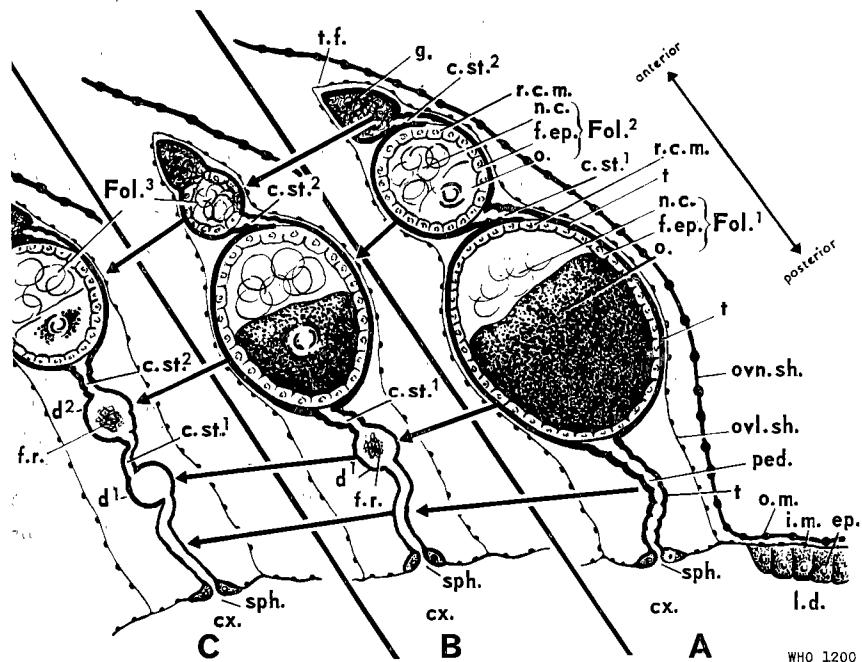
Each ovariole consists of the following elements:

- (1) A *follicular tube* formed by a thin structureless elastic membrane, the tunica, within which the eggs originate, as described below.
- (2) An elastic *ovariole sheath*, which closely invests the follicular tube on its outer surface. It is not dissimilar in structure to the nucleate and muscled ovarian sheath. Indeed, fine muscle strands cross between these two structurally similar sheaths.

The contents of the follicular tube and the changes they undergo in oogenesis are of first importance.

The anterior, tapered end of each follicular tube contains the germarium, a zone of undifferentiated nucleated tissue—possibly a syncytium. An egg originates in the germarium from a primordial germ cell, or oogonium, budded from this tissue. The oogonium divides into eight daughter cells of which one is the uninucleate female germ cell, or oocyte (ovum), which will develop into an egg, while the remaining seven will accompany it as nurse cells (trophocytes) during its growth. As the oocyte and its nurse cells pass backwards from the germinal tissue, they become completely enclosed in an epithelial sac composed of small cells also derived from the germinal zone. This sac and its contents are the follicle and its cellular

FIG. 2. RELATIONSHIP OF STRUCTURES IN AN OVARIOLE DURING SUCCESSIVE GONOTROPHIC CYCLES



WHO 1200

- A From nulliparous female, with primary follicle (Fol¹) at early stage III and second follicle (Fol²) shown at stage I. No dilatation.
- B From parous female, after first oviposition (uniparous) and former primary follicle represented by dilatation (d¹) containing follicular relict (f.r.). Second follicle at stage II.
- C From parous female, after two ovipositions (diparous) showing two dilatations (d¹ and d², the latter only containing a follicular relict) representing, respectively, positions of former primary (first) and second follicles. Third follicle (Fol³) at early stage II.

c.st. ¹ , c.st. ²	First connecting stalk, second connecting stalk
cx	Calyx
d ¹ , d ²	Dilatations representing former primary (first) and second follicles
ep.	Epithelium of lateral oviduct wall
f. ep.	Follicular epithelium
Fol ¹ , Fol ² , Fol ³	Primary (first) follicle, second follicle, third follicle
f.r.	Follicular relict
g.	Germarium
i.m.	Inner longitudinal muscle layer of lateral oviduct wall
l.d.	Lateral oviduct
n.c.	Nurse cell region
o.	Oocyte (stippling indicates yolk granules; nucleus a central clear area)
o.m.	Outer circular muscle layer of lateral oviduct wall
ovl. sh.	Ovariole sheath
ovn. sh.	Ovarian sheath
ped.	Pedicle
r.c.m.	Position of rosette cells (Nicholson) from which micropyle is formed in fully developed oocyte
sph.	Sphincter composed of rosette cells (Giglioli)
t.	Tunica (a thin membrane, but shown thick in figure to emphasize its function as wall of follicular tube)
t.f.	Terminal filament (Tracheoles omitted)

wall is the follicular epithelium. In a young, unfed female which has yet to develop its first batch of eggs, this first follicle in each ovariole grows a little, but becomes quiescent as a bulbous swelling about $60-80\mu$ long (Fig. 1B) if the female remains unfed. Occupying much of the follicular tube, it remains linked by a short, narrow, asymmetrically disposed connecting stalk (funicle) with the small germarium. Posterior to the follicle (cf. Fig. 2A), the follicular tube again narrows—as it does in the connecting stalk—to form the wall of the pedicel (terminal pedicle, terminal funicle), which extends, from a slightly lateral position on the follicle, to the calyx. The pedicel and connecting stalks have, at least before an oocyte passes through them, an imperfectly beaded appearance due to regularly repeated constrictions of the wall of the follicular tube. In *Anopheles maculipennis*, the pedicel is longer than any connecting stalks. Within the follicular epithelium of the first, or primary, follicle the nurse cells and oocyte are distinct, the latter posterior to, and taking up rather less space than, the nurse cells. This primary follicle is the first of a series of follicles destined to be budded off progressively in time from the germarium during the mosquito's reproductive activities. Indeed, the second follicle may already be recognizable at an earlier, and smaller, stage of its formation anterior to the first, an asymmetrically disposed connecting stalk intervening between them. This small follicle, together with the germarium, appears as a small cap mounted, often sideways, on the anterior end of the relatively large primary follicle.

The internal structure of the narrow parts of the follicular tube, the connecting stalks and pedicel, is not well understood. Generally accepted as necessarily tubular, it may be that they have a solid cellular core,¹ at least until they are breached by the passage backwards of a fully developed oocyte (egg) into the calyx. In *Anopheles gambiae*, Giglioli² observed the pedicel (terminal funicle) as being tubular; it opens into the calyx through a rosette of cells (the funicle rosette) which form a sphincter round the orifice. The rosette cells are continuous peripherally with the calyx wall.

The delicate ovariole sheath, which in fact commonly disappears in dissections, and the thin tunica it so closely invests are difficult to differentiate in dissected material (Fig. 1B). The ovariole sheath if still present may, however, be perceived by the existence of occasional protuberances, its nuclei, on the outer surface of a follicle, where it overlies the fine inapparent tunica, or as a delicate membrane (with, perhaps, one or two dot-like nuclei) stretching across from one follicle to another at the level of a connecting stalk. Here it is separated from the tunica, which still defines the wall of the connecting stalk. This has been shown by Lewis.³

¹ Nicholson, A. J. (1921) *Quart. J. micr. Sci.*, **65**, 395.

² Giglioli, M. E. C. (1959) *Trans. roy. Soc. trop. Med. Hyg.*, **53**, 310.

³ Lewis, D. J. (1958) *Trans. roy. Soc. trop. Med. Hyg.*, **52**, 456.

In the region of the pedicel the ovariole sheath is a wider, looser sleeve, which continues into the calyx wall as its elastic muscular layer. Centrally within it is the much narrower pedicel (cf. Fig. 2A).

There appears to be some attachment of the ovariole sheath to the tunica of the follicular tube in the region of the germarium. On occasions, during dissections to expose the follicular tube, the ovariole sheath may be stripped back from its position ensheathing the tube. It may then be found as a strand of tissue adherent to the germarium region, rather like the finger of a glove peeled back from a hand but still attached to the finger tip.

The Walls of the Calyx and Lateral Oviduct (Fig. 1A and 2A)

At the junction of the calyx and lateral oviduct, the muscular elastic layer of the calyx wall continues backwards as the inner longitudinal muscle layer of the wall of the lateral oviduct. This is overlaid by an outer circular muscle layer continuous anteriorly as the ovarian sheath. According to Nicholson¹ in *A. maculipennis*, but possibly not in *A. gambiae* (Giglioli, personal communication), the lateral oviduct has an internal epithelial layer. It is not certain if an epithelium lines the calyx.

Oogenesis and Ovarian Changes (Fig. 2A, B and C)

A female which has yet to lay its first batch of eggs is said to be nulliparous, or a nullipar. A parous female, on the other hand, has laid at least one batch of eggs. Pauciparous is considered appropriate for a female which has laid one or two batches, as opposed to multiparous (or a multipar), when more than two batches have been formed. The suggestion was also put forward during the London Course that, if the exact number of layings is known, terms such as 1-parous, 2-parous, etc., would be a suitable notation in print, although for verbal reference uniparous and biparous, etc., would seem more convenient. Such terms as primiparous, or a primipar, should be avoided, since, in their usage in human obstetrics, they denote a woman who is bearing, or has borne, her first offspring. Their use for mosquitos could cause considerable ambiguity as to whether a nulliparous or uniparous (1-parous) state was under discussion. In so far as a uniparous female is a particular instance of the parous state, ambiguity would arise concerning two readily separable and useful groups of a mosquito population—viz., the nulliparous and the parous females.

¹ Op. cit.

The ovary in the nulliparous female (recently emerged and unfed) is usually a little more than 1 mm long, translucent, and contains as the obvious part of each ovariole the quiescent first follicle, as already described. No further development takes place until the mosquito obtains a suitable meal, commonly a blood-meal. Development of the first follicle in the ovarioles then proceeds as the meal is digested with great enlargement of the ovary as a whole, and culminates in oviposition. Two blood-meals may be necessary for the completion of this first gonotrophic cycle. One meal normally suffices in subsequent cycles, which may take no longer than 48 hours in tropical conditions.

The progressive changes in the ovary and ovarioles during oogenesis may be divided into five arbitrary phases—viz., stages I-V of Christophers.¹ *Stage I*, the initial quiescent phase found only in the unfed, nulliparous female, is as described above. The follicle is about 60-80 μ in length, the ovary about 1 mm long. Absence of yolk granules from the oocyte is an important feature. A meal initiates further development into *stage II*, which begins with the appearance of granules of yolk round the nucleus of the oocyte. During this stage, the oocyte grows to occupy the posterior half of the follicle and, with its cytoplasm becoming uniformly opaque with yolk granules, it is distinctive from the transparent area of the nurse cells. The oocyte nucleus remains visible. The follicle is oval and attains a length of about 100 μ or so.

Stage III begins when the oocyte nucleus becomes obscured by the accumulating yolk. The opaque, yolk-laden oocyte takes up about three-quarters of the follicle, which becomes broadly oval and about 200 μ long. In *stage IV* rapid growth and elongation of the oocyte and, with that, of the follicle occur until the fusiform shape and length (0.5-1 mm) of an egg is attained. The white, opaque oocyte now fills almost the entire follicle, the nurse cells being restricted to a small transparent area of its anterior end. *Stage V* begins with the recognition, in anophelines, of the first striations marking the appearance of egg floats. The egg-shell (chorion) and its various cuticular details are completed. This stage lasts until the oocyte, as a fully formed egg, is oviposited. Some refinements to this classical recognition of five stages were found useful by Mer² and Macan³. These refer to the degrees of yolk distribution in stages II and III, except a stage N, preceding stage I, which Mer recognized as having a cuboidal follicular epithelium but eight still undifferentiated cells within the follicle.

Throughout this development, the follicular epithelium becomes progressively thinner. It is finally reduced to groups of disintegrated cells difficult to detect, but, prior to this, plays an important part in forming

¹ Christophers, S. R. (1911) *Paludism*, 2, 73.

² Mer, G. (1932) *Bull. ent. Res.*, 23, 563.

³ Macan, T. T. (1950) *Mem. Lond. Sch. Hyg. trop. Med.*, 7, 109.

the egg-shell, its floats and other cuticular structures. In this connexion, it may be noted that the connecting stalk from the next follicle in the chain joins the developed follicle at a slightly antero-lateral position (= antero-ventral position on the egg when laid). Here the micropyle is formed, a small porous structure in the egg-shell through which spermatozoa later enter to fertilize the oocyte nucleus. In *A. maculipennis* a rosette of cells of the follicular epithelium at this point, associated with the residual nurse cells which by now are extruded beyond the egg-shell, take part together in the formation of the micropyle (Nicholson¹). These rosette cells concerned with micropylar formation are transient, occurring afresh in successive follicles as part of the follicular epithelium, which finally disintegrates. The cells of the funicle rosette (Giglioli² for *A. gambiae*) are located as a sphincter only at the orifice of the pedicel into the calyx and are permanent. When the egg-shell is fully formed and the follicular epithelium no longer apparent as a discrete layer of cells, the now egg-like oocyte is bounded only by the virtually imperceptible thin, stretched tunica and the overlying ovariole sheath.

Since all, or most, of the ovarioles develop synchronously in this way, the ovaries before oviposition become conspicuous bodies about 3-4 mm long, one to each side of the abdomen, their mass of overlapping ovarioles resembling a bunch of tightly packed white bananas occupying most of the abdominal space. The elasticity of the ovariole sheath, tunica, calyx wall and ovarian sheath permits this distension. In dissection at stages IV and V, these taut membranes may rupture, readily setting free the egg-like oocytes, some of which may bear a "cap" of follicular tube consisting of a chain of the next one or two follicles and the germarium, possibly including the overlying ovariole sheath. Little blood now remains in the stomach.

Ovulation, Fertilization and Oviposition

Ovulation is the process in which the fully developed oocytes—they are now commonly referred to as eggs—leave their follicular tubes and pass into the calyx. There is some evidence to suggest that they may protrude from the follicular tube into the calyx for some time before passing free into the calyx. As regards the route taken at ovulation, there seems general agreement that, at least during the course of the ovulation of the first batch of fully developed oocytes, the pedicel must become a tube through which the oocyte passes to the calyx, and that it remains patent thereafter. This is consistent with expectation, and appears to be substantiated by Giglioli² in *A. gambiae*. The ovulated oocytes (eggs) proceed in sequence through the lateral and common oviducts, being fertilized as

¹ Op. cit.
² Op. cit.

they pass the spermathecal duct immediately before oviposition. Maturation of divisions of the oocyte nucleus with the formation of polar bodies and subsequent embryonic development of the larva is completed after oviposition. Once the egg is laid the shell darkens rapidly.

The Ovaries and Ovarioles after Oviposition (Fig. 2B and C)

It is not the purpose of this Annex to discuss in detail the changes in the ovary and ovarioles following oviposition, but to consider briefly only certain anatomical relationships involved in terms of the preceding descriptions. From the time when Mer¹ described the increase in the diameter of ovarian ampullae after ovulation, attention has been drawn to the possibility of determining the age of female mosquitos from the anatomical changes in their genital tract. The discovery of the significance of dilatations of the follicular tube referred to below is due to Polovodova.² Details are given by Gillies³ and more fully in this monograph. As the nulliparous female completes her first ovulation and oviposition, the elasticity of the various membranes allows the ovary and ovarioles to contract towards, although not quite to, their original dimensions. At first, a follicular tube in the region occupied by the former follicle is capacious, sac-like and of the dimensions of the egg just laid. It contains cellular debris of the follicular epithelium. The wall of this sac is the tunica, overlaid closely by the investing ovariole sheath. The sac is in communication with the calyx through the now relatively short pedicel, which is also distended as part of the sac. Over the next few hours, the sac shrinks and shortens to narrow tubular shape, but the original position of the former follicle remains apparent as a small persisting distension of the tunica, called a dilatation (thickening). Usually, but not inevitably, some cellular remainder of the follicular epithelium is confined within the dilatation. This is known as the follicular relic (corpus luteum, follicular residue body). Anteriorly to this dilatation of the follicular tube, its wall, the tunica, forms the connecting stalk to the next follicle, while posteriorly to the dilatation it forms the wall of the pedicel, again relaxed to its narrow, tubular form. The ovariole sheath still closely invests the follicular tube, with the exception that from the posterior curvature of the new current follicle it runs back as a broad sleeve, embracing loosely not only the pedicel but also the dilatation and the stalk linking it to the new follicle. It may be noted that the next follicle is already at stage II, yolk granules being present round the oocyte nucleus. This is always the case with the next follicle of the chain by the time a batch of eggs is laid—that is, at

¹ Op. cit.

² Polovodova, V. P. (1941) *Med. Parazit. (Mosk.)*, **10**, 387.

³ Gillies, M. T. (1958) *Trop. Dis. Bull.*, **55**, 713.

the end of a gonotrophic cycle. The nulliparous female has now become parous, or, more precisely, 1-parous or uniparous.

Detinova¹ reported that the appearance of the tracheolar skeins of the ovary is permanently altered after the first oviposition. The tightly coiled skeins of the nulliparous female are different from the loose coils of the parous ovary. This important observation allows for easy distinction between the two groups and gives some indication of the age composition of a mosquito population.²

The Ovariole in Multiparous Females (Fig. 2C)

This process of follicular development—with great distension of the follicular tube, followed by its subsequent contraction to narrow tubular form but leaving a dilatation of the tunica to mark the site of the recently ovulated follicle—is repeated in each gonotrophic cycle. Moreover, the dilatation for each cycle persists despite subsequent cycles. Thus, a chain of dilatations of the tunica intercommunicating by narrow, tubular, connecting stalks extends between the currently formed follicle and the tubular pedicel of the multiparous (here including 2-parous or pauciparous) females. A follicular relic of cellular debris may be present in some or all of the dilatations. Throughout the zone of dilatations the ovariole sheath remains as a wide outer sleeve. Unlike the serially dilated tunica, it is without significant malformation to indicate the position of former follicles. In dissections to expose the dilatations, the ovariole sheath may be disrupted and removed inadvertently, isolating the follicular tube with all or part of its posterior chain of dilatations attached. The length of the pedicel relative to the shorter stalks appears to be potentially useful in deciding if a chain is complete or not.

There is some evidence that in the parous females this serial arrangement of discrete dilatations, each representing a former follicle, may be, in some species of mosquito (e.g., *A. gambiae*), less clearly demarcated or even non-existent, although the phenomenon is already established beyond question for other species (e.g., *A. maculipennis*, *Mansonia* spp.). When it is proposed to apply in the field this principle of aging mosquitos to a species not hitherto studied in this way, it would be well, if possible, to confirm the practicability of the technique for the species in question by studies with laboratory colony material.

The foregoing descriptions clarify, it is hoped, the relationships and changes in the ovarioles of a mosquito during reproduction in a way which may be helpful to those aspiring to proficiency and confidence in the quite delicate dissection required for their demonstration.

¹ Detinova, T. S. (1945) *Med. Parazit. (Mosk.)*, 14, 45.

² Detinova, T. S. (1946) *Med. Parazit. (Mosk.)*, 15, 52.

GLOSSARY

Ampulla (see under **Lateral oviduct**)

Anterior (proximal, distal) end of ovariole

The more forwardly disposed, tapering end of an ovariole containing the germarium.

Calyx (inner or internal oviduct, lumen of ovary)

The central tube in an ovary into which the fully developed oocytes (eggs) of the ovarioles pass at ovulation.

Connecting stalk (funicle, interfollicular stalk, interfollicular constriction or interfollicular tissue)

The narrow parts of the follicular tube between the germarium and the most recently produced follicle, between follicles, and between dilatations. Between dilatations they are tubular, but possibly have a cellular core in the more anterior region of the follicular tube where follicles are present. They may be designated first, second, etc., the first one being that anterior to the primary follicle, or to the dilatation representing it in a parous female.

Degenerate follicle

A follicle in which oocyte, nurse cells and follicular epithelium fail to develop normally and disintegrate to cellular debris forming what constitutes a follicular relic, although of abnormal origin.

Dilatation (ovariolar thickening, pigmented thickening, thickening, bead, structureless bag, sac; old follicle, relic or remnant of follicle)

A distension of the tunica, the thin membranous wall of the follicular tube, at the position of a former follicle in a parous female. It may be empty or include cell debris of a follicle, i.e., a **follicular relic** (see below).

Note: Alternative terms for a dilatation, as defined here, and for a follicular relic are not always sharply separable, since, in the literature, these two elements of a locus of former oogenesis have not always been clearly differentiated. Most of the alternatives given above indicate, or imply, the distension of a dilatation rather than its content. Under **follicular relic**, defined below as cell debris of the follicular epithelium or its contained cellular elements, alternatives given include terms clearly referring to, or implying, the contents of a dilatation. It will be apparent, however, that some of the

alternatives may, in fact, be interpreted either way. In general, this may not obscure the sense of a particular text, but it seems appropriate to remove such ambiguities by using, as proposed here, separate terms for the two discrete elements of a former locus of oogenesis—viz., "dilatation" for the distended wall, or tunica, of the follicular tube, and "follicular relic" for the disintegrated traces of cellular follicle material which may still be found in it.

Egg (oocyte, ovum)

Loosely used, often with reference to the later stages of the development of the oocyte when its size and shape are approaching that of the typical egg, and its covering membrane (egg-shell, chorion) is well formed.

Follicle

An epithelial sac lying within the follicular tube and containing an oocyte and its nurse cells. Persists until disintegration of its wall, the follicular epithelium, after this has been concerned in forming the egg-shell round the fully developed oocyte. The latter then lies free within the distended tunica, which is the wall of the follicular tube.

Follicular epithelium

The epithelial wall of a follicle surrounding the oocyte and nurse cells within the follicle. It almost completely disappears on formation of the egg-shell, but traces of cellular debris may persist as a follicular relic within a dilatation of the tunica.

Follicular relic (yellow body of ovariole, corpus luteum, pigmented thickening, follicular residue body; degenerate or old follicle, relic or remnant of follicle)

The cellular debris of the follicular epithelium of a former follicle present in some, or all, of the dilatations of the follicular tube in a parous female. The follicle may have developed normally or degenerated prematurely. In the latter case the follicular relic includes also degenerate oocyte and nurse cell material. (See also **Dilatation**, above.)

Follicular tube (ovariole, ovarian tube, egg-tube, egg-string, chain of ovarian follicles)

The tube formed by the thin, structureless, elastic membrane known as the tunica (tunica propria) and which contains and connects by stalks (funicles) the germarium and successive follicles and any follicular relics, and terminates posteriorly as the pedicel opening into the calyx.

Funicle (see Connecting stalk)

Germarium (end-chamber, terminal chamber, rudimentary zone, pre-follicular tissue)

The anterior, tapering end of an ovariole consisting of the germinal epithelium, possibly a syncytium, giving rise to (a) primordial germ cells (oogonia) differentiating into oocytes and nurse cells, and (b) other cells which become the follicular epithelium.

Intima (see Tunica, Ovariole sheath)**Lateral oviduct** (ovarian tube, oviduct)

The tubular, posterior continuation of the calyx of an ovary, having possibly an internal cellular epithelium, an overlying longitudinal muscle layer (continuous with the muscular nucleated membrane which forms the *ovariole* sheath, and an outer, circular, muscle layer (continuous with the muscular, nucleated membrane which forms the *ovarian* sheath). Dilated towards its junction with its fellow from the other ovary to form an *ampulla* in anophelines and some culicines.

Micropyle

A small porous area in the egg-shell in an antero-ventral position, and formed by a rosette of cells of the follicular epithelium, together with the residual nurse cells extruded into this region of the epithelium. The micropyle provides for the entry of spermatozoa into the oocyte at fertilization.

Nurse cell (trophocyte, nutritive cell, nourishment cell)

Each of seven cells derived, together with one oocyte, from a primordial germ cell or oogonium, and accompanying the oocyte in its follicle until they are discharged, at a late stage in the formation of the egg and its shell (chorion), to be absorbed by the follicular epithelium just anterior to the position of the micropyle of the egg (i.e., at the point where the secondary stalk joins the follicular epithelium). Concerned mainly with the nutrition of the single oocyte.

Oocyte (ovum, egg)

The uninucleate female germ cell prior to maturation divisions, which are not initiated until fertilization just before oviposition. Derived from division of a primordial germ cell (oogonium) of the germarium into one oocyte and seven nurse cells, it fills with yolk granules and forms the opaque, fully developed "egg" with its egg-shell, or chorion, in the final stage of its growth.

Oogonium

A primordial female germ cell in the germarium which divides into one oocyte and seven nurse cells.

Ovarian sheath (outer membrane of ovary, intimal sheath of ovary, investing membrane, elastic membrane, peritoneal sheath)

The thin, elastic, membranous outer wall of the ovary within which lie the many ovarioles, each within its own ovariole sheath. Nuclei, distributed over this membrane, are connected by an anastomosing system of fine muscle strands, some of which cross to the similar nucleated and muscled ovariole sheaths.

Ovariole

A tapered tube comprising (*a*) the follicular tube in which oogenesis takes place, and (*b*) the ovariole sheath, which closely invests the follicular tube on its outer surface.

Ovariole sheath (ovariolar sheath, inner or internal membrane, follicular tube membrane, intima, epithelial sheath, ovariole limiting membrane, connective tissue layer or coat)

A thin elastic membrane closely apposed externally to the tunica, the wall of the follicular tube; with scattered nuclei and fine anastomosing muscle strands. Posteriorly to the currently developing follicle, it forms a wider sleeve round the pedicel of the follicular tube and continues into the calyx as its nucleated, muscled wall.

Ovary

The female reproductive gland or organ, composed of numerous ovarioles opening into its lumen, or calyx, the whole encased within a nucleated, muscled membrane—the ovarian sheath.

Oviposition

The act of laying eggs through the external genital opening.

Ovulation

The passage of an oocyte (egg) from the follicular tube into the calyx.

Ovum (see Oocyte, Egg)**Pedicel** (terminal pedicle; terminal, first, or distal funicle; distal or terminal stalk; terminal part of ovariole tube; short length of connective tissue)

The portion of the follicular tube between the calyx and the first, or primary, follicle during its oogenesis, or, after its ovulation, between the calyx and the dilatation marking the original position of the primary follicle. The pedicel is reported as longer than any connecting stalks. It is tubular, at least during and after the first ovulation.

Posterior (distal, proximal) end of ovariole

The broader, hinder end of an ovariole connecting to the calyx of the ovary.

Rosette cells

(1) **Rosette cells** (Nicholson). Cells of the follicular epithelium which arrange themselves in a rosette pattern at the point of attachment of the connecting stalk to the antero-ventral aspect of an almost fully developed oocyte and which, together with residual nurse cells, take part in forming the micropyle. Transient and recurring afresh with each successive follicle.

(2) **Funicle rosette** (Giglioli). Cells arranged in a rosette pattern to form a muscular sphincter round the opening of the pedicel into the calyx. Peripherally linked to the nucleated, muscled wall of the calyx. One permanent rosette to each ovariole.

Sac

A dilated elongation of the posterior part of the follicular tube, caused by the growth of a follicle and by ovulation. Persists for some hours after ovulation, but subsequently shrinks to a narrow tube, which shows one dilatation between the pedicel and the next formed follicle for each ovulation that has taken place.

Skeins (leashes, bundles, coils) of tracheae

The mass of fine tracheae, and tracheoles, folded closely together in between ovarioles, and representing the distal branchings of the two tracheae supplying each ovary.

Stalks (see Connecting stalk)**Suspensory ligament (apical filament)**

Anterior, tapering, prolongation of the *ovarian* sheath.

Terminal filament

Anterior point of the *ovariole* sheath--may be small and inapparent.

Trophocyte (see Nurse cell)**Tunica (tunica propria, intima)**

The thin, structureless (a few nuclei reported), elastic membrane forming the wall of the follicular tube. Inapparent round follicles, but perceptible as wall of connecting stalks and pedicel. Distended in parous females in the region between the current follicle and the pedicel, each dilatation being the site of a former follicle.

Vitellarium

The zone of a follicular tube in which follicles grow during oogenesis.

Yolk (yelk)

Nutritive, granular material which appears in the cytoplasm of the oocyte from stage II onwards and imparts the white opacity to the developing oocyte, eventually making up the bulk of the fully developed egg-shaped oocyte and, finally, of the egg within the egg-shell.

ACKNOWLEDGEMENTS

Thanks are due to Dr D. J. Lewis for discussing and reading this Annex; to Dr M. E. C. Giglioli in the Gambia for valuable exchanges of information; to Mr W. A. Samarawickrema, Dr T. S. Detinova, Dr L. J. Bruce-Chwatt, and other colleagues and members of the London Course for their views on the original glossary.

INDEX

INDEX

- Accessory gland in *Anopheles maculipennis*, 22, 23-24
Aedes aegypti, neurosecretory regulation of ovarian development, 37
role of corpora allata, 32, 33
Age changes, in *A. maculipennis*, digestive processes, 57-59
excretory processes, 53-57
reproductive system, 24-29
in Diptera, definition, 13-14
Age composition, of *Anopheles*, as criterion in evaluating control measures, 151-168
of *A. maculipennis*, as indication of epidemiological importance, 122-150
determination in relation to physiological age, 101-121
of *Musca domestica*, as criterion in evaluating control measures, 168-169
of *Phlebotomus*, as criterion in evaluating control measures, 169
of vector populations, definition, 95
seasonal changes in, 97-101
Age, physiological, *see* Physiological age
Agglutination reaction, use in determining presence of human blood in mosquitos, 139
Ampullae in *A. maculipennis*, age changes in, 24
functional changes in, 23
morphology and function, 21
qualitative changes in, as indication of physiological age, 72-73
size of, as indication of physiological age, 72
Anopheles, age composition, as criterion in evaluating control measures, 151-168
autogenous ovarian development, 37-38
determination of degree of contact with man, 139
endophily and exophily, 106-107
gastric tracheation, 76-77
ovarian tracheation, 73-74
Microsporidia infestation, 34-36, 42-44
potentially dangerous females,
see Potentially dangerous females
A. gambiae, seasonal fertility, 64
A. maculipennis, determination of epidemiological importance by age composition, 122-150
digestive processes, age changes in, 57-59
excretory processes, age changes in, 53-57
experiments to determine physiological age, 26-27
fertility in relation to physiological age, 59-65
gastric tracheation, 51-53
oogenesis, 30-47
ovarian tracheation, 48-51
reproductive system, age changes in, 24-29
functional changes in, 23-24
structure, 16-23
seasonal changes in age composition, 97-101
study of age composition by determination of physiological age, 101-121

BHC, *see* DDT and BHC
Black-flies, *see* Simuliidae
Blattodea, presence of "corpora lutea" in ovaries, 25
Blood-meal, completeness of, effect on ovarian development in *Anopheles*, 33-39
determination of presence or absence by appearance of gastric tracheation in *Anopheles*, 76-77
Body size, relationship to fertility in *A. maculipennis*, 64-65

Cattle, insecticide treatment in malaria control, entomological evaluation, 164-167
presence in settlements, effect in reducing number of mosquitos attacking man, 139
Christophers' classification of ovarian development, 17, 201
Cimex lectularius, role of corpora allata, 32
Climatic conditions, effect on age composition of *A. maculipennis*, 113-121
effect on duration of malaria transmission season, 123, 142, 145

- Climatic conditions (*continued*)
 effect on epidemiological importance of
 A. maculipennis, 140-150
 See also Meteorological conditions; Temperature
 Corpora allata, functional changes in, 31
 role in gonotrophic cycle, 31-33
 "Corpora lutea", use of term to describe
 ovariole dilatations, 25
 See also Yellow bodies
Culex, autogenous ovarian development,
 37-38
 role of corpora allata, 33
C. pipiens, observations on spermathecal
 duct, 22
- DDT, and BHC, barrier spraying, entomological evaluation, 162-163
 complete spraying coverage, entomological evaluation, 153-162
 selective spraying, entomological evaluation, 163-164
 treatment of cattle, entomological evaluation, 164-167
 effect of sublethal doses in producing follicular degeneration in *Musca domestica*, 46
- Digestive processes in *A. maculipennis*, age changes in, 57-59
- Dilatations in ovarioles, determination of physiological age by, 25-29, 71-72, 74-76, 204
- Egg retention, as indication of physiological age in *Anopheles*, 70
- Endophily and exophily in *Anopheles*, 106-107
- Epidemiological importance of *A. maculipennis*, determination by age composition, 122-150
- Excretory processes of *A. maculipennis*, age changes in, 53-57
- Fertility of *A. maculipennis*, in relation to physiological age, 59-65
- Follicular degeneration, 40-47
- Functional changes in reproductive system, of *A. maculipennis*, 23-24
 of Diptera, definition, 13
- Gastric tracheation in *Anopheles*, examination of, as means of determining presence or absence of blood-meal, 76-77
- Gastric tracheation in *Anopheles* (*cont.*)
 structure and functional changes in, 51-53
- Geographical conditions, effect on age composition of *A. maculipennis*, 113-121
 effect on epidemiological importance of *A. maculipennis*, 140-150
- Glossary of terms relating to mosquito reproductive system, 205-210
- Glossina*, determination of physiological age, 86-88
- Gonotrophic concordance, definition, 14
- Gonotrophic cycle, duration, 96, 132-134
 measurement of first phase, 132-134
 measurement of second phase, 130-132
 measurement of third phase, 135-136
 neurosecretory regulation, 30-36
 role of corpora allata, 31-33
- Haematobia stimulans*, determination of physiological age in, 82-84
- Hibernating places of *A. maculipennis*, 99
- Hippobosca capensis*, determination of physiological age, 84-86
- Houseflies, see *Musca domestica*
- Hydrachnidae infestation, as indication of physiological age in *Taeniorhynchus (Mansonoides) africanus*, 71
- Insecticides in malaria control, entomological evaluation, 151-167
- Larval control, entomological evaluation, 168
- Loxostege sticticalis* L., presence of "corpora lutea" in ovaries, 25
- Hyperosia irritans*, determination of physiological age, 82-84
- Malaria control, age composition of *Anopheles* as criterion of effectiveness, 151-168
- Malaria transmission season, differences in duration caused by climatic factors, 123, 142, 145
- Malpighian tubes, excretory processes, 54-57
 "Matting plugs", as indication of physiological age in *Anopheles*, 71
- Meconium in stomach, as indication of physiological age in *Anopheles*, 70
- Mer's classification of ovarian development, 17, 201

- Meteorological conditions, effect on speed of blood digestion, 57
See also Climatic conditions; Temperature
- Microsporidia infestation in *Anopheles*, effect on ovarian development, 34-36, 42-44
- Mortality rate in mosquitos, 96-97
- Moshkovsky's method for determining duration of sporogony, 126, 127
- Mosquitos, mortality rate, 96-97
- reproductive system, glossary of terms, 205-210
 - morphology and function, 195-205
- See also under individual species*
- Multiparous mosquitos, ovarioles in, 204
- Musca domestica*, age composition, as criterion in evaluating control measures, 168-169
- determination of physiological age, 88-93
- follicular degeneration, 46
- presence of "corpora lutea" in ovaries, 26
- Nervous system of reproductive organs in *A. maculipennis*, 19-20
- Neurosecretory regulation of gonotrophic cycle, 30-36
- Nosema* infestation of mosquitos, 34
- Nulliparous mosquitos, deficit in house-sheltering populations, 103
- definition, 200
- Oganov-Rayevsky method for determining duration of sporogony, 126-129
- Onchocerca* infestation, effect on Malpighian tubes of *Simulium damnosum*, 55
- Oogenesis, in *A. maculipennis*, 30-47
- in mosquitos, physiological stages, 20-21, 201-202
- Ovarian development, Christophers-Mer classification, 17, 201
- effect of completeness of blood-meal on, 33-39
 - effect of Microsporidia infestation on, 34-36, 42-44
- Ovarian tracheation, in *Anopheles*, as indication of physiological age, 73-74
- structure and functional changes, 48-51
- in *Musca domestica*, as indication of physiological age, 90-93
- Ovaries of mosquitos, changes after oviposition, 203-204
- morphology and function, 196-197
- Ovarioles of mosquitos, changes after oviposition, 203-204
- changes during ovulation, 24-29
 - in multipars, 204
 - morphology and function, 197-200
- Overwintered *A. maculipennis*, age composition, 114-116
- epidemiological importance, 140-141
- Ovulation, in mosquitos, 202-203
- Pauciparous mosquitos, definition, 200
- Perry's method of determining physiological age in *Anopheles*, 69-70
- Phlebotomus*, age composition, as criterion in evaluating control measures, 169
- determination of physiological age, 81-82
- follicular degeneration, 45
- P. papatasii*, autogenous ovarian development, 37-38
- Physiological age, definition, 10
- determination, in *Anopheles*, 69-77
 - in *Glossina*, 86-88
 - in *Haematobia stimulans*, 82-84
 - in *Hippobosca capensis*, 84-86
 - in *Lyperosia irritans*, 82-84
 - in *Musca domestica*, 88-93
 - in *Phlebotomus*, 81-82
 - in *Simuliidae*, 78-81
 - in *Stomoxys calcitrans*, 82-84
- effect on follicular degeneration in *Anopheles*, 44
- importance in determining age composition of *A. maculipennis* populations, 101-121
- relationship to fertility in *A. maculipennis*, 60-65
- Physiology of different age-groups of *A. maculipennis*, 66-68
- Plasmodium vivax* and *P. falciparum*, effect of temperature on sporogony, 125-129
- Polovodova's method of determining physiological age, 71-72
- Potentially dangerous females, determination of absolute numbers, 137-139
- determination of age, 136-137
 - seasonal differences in epidemiological importance, 146-150
- Precipitation reaction, use in determining presence of human blood in mosquitos, 139
- Rhodnius*, neurosecretory regulation of moulting process, 37

- Sandflies, see *Phlebotomus*
Seasonal changes in age composition of
A. maculipennis populations, 97-101
Seasonal changes in fertility of *A. maculi-*
pennis, 61-64
Sella's stages of blood digestion and ova-
rian development in *Anopheles*, 57-58
Simuliidae, determination of physiological
age, 78-81
 follicular degeneration, 42
Simulium damnosum, Malpighian tubes in,
 55
Spermatheca, function, 22
Spermathecal gland in *A. maculipennis*, 22,
 23-24
Sporogony, determination of duration,
 125-129, 142-145
Stomach, distension of, effect on ovarian
 development in *Anopheles*, 33-36
 tracheal system, in *Anopheles*, see Gastric
 tracheation in *Anopheles*
Stomoxys calcitrans, determination of phy-
 siological age, 82-84
Summer generations of *A. maculipennis*,
 age composition, 116-121
 epidemiological importance, 141
Taeniorhynchus (Mansonioides) africanus,
 infestation with Hydrachnidae, as in-
 dication of physiological age, 71
Temperature, effect on follicular degener-
 ation, 45
 effect on hibernating mosquitos, 99
 effect on sporogony, 125-129
 See also Climatic conditions; Metereo-
 logical conditions
Thelohania infestation of mosquitos, 34
Tracheation, gastric, in *Anopheles*,
 see Gastric tracheation in *Anopheles*
ovarian, see Ovarian tracheation
Tsetse flies, determination of physiological
age, 86-88
Wing scaling, as indication of physiological
age in *Anopheles*, 69-70
Yellow bodies, discovery in insect ovaries,
 25
 in Cyclorrhapha, as indication of physio-
 logical age, 82-84
 in *Musca domestica*, as indication of
 physiological age, 89-90
 See also "Corpora lutea"