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A Study of the Blood-feeding Patterns of *Anopheles* Mosquitos through Precipitin Tests *

Results of Collaborative Work for the Period 1955-59 and Their Application to Malaria Eradication Programmes

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The success of malaria eradication campaigns depends on the use of all methods which make for a better understanding of the biology and behaviour of mosquito vectors. One such method is precipitin testing, by which it is possible to identify the human or animal origin of blood meals of mosquitos and thereby to determine their host preferences and vectorial importance, both generally and locally.

In 1955, the World Health Organization in agreement with the Lister Institute of Preventive Medicine, Elstree, England, set up a precipitin test service related to entomological surveys in malaria eradication programmes and available to national research and WHO field personnel. The purpose was to stimulate interest in the study of bionomics of Anopheles *species, to facilitate the identification of blood meals of* Anopheles, *to eliminate experimental errors by the use of a standardized technique and highly sensitive antisera, and finally to apply the results in the strategy of malaria eradication.*

The results obtained over the past five years are summarized in tabular form. The study—the largest ever undertaken—included 51 species of Anopheles *and 56 377 tests, of which 93.9% yielded positive results, are reviewed. The available knowledge of the vectorial importance of 39 species of* Anopheles *is compared with their human blood ratio, this term being used to express the percentage of human blood in relation to all precipitin tests found positive.*

INTRODUCTION

The cyclical transmission of malaria infection from man to man by a female *Anopheles* requires that the mosquito should take at least two human blood meals. The two feeds must be suitably spaced in time to allow for the completion of sporogony to the stage of invasion of the salivary glands by the sporozoites. Apart from the variation in the inherent susceptibility of species and strains of *Anopheles* to the infection, the importance of any particular *Anopheles* depends on the degree of its contact with man. The study of the feeding habits of malaria vectors was limited at first to recording the place of capture of blood-fed females. As early as the beginning of this century Koch recognized the importance of determining the source of blood ingested by the insects; he had recourse to microscopic examination to ascertain whether *Glossina palpalis* fed on crocodiles.[1] Later the reliable identification of blood specimens was made possible thanks to the method of precipitin testing devised by Uhlenhuth, Weidanz & Angeloff (1908). This method was based on Nuttall's (1904) work on the antigenic relationship of animal sera and its correlation with zoological classification.

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[1] Missiroli, A. & Hackett, L. W. (1929) *The precipitin test as a means of determining the source of anopheline blood-meals* (multigraphed League of Nations document C.H./Malaria/131).

The method of precipitin tests for the determination of host selection by mosquitos became of particular interest in the 1920's, when Roubaud (1921) proposed a new theory of the competition among anophelines for food. Writing of *A. maculipennis* Roubaud postulated the existence of two physiological races with different feeding habits, one " anthropophilic ", which fed only on man, and the other " zoophilic ", more or less definitely preferring animals. Roubaud also emphasized the epidemiological importance of " animal deviation " in the spontaneous disappearance of malaria. This theory aroused considerable discussion, and a method of precipitin testing of the stomach contents of mosquitos was first used in 1922 [1] by Grassi, and, independently, by King & Bull (1923) and then more widely by Darling (1925) and by Boyd (1930) in the United States of America. It was subsequently employed in the 1920's in the Netherlands (Swellengrebel & de Buck, 1938), in Italy (Missiroli & Hackett, 1927), in Indonesia (Walch & Sardjito, 1928), in Argentina (Davis & Shannon, 1928) and in Jamaica (Boyd & Aris, 1929). During the 1930's the method of precipitin testing gained increasing popularity. The real possibilities of this method were, however, shown by Rice & Barber (1935) during their studies in Greece. These two authors introduced a modified technique, suitable for large-scale field investigation, and tested 18 000 blood meals of the *A. maculipennis* complex.

The application of precipitin tests to malaria surveys was subsequently extended to many parts of the world with varying degrees of success, and provided increasing evidence that Roubaud's theory of physiological races of *Anopheles* was too rigid and could not be confirmed in its entirety (Toumanoff, 1936).

Most of the information available ten years ago on the degree of preference of various anopheline species for human or animal hosts was collected by Boyd (1949). Boyd's records cover the results obtained during the period 1930-44 in over 48 000 *Anopheles* of 39 species from about 30 countries. The results indicated that not a single anopheline species could be called absolutely dependent on man, although some species [2] showed a definite preference for human blood even in the presence of cattle.

During the late 1940's interest in precipitin tests as a research method waned somewhat, perhaps on account of conflicting results obtained in various parts of the world. A number of authors abandoned precipitin tests in favour of a comparison of the relative densities of different species in human bait traps and cattle sheds. We may note that in the attempted eradication of malaria vectors from Sardinia (1946-50) the technique of precipitin testing was not employed for the study of the habits of *A. labranchiae* (Aitken, 1953).

The value of precipitin tests in research on tropical *Anopheles* was reviewed by Muirhead-Thomson (1951), who pointed out the difficulties of the interpretation of some of these tests and emphasized the importance of collecting mosquitos from outdoor haunts in which the bias of the type of the indoor shelter is eliminated. It has been suggested by Gabaldon (1953) that *Anopheles* are primarily " zoophilic " and that they depend on the availability of human blood only at the periphery of their area of geographical distribution.

A renewed interest in the precipitin test as a tool of field research on the bionomics of malaria vectors dates from 1950 and is largely due to the late Professor P. Buxton, who organized a joint investigation in which a number of entomologists and the special serology laboratory of the Lister Institute of Preventive Medicine participated with valuable results.

A considerable amount of newer data on the results of precipitin tests on *Anopheles* was collected by Horsfall (1955) in his comprehensive study of the bionomics of mosquitos in relation to disease. Out of 115 anopheline species or species-complexes, the habits of which Horsfall describes in detail, information on the origin of ingested blood is available in respect of 57.

The decision of the Eighth World Health Assembly in 1955 to strive for global malaria eradication indicated some requirements in the planning for this endeavour. There was need for a better knowledge of the bionomics of malaria vectors in many parts of the world. The degree of epidemiological contact between man and the *Anopheles* can be assessed by identifying the origin of the last blood meal of the female mosquito. The judicious use of this method fits into the general plan of entomological research, providing that we know most of the variables which determine the movements of *Anopheles* during their gonotrophic cycle. Even before the historical date of 1955, when the aim of malaria eradication was approved, the World Health Organization considered

[1] Missiroli, A. & Hackett, L. W. (1929) *The precipitin test as a means of determining the source of anopheline blood-meals* (multigraphed League of Nations document C.H./Malaria/131).

[2] *A. sundaicus* of Indonesia; *A. hyrcanus sinensis* of mainland China, Indonesia, Malaya; *A. fluviatilis* of India; *A. gambiae* of West and East Africa; *A. minimus* of Assam and Viet Nam.

that there was a good case for making precipitin testing freely available to interested field and research workers.

IDENTIFICATION OF BLOOD MEALS OF MALARIA VECTORS AS A METHOD OF STUDY OF THEIR HABITS

The best way of assessing the value of precipitin tests in the study of malaria vectors will be to describe the changing trends concerning a few species of *Anopheles*.

The results of the work carried out in Europe in the early 1930's on the *A. maculipennis* complex were brilliantly summarized by Hackett (1937) and by Bates & Hackett (1939). The theory of two distinct physiological races of "*A. maculipennis*", one "anthropophilic" and a vector and the other "zoophilic" and a non-vector, had to be greatly modified. The method of precipitin testing showed that the *A. maculipennis* complex consists of at least six distinct populations (=subspecies) with different habits and host preferences; these differences are relative, however, and each population shows a varying degree of preference for man or for animals. Thus, *A. maculipennis atroparvus*, *A. m. labranchiae* and *A. m. sacharovi* feed on man or animals almost equally readily. On the other hand, *A. maculipennis typicus*, *A. m. messeae* and *A. m. melanoon* are reluctant to feed on man and feed more readily on cattle. The difference between the two groups is relative and even those that do not favour man as a host might in some instances be malaria vectors.

The main lesson was that there are few hard-and-fast rules in biology and that differences in biological characters must be expressed in terms of degrees.[1]

Although the relativity of host preferences of mosquitos was generally conceded, most malariologists in the tropics maintained that there was a direct correlation between malaria transmission and a high human blood ratio in anophelines. Early work in the highly malarious areas seemed to support the conclusion that *Anopheles* species incriminated as malaria vectors (by the presence of sporozoites in the salivary glands) had a very marked preference for human blood. Nevertheless there was an increasing number of investigations in which it was difficult to interpret the mosquito's feeding habits in relation to the vectorial importance of the species.

This pattern was found with regard to *A. minimus* in South-East Asia. The first studies in Assam (Ramsay et al., 1936) showed a clear preference for human blood; this was partly corroborated in Viet Nam (Toumanoff, 1936) and later in Burma (Yofe & Fox, 1946). However, in the Philippines *A. minimus* (probably *flavirostris*) collected outdoors showed a low human blood ratio (Laurel, 1934), while later work in Assam (Muirhead-Thomson, 1941) showed that *A. minimus* (*sensu stricto*) also fed on cows, goats, fowls and dogs. Chow (1948) found that the Yunnan form had a preference for cattle, while Jackson (1951) reported that *A. minimus* in Hong Kong would feed equally well on pigs, goats and cattle. It is probable that *A. minimus* of Assam maintains itself entirely on animals in the absence of man (Muirhead-Thomson, 1951).

A similar development took place in the investigation of *A. maculatus*, which is an important vector in Malaya but considered to be of little or no importance in all the other parts of its wide range (Assam, Borneo, Philippines). The results of precipitin tests carried out in Malaya during the 1930's showed that *A. maculatus* was predominantly "anthropophilic". Wharton (1953) showed that the interpretation of these early studies was not faultless since the collection of those *Anopheles* was inevitably biased in favour of human dwellings. A new investigation in which the collection of *Anopheles* represented a cross-section of all possible resting-places (including outdoor shelters) revealed that the Malayan *A. maculatus* feeds equally well on animals though it shows a slight preference for man even when cattle are available. On the other hand, *A. maculatus* of Hong Kong and Viet Nam attacks either cattle or man, whichever is the more numerous (Jackson, 1951), while in the Philippines the local *A. maculatus* seems to feed largely on cows (Ejercito, 1934).

The same trend was noted in tropical Africa with *A. gambiae*, where precipitin tests carried out in Nigeria (Davis & Philip, 1931), in Kenya (Symes,

[1] The use of the terms "anthropophily" ("anthropophilia", "anthropophilism") or "zoophily" ("zoophilia", "zoophilism") for preferred nutritional tropisms of mosquitos is misleading and should be avoided altogether. As all *Anopheles* have a preference for several hosts, of which one may be man, "anthropophily" is too exclusive, while "zoophily" includes arbitrarily all animals irrespective of their species. There may, however, be one most preferred which can be called the "host of predilection". The degree of relationship of vector to man has often been referred to as the "anthropophilic index" but it is probably better expressed as a high or low "human blood ratio" (Covell, Russell & Swellengrebel, 1953). The latter term refers to the proportion of freshly fed *Anopheles* giving a positive precipitin reaction for human blood in the particular conditions in which the captures have been made.

Naturally this proportion must be calculated in relation to the total number of positive tests, including those for which the host has not been identified, because of the limited number of antisera used for this purpose.

1932; Kauntze & Symes, 1933), in Uganda (Gibbins, 1933) and in Mozambique (de Meillon, 1938) indicated a pronounced preference of *A. gambiae* for man even where man and cattle lived together under the same roof (as in Kenya). For many years *A. gambiae* was regarded as a species closely associated with man and typically "anthropophilic", even though some observers reported that *A. gambiae* could be found in uninhabited areas. The discovery by Haddow et al. (1947) that in East Africa extraordinary numbers of *A. gambiae* could be found in the uninhabited Semliki forest indicated that man is not necessarily a normal host of this species. This was confirmed experimentally in Southern Nigeria by Muirhead-Thomson (1948), who showed that the *A. gambiae* complex (including *A. melas*) may feed almost indiscriminately on man, goat, pig and cow and that the proportions of feeds on these animals depended on the relative numbers of hosts available, and to some extent on their distribution in houses. Similar observations were also made by Bruce-Chwatt [1] in the Lake Chad area, where *A. gambiae* feeds on man and on domestic animals, particularly horses.

Holstein (1952) working in French West Africa, found that one race of *A. gambiae* (paucidentate) was attracted by man while another race (multidentate) showed an equal degree of attraction to man or animals. Among the *A. gambiae* collected in the bush, Holstein found that as many as 40% had fed on antelopes. Other observations (Bruce-Chwatt and co-workers; [2] Gillies, 1956; Hamon et al. 1958), confirmed the fact that *A. gambiae* (without reference to any of its hypothetical races or strains) can feed more or less indiscriminately on man or on animals.

Thus it became obvious that in many parts of Africa a proportion of *A. gambiae* maintains itself on animals independently of the presence of man.

The study of host preferences of *A. darlingi*, the most important malaria vector in South America, showed similar trends, well summarized by Rachou (1958). While a number of workers in the early 1940's maintained that *A. darlingi* is an important vector because of its high "anthropophily", later work by Deane, Vernin & Damasceno (1949) and by Rachou (1958) indicated that the human blood ratio of this species varies considerably in relation to the type of human dwellings and to the availability of alternative hosts, such as cattle and horses. On the other hand, Gabaldon (1953) and Giglioli (1956) pointed out that at the periphery of its distribution *A. darlingi* shows a very definite preference for human blood.

Early investigations of blood preferences of *A. aquasalis* pointed to man as a favourite host, but later findings in British Guiana and elsewhere were different and showed that this mosquito fed readily on man and on livestock. In Trinidad, Senior White (1952) showed that cattle and horses are preferred hosts and that man is only seriously attacked when there is a lack of sufficient "deviation" by these animals. These findings have been largely confirmed in Brazil (Rachou, 1958).

Information on the host preferences of *A. culicifacies*, which extends from south-eastern Arabia and Pakistan to Indochina and south across India to Ceylon, is very confusing. It was particularly puzzling to find that in those parts of India where *A. culicifacies* was considered the main local vector, the results of precipitin tests positive to man did not exceed 2% of all positive tests (Russell et al., 1938; Afridi et al., 1939). Afridi & Puri (1940) showed that the ratio between numbers of cattle and man influenced the number feeding on man, while Senior White & Rao (1943) found that the proportion of precipitin tests on *A. culicifacies* positive to man or to cattle varied in relation to the type of shelter in which the mosquitos were collected. Senior White et al. (1945) believed that, in Delhi, some factor other than the availability of blood was operative in host selection.

The precipitin tests made on Australian *Anopheles* collected in their natural resting-places were tabulated and discussed by Mackerras (1947), who showed some striking differences between the human blood ratio of *A. punctulatus punctulatus* (95%) and *A. punctulatus farauti* (19%). This was confirmed in laboratory trials. Nevertheless, *A. punctulatus farauti* is as dangerous as the type form because of its high population densities. Apparently man is the preferred host in areas where there are no large domestic animals. The local differences are well brought out by Black (1955), who found that the great majority (92%) of *A. farauti* in the Trobriand Islands resting indoors fed on man while the same species in the Minj area of New Guinea fed equally well on pigs and man.

The examples of these important malaria vectors, the natural feeding preferences of which are only

[1] Unpublished report (1950) to the Director of Medical Services, Nigeria.

[2] Bruce-Chwatt, L. J. (1955) First Annual Report on the Western Sokoto Pilot Project; Bruce-Chwatt, L. J., Archibald, H. M. & Haworth, J. (1956, 1957) Second and Third Annual Reports on the Western Sokoto Pilot Project (mimeographed documents).

loosely linked with the host of the human malaria parasite, could be extended to other *Anopheles* species such as *A. quadrimaculatus* of the United States of America, *A. stephensi* of India, *A. funestus* of Africa, *A. hyrcanus sinensis* of China and others (Muirhead-Thomson, 1951; Wharton, 1953; Horsfall, 1955).

Need for critical evaluation of results of precipitin tests

The results obtained by various authors using the precipitin test as a working tool have shown the necessity of critically assessing the possibilities of this method. Two important conclusions have become obvious:

1. The presence of a specific type of blood in the stomach of *Anopheles* found in a given shelter does not mean that the feeding took place in this shelter on the available host.

2. The analysis of collections of blood-meal smears may be influenced by a large number of variables such as: (*a*) the availability to the biting *Anopheles* of the main or an alternative host; (*b*) the characteristic behaviour of the anopheline population represented by the sample; (*c*) the density of the anopheline population; (*d*) the season of the year,[1] the general climatic conditions and the time of collection of mosquitos; and (*e*) the conditions of the collection site and its microclimate.

It might be of interest to discuss some of these variables.

The *Anopheles* probably seek out their hosts with the aid of various mechanisms about which we still know very little.[2] The final choice of host by a hungry mosquito may depend on extrinsic or intrinsic factors. Extrinsic factors—such as availability of the host, its range of flight and resting habits, etc.—may influence the selection, but the mosquito is still able to exert a free choice when extrinsic factors lead it to a number of possible hosts (e.g., in huts where humans and animals live together). Extrinsic factors such as the sleeping habits of the hosts, the odours given out by them, the large exposed area of warm skin, the accessibility of the capillary blood vessels, the palatability of the blood, or possibly simple random selection lead to the final choice. However, intrinsic factors—e.g., a genetic requirement of blood from a certain host or a particular capacity to respond to some impulses only—may override these tendencies. In addition to this, certain characteristics of behaviour may be due to an interplay of both intrinsic and extrinsic factors. This is the case of the difference between the indoor- and outdoor-biting behaviour. *Anopheles* which enter houses (endophilic species) will have better opportunities for feeding on man than those which remain out of doors (exophilic species).[1] It might also be pointed out that according to recent research by Soviet entomologists (Detinova, 1953, 1959 [2]) the physiological age of female *Anopheles* has some bearing on their habits inasmuch as younger females (of *A. maculipennis*) have more pronounced exophilic tendencies than the older females, which prefer the indoor resting-places. This might be of importance in the feeding preferences of certain vector populations in some local conditions. Obviously a knowledge of the behaviour of mosquitos (dispersion, gonotrophic cycle, nightly turnover, resting-habits) is most useful for the study of feeding preferences in malaria vectors, and therefore of their epidemiological importance. Conversely, the use of precipitin tests as a marker of the previous blood-meal is of value in the study of bionomics.

The value of any method used for the study of mosquito bionomics depends, naturally, on the number of specimens collected. The largest collections of blood-fed mosquitos are usually made in houses or cattle sheds, where many females rest while digesting their blood-meal. But in collecting only indoors one selects a biased sample of the given mosquito population, leaving out of account the

[1] The seasonal variation of the human blood ratio in *A. maculatus* and *A. philippinensis* in Malaya was investigated by Wharton (1953), but there was no evidence of a significantly greater frequency of feeds on man during the peak of the transmission season. Incidentally, this study of Wharton's illustrated the danger of accepting the precipitin test as a guide to feeding preferences of malaria vectors without taking into account the place where the mosquitos were collected and without checking whether anophelines found resting in houses have obtained their blood from the occupants or could have fed elsewhere.

[2] Some progress in the study of the host-seeking behaviour of *Anopheles* was achieved recently by Laarman (1955), who in a series of ingenious experiments showed that females of *A. atroparvus* are able to localize very sharply either man or the rabbit. Laarman showed that the body of the host emits odours which attract the female mosquito.

[1] Exophily implies a tendency for the mosquito to rest outside rather than in man-made shelters during the daytime (Senior White, 1954). Exophily can be (*a*) obligatory, when there are no houses in the area ; (*b*) facultative, when the preferred host is outside but where human or animal shelters abound in the vicinity; or (*c*) deliberate, when feeding takes place inside the man-made shelter and yet the mosquito prefers to seek natural resting-places outside the house (Gillies, 1956).

[2] Detinova, T. S. (1959) *III. Age-grouping methods in diptera of medical importance.* In: *Course in advanced entomological techniques applied to malaria eradication* (unpublished working document WHO/Mal/238).

food preferences of those which feed and rest outdoors and are more difficult to find.

The influence of environmental conditions when the *Anopheles* are feeding is so disturbing for the study of host selection that several authors have attempted to obtain a better idea of this characteristic by using experimental methods, such as a " free choice " device in which a mosquito contained in a small cage is offered a choice between two hosts. It was shown by van Thiel (1939) that host selection in such artificial conditions can give only a doubtful indication of what happens in nature.

In à rather different method of investigating feeding preferences the mosquitos are released in a large, screened room containing various possible hosts housed in identical conditions. This method was first used by Hu & Yu (1936) in China and then by Livadas & Sphangos (1940-41) in Greece. The latter authors confirmed the relativity of food preferences of the investigated vectors species (*A. sacharovi*, *A. superpictus*.)

Huts baited with different hosts were used in Puerto Rico by Weatherbee (1944) on *A. albimanus* and *A. darlingi*, and huts provided with window-traps were used in Nigeria by Muirhead-Thomson (1945) in studying *A. gambiae*.

Deane, Vernin & Damasceno (1949) carried out " multiple host selection " tests with *A. darlingi* and *A. aquasalis* and showed considerable differences between the response of laboratory-bred mosquitos and those caught in houses.

It was pointed out by Muirhead-Thomson (1951) that any further advances in our knowledge of the bionomics of malaria vectors must depend on a wider and more critical use of precipitin tests on the blood meals of *Anopheles* collected outdoors and indoors, together with some new devices such as baited trap-huts to follow up the movements of mosquitos.

It was suggested by C. Garrett-Jones in an unpublished report (1958) to the World Health Organization that a good example of the need of such studies is the case of *A. sergenti* in the Jordan valley. This species has been collected by the hundred during night-catches on human bait but is rarely found resting in houses or tents during the day; it shelters chiefly in caves and rock crevices. Although it is found infected in nature and maintains a low degree of malaria transmission in Israel, Jordan and Syria, its feeding preferences have not been sufficiently investigated.

Another interesting case is that of *A. gambiae* in Mauritius. This mosquito is rarely found resting in houses or huts in spite of the fact that it enters houses at night, bites freely and shelters out of doors, between rocks piled up to form walls. A small number of blood-meal samples collected in houses or in cattle sheds gave positive precipitin results related to the hosts present in each shelter, but a thorough investigation is still needed in Mauritius of the blood meals of *A. gambiae* found out of doors or given a free choice of host.[1]

The precipitin test is of particular importance in the presence of large numbers of mosquitos with a very low infectivity rate. A high human blood ratio found in an *Anopheles* population in such conditions indicates the importance of the species as a vector, and a low ratio indicates the reverse.

Finding a very low (or nil) human blood ratio in a species of *Anopheles* is often most useful when the number of specimens tested is high. The value of this observation in the field was commented by Mackerras (1947).

It might be worth recalling here that females normally seek a blood meal within 24 hours of emergence. The frequency of subsequent feeds is determined by the duration of the gonotrophic cycle and by the rate of digestion, which is more rapid in hot, humid weather.

The amount of blood that has been ingested by fully fed female *Anopheles* varies according to the size of the species, but there is also some variation between individual females. The average is about 1.0 mm^3 for smaller species such as *A. minimus* or *A. albimanus and* 2.0-2.5 mm^3 for *A. maculipennis and A. quadrimaculatus*. Soon after the ingestion of the blood there is a significant degree of separation of the plasma from the cellular elements. Plasma is excreted through the anus while the density of red blood cells in the stomach contents is approximately doubled.

It has been pointed out above that the difficulty of capturing blood-fed mosquitos arises particularly among those species which rest outdoors after feeding. A large proportion of such insects will be found either empty or with blood in an advanced stage of digestion, so that the number containing identifiable specific proteins is often small.

Weitz & Buxton (1953) did not find any significant difference in the rate of digestion of mosquitos in cages and those found in nature. In wild *A. aquasalis* high rates of positive precipitin tests occurred up to 12-16 hours after feeding; only 26% were

[1] P. Issaris & J. Hadjinicolaou—unpublished report to WHO, 1958.

positive after 20 hours and none after 40 hours. On the average it seems that mosquitos captured within 24 hours of feeding will give satisfactory results. Environmental temperature and humidity play an important part in the rate of blood digestion by mosquitos. In *Aëdes aegypti* kept at 27°C the rate of positive precipitin tests decreased from 100% after 24 hours to 8.3% after 48 hours and 0 after 72 hours; on the other hand, these mosquitos kept at 11°C had 100% of positive precipitin tests for as long as eight days after the blood meal (West & Eligh, 1952). It is also possible that the process of digestion is faster in some species (*A. maculipennis*) than in others (*A. superpictus*), according to Rice & Barber (1937). It was observed in India that during the hot, dry weather the rate of blood digestion of mosquitos was considerably slowed down. Blood digestion in females of *A. maculipennis atroparvus* in the temperature range 20°-26°C took 55-65 hours when the ovaries of mosquitos were undeveloped. On the other hand in gono-active females with developing ovaries the duration of blood digestion was between 73 and 87 hours in the same temperature range (Detinova, 1953).

A study of the speed of blood digestion in *A. sacharovi* by Storozheva (1957) in Turkmenistan showed that at a temperature of 25°-26°C the blood is digested in 3-4 days, while between 6°C and 18°C the blood digestion lasts for 8-27 days. Below 6°C the process of digestion ceases.

Another important point should be mentioned here. The influence of the work on different forms of the *maculipennis* complex in Europe was such that many entomologists have attributed any discrepancies in the feeding preferences of various populations of a given species to the presence of races (crypto-species) with distinctive feeding habits. It seems that the evidence for such races is not fully convincing since the variations in feeding habits are often due to other, mainly environmental, variables (Lamprell, 1936; Muirhead-Thomson, 1951).

It must be confessed that our present knowledge is still inadequate. It is hoped that an increased amount of critical work on the bionomics of malaria vectors, using the precipitin test as a natural marker of one important phase in the life cycle of *Anopheles*, will be forthcoming.

EPIDEMIOLOGICAL IMPORTANCE OF HOST SELECTION BY MALARIA VECTORS

The transmission of malaria by mosquitos depends *grosso modo* on: (*a*) the inherent susceptibility of the species (and strain) of *Anopheles* to the infection with the malaria parasite, (*b*) the presence in man of infective stages of the parasite, and (*c*) the repetitive biting of man in relation to the intrinsic cycle of development of the parasite in the vector.

One of the weakest links in the epidemiological chain of malaria infection and one that especially limits the chance of transmission of the parasite is the requirement that the vector must feed repeatedly on man (Hackett et al., 1938). Fifty years ago Ross (1910) estimated that even in a favourable environment only about 4% of an otherwise efficient vector species had an opportunity of feeding a second time.

Considering the tenuous links of probability which govern the laws of transmission of malaria, and remembering that this disease has maintained itself for thousands of years, one shares with Sir Charles Sherrington (1955) his feeling of awe and wonder at a genetic system which in meeting a " need " for a speck of life provided such a mechanism for its survival at an incalculable cost in human misery.

There are some gaps in our knowledge of the inherent susceptibility of anophelines to parasites of human malaria owing to the varying results obtained experimentally with different species of parasites and even with different strains of the same parasite species. Some *Anopheles* species may be highly susceptible to infection with malaria and yet be of little importance since their contact with man is infrequent. As pointed out by Macdonald (1957), wherever there is any degree of susceptibility of a given *Anopheles* species to infection with human plasmodia, the most important epidemiological factors are the mean longevity of the vector and its man-biting habits. The latter factor is composed of two variables: the frequency of feeding and the selection of host. The frequency of feeding depends on the duration of the gonotrophic cycle, which varies from species to species and is influenced even more by the environmental conditions, such as temperature and humidity. In tropical conditions the frequency of feeding of mosquitos varies between two and three days. The choice of host, as already discussed earlier in this paper, can be investigated by means of precipitin tests provided that the results are critically assessed.

The epidemiological importance of the man-biting habits of mosquitos will be better assessed if it is considered in the light of Macdonald's (1957) equation in which h represents the inoculation rate or, in other words, the mean daily number of bites inflicted on one individual by infected vectors.

This rate h equals $mabs$, where m is the anopheline density, a the man-biting index, b the proportion of *Anopheles* with infective sporozoites, s the sporozoite rate.

The man-biting index in this expression indicates the probable average number of bites per man per day and is a compound of the frequency of blood meals taken by the *Anopheles* vector and of the choice of man as host. For example, if a mosquito feeds every two days (0.5 per day) and if 30% (0.3 as a proportion) of its feeds are on man, then the man-biting index is 0.15 (0.5×0.3).

If in the expression $h = mabs$ the sporozoite rate is substituted in its expression $s = \frac{p^n ax}{ax - \log_e p}$, then the inoculation rate h equals $\frac{ma^2 bxp^n}{ax - \log_e p}$ (Macdonald, 1957).

This expression makes it obvious that the transmission of malaria varies with the square of the man-biting index and that even small changes in the frequency of feeding or in the human blood ratio have a great effect on the degree of transmission.

Nevertheless it should be remembered that in the mathematical expression quoted above the square of the man-biting index is preceded by the density of *Anopheles* (m). Should this density be very great then even the " zoophilic " vectors may cause explosive outbreaks of malaria, as has happened with *A. culicifacies* in Madras and Ceylon or *A. messeae* in Europe.

ORGANIZATION OF THE WHO PRECIPITIN TEST SERVICE AND ITS SCOPE

Five years ago the World Health Organization organized a collaborative study of host preferences of *Anopheles* mosquitos by concluding an agreement with the Lister Institute of Preventive Medicine, Elstree, England, for the setting up of a precipitin test service related to entomological surveys in malaria eradication programmes and available to all national research and WHO field personnel.

The main purposes which prompted the World Health Organization to arrange this centralized service were the following:

1. Stimulation of interest in the investigation of the vectorial importance and bionomics of a number of *Anopheles* species.

2. Provision of facilities for the identification of blood meals of *Anopheles*.

3. Elimination of experimental errors by the use of a standardized, dependable, serological technique and a wide choice of highly sensitive antisera.

4. Application of the results of this technique to the improvement of the planning of malaria eradication in geographical areas with the greatest gaps in knowledge of the habits of local malaria vectors.

Some points mentioned above require comment. There is little need to emphasize that knowledge of the habits of malaria vectors is often the key to understanding the epidemiology of malaria. In the early flush of enthusiasm for residual insecticides the attention given to this part of entomological work waned, especially as some of the most successful programmes were due rather to good organization of spraying than to knowledge of the habits of the vector. With the expansion of malaria eradication activities the disappointing results of some projects were obviously linked with ignorance of the behaviour of the vector. The need for investigation of the behaviour of *Anopheles* on a large scale was obvious, and a better co-ordination of these investigations was of importance at a time when global malaria eradication became the avowed aim of governments and of the United Nations agencies.

Three lines of inquiry may provide information on the host preferences of malaria vectors: precipitin tests on blood meals collected in the field, observation of feeding habits in nature, and study of feeding behaviour in the laboratory.

Many problems concerning the natural behaviour of a number of malaria vectors and changes in their behaviour resulting from residual spraying remain unsolved or are only dimly perceived. With some notable exceptions (Beklemishev & Shipitzyna, 1956), little is known about the country-wide pattern of movements of these vectors during the gonotrophic cycle and at different seasons of the year; about the length of time they stay either in human or in animal shelters; about the preferential resting places inside these shelters; about the irritant effect of DDT and other residual insecticide deposits and the relationship of this effect to the amount of transmission; about the importance of a few untreated premises within an area of " total coverage "; or about the changes in the human blood ratio in local vector populations following the spraying of human dwellings to the exclusion of cattle sheds, etc.

In investigations of all these points and in many more studies a judicious use of precipitin tests could be helpful, the blood being a marker indicating the

type of host on which the female *Anopheles* has fed during the past 24-72 hours.

The ideal condition for the use of precipitin tests is present when the collection of blood-fed local *Anopheles* represents a fairly true sample of their resting habits and especially when there is a choice of two hosts under similar ecological conditions.

The provision of ready facilities for carrying out the test was of obvious importance. It is, of course, perfectly true, as pointed out by Macdonald (1957), that the malariologist may wish to know only whether the mosquito feeds on man or not, while the entomologist pursuing special studies would also be interested in knowing what animal was chosen by the mosquito as an alternative to man.

In the first case the field worker will have to rely mostly on his own resources, ingenuity and enthusiasm. In many conditions it is quite satisfactory to use the simple technique of precipitin testing developed by Barber & Rice (1935) and described in numerous textbooks (Christophers, 1941; Boyd, 1949; Russell, West & Manwell, 1956; Macdonald, 1957). Excellent proof that this can be done even in field conditions can be found in Holstein's (1952) monograph on *A. gambiae*.

It was thought that wherever the conditions are such that the carrying out of simple precipitin tests in the field becomes too difficult or time-consuming, the facilities offered by the WHO precipitin test service would be of value. These facilities have been so designed that they can take care of both types of interested persons mentioned by Macdonald.

Should only the human blood ratio be of interest to the malariologist and entomologist of an eradication programme, in which the field staff cannot be expected to devote much time to the performance of precipitin tests or have no other facilities near by, then the test requested can be limited to the determination of the only important host—man.

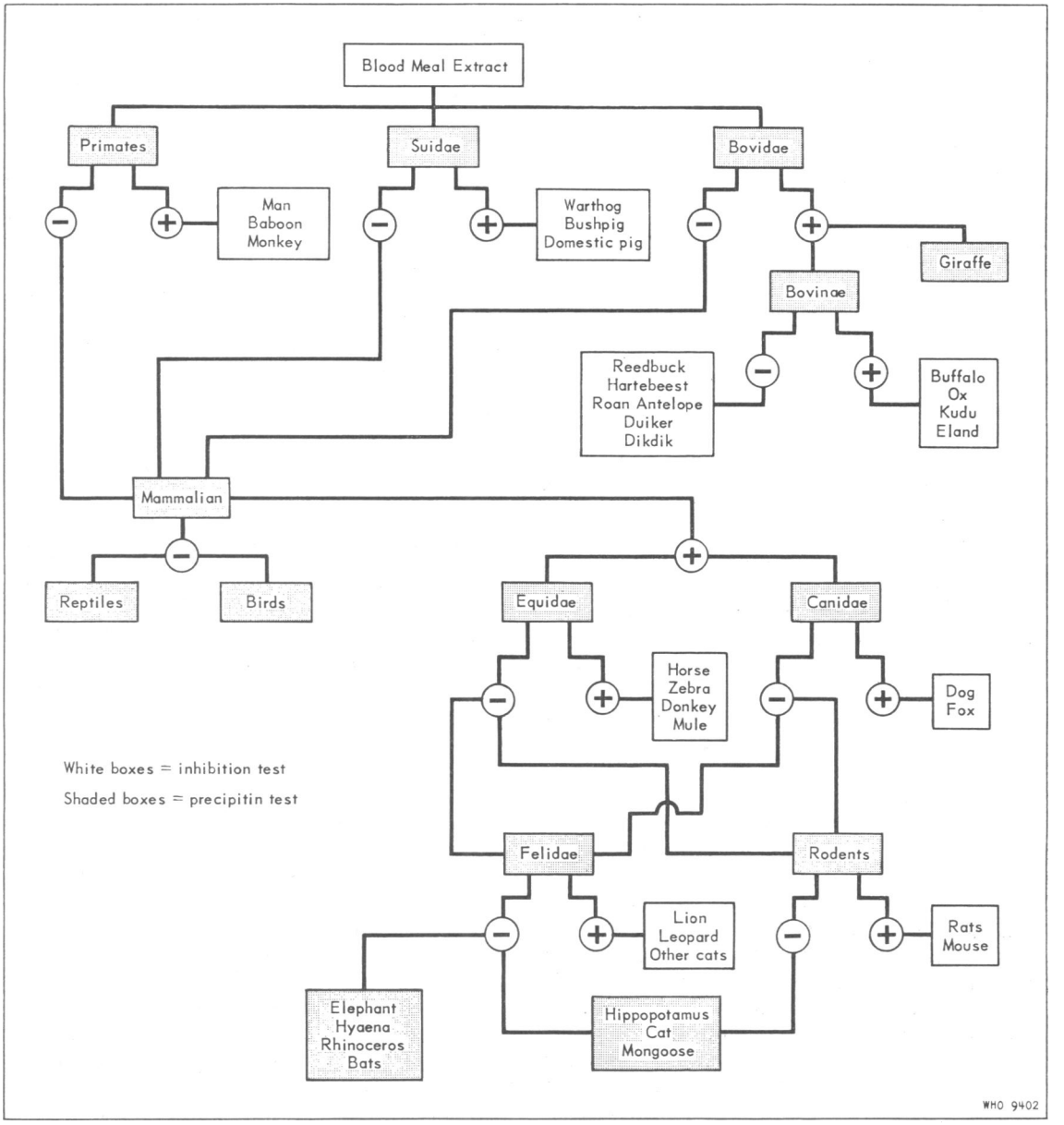
On the other hand, closer identification of blood meals taken on hosts other than man can be easily obtained on demand. The need for such tests is probably greater than was previously assumed. In many series of simplified precipitin tests there is a large proportion of negative results, the reason for which will be obscure unless the relationship between the place of capture and the identity of the blood meal is better known. Moreover, the need for a more precise study of the origin of blood meals became greater with the recent discoveries of transmission by several *Anopheles* species of mammalian malaria parasites other than the human ones. It was pointed out by Green (1935) that the presence of malaria parasites of monkeys, fruit-bats, birds, water buffalo, squirrels, etc. in *Anopheles* may lead to confusion with the human plasmodia. Plasmodia of birds in part of the USA have been mistaken for human parasites in mosquitos probably more often than has been suspected (Hunninen et al., 1950). It is known today that at least five *Anopheles* may be found with salivary glands containing sporozoites of animal malaria: *A. dureni* in the Belgian Congo transmits *P. berghei* and probably *P. vinckei*; *A. smithi rageaui* in the Cameroons transmits the plasmodium of porcupine; *A. machardyi* of the Amani area in Tanganyika transmits a probably unknown malaria parasite of antelopes; and finally *A. van thieli* transmits *P. atheruri* in the Belgian Congo. It is also possible that in Sarawak the sporozoites found in *A. letifer* are of non-human origin (de Zulueta, 1956). The sporozoite rate has been used by malariologists as a criterion of the importance of different species of *Anopheles* vectors of human malaria. Today we cannot discount the probability that the natural infection of some *Anopheles* species may be due to animal malaria, and the importance of identifying blood meals from various hosts is greater than formerly supposed.

In addition, recent surveys have revealed that the vectorial importance of less prominent, less well known *Anopheles* species has been underestimated, especially in tropical Africa. Secondary vectors such as *A. nili*, *A. moucheti*, *A. rufipes* and *A. pharoensis* might maintain a low degree of transmission even when malaria carried by *A. gambiae* and *A. funestus* has been interrupted. The identity of some malaria infections (such as in *A. coustani* in East Africa) remains unsolved.

The organization of a centralized precipitin testing service available to all field workers was made possible thanks to the setting up in 1952 of a special laboratory at the Lister Institute of Preventive Medicine, Elstree, England. This created entirely new and favourable conditions for large-scale identification of blood samples, as antisera of uniformly high specificity, sensitivity and purity became available (Weitz, 1956). Moreover, the introduction of an automatic dispenser for multiple serological titration permitted a great increase in the number of tests performed (Weitz, 1957). The availability of a highly dependable and standardized method of carrying out precipitin tests using sensitive and specific antisera distinguishes the present series of precipitin tests from any other large series hitherto recorded.

FIG. 1

PROCEDURE FOR THE IDENTIFICATION OF BLOOD MEALS IN TSETSE FLIES



Reproduced from Weitz (1956).

In order to economize material, the procedure outlined in this figure can usefully be followed. First test the blood meal with antihuman, antipig and antibovid serum. If the result is negative, test further with antimammalian and antiavian serum. If the result is positive, test for further identification with specific sera in the mammalian groups indicated in the chart; if it is negative, the sera are either of reptilian origin or unsuitable for testing.

In 1955 a number of national entomologists and WHO field staff were apprised of the facilities available and the scheme commenced at the end of that year. It must be admitted that during the early stage of this investigation not enough attention was paid to the planning of the testing procedures, so that the interpretation of results was at times doubtful. A new set of instructions has been devised to obviate these difficulties and during 1958-59 the information on the site of collection of *Anopheles* tested and on the spraying history of the locality was increasingly satisfactory.[1]

A full description of the serological technique used for the identification of blood meals at the Lister Institute was given by Weitz (1956) and need not be repeated here. The antisera obtained from suitably immunized rabbits contain the homologous and the heterologous group of precipitating antibodies. The heterologous antigens are removed by absorption and homologous sera are used for the identification of human blood and of a number of main groups of animals. For identification of animal species within these groups agglutination-inhibition tests could be used, but this is hardly ever needed in the study of malaria vectors.

A recent detailed review of the feeding habits of blood-sucking arthropods, based on the precipitin testing technique, has been prepared recently (Weitz, 1960).

The procedure for the identification of the five main groups of hosts of interest to malariologists (man; bovid;[2] horse and donkey; sheep and goat; pig) was to identify all human and bovid feeds first. Depending on the quality of the feeds other tests were applied, e.g., sheep and goat and/or horse, pig, avian. Meals which were not identified at this stage were tested with an antimammalian serum, where the quality of the meals permitted. If the result was positive further identification could be carried out for horse and donkey or for dog or other animals. If the result was negative the sera were either of avian or reptilian origin or else unsuitable for testing. The procedure used is similar to that shown diagramatically in Fig. 1, which is taken from Weitz (1956) and in fact applies to the identification of blood meals in tsetse flies.

It should perhaps be pointed out that negative results might be due to one of three causes:

(1) Tests were requested for human blood only and were not carried out beyond this stage if the result was negative.

(2) Tests were negative to any of the five or more groups of antisera, and the blood was that of an animal belonging to an untested group.

(3) The smear was of poor quality or contained little or no blood.

It will be seen in the next section that the over-all number of negative precipitin tests has not been unduly large (6.1%) and this testifies to the satisfactory technique of the collection of blood feeds in the field and their processing in the laboratory.

Since 1958 the results of precipitin tests received at the WHO Division of Malaria Eradication in Geneva have been recorded on punch cards and sorted manually. The design of the punch card is shown in Fig. 2.

REVIEW OF CONSOLIDATED RESULTS OF PRECIPITIN TESTS CARRIED OUT DURING 1955-59

The results of each series of precipitin tests were communicated to the individual research workers and used by them for their entomological and epidemiological studies. The classified results of all precipitin tests are, however, of such interest that they deserve an over-all tabulation and discussion. The results of the precipitin tests for the first five years can now be summarized and reviewed. In interpreting the data obtained one has to take into consideration the fact that at the beginning of the study very inadequate information was conveyed to WHO Headquarters pertaining to the circumstances of capture of blood-fed specimens. Only during the past two years has this been information forthcoming with greater regularity and in more detail.

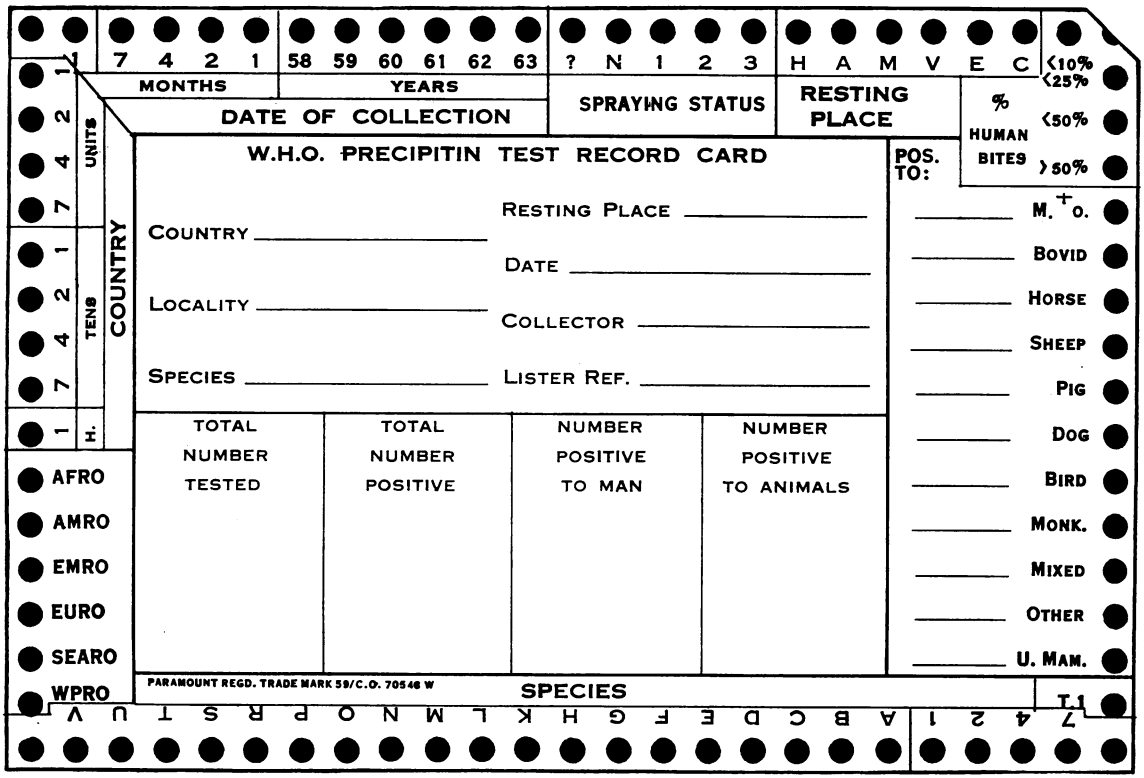
It is not known whether stables and human habitations were searched with equal thoroughness. It seems likely that some of the collections were made only from those places offering the greatest chance of collecting an adequate number of specimens. It is well realized that these factors may have caused a bias from the beginning.

[1] These instructions were appended to the preliminary report issued as mimeographed document WHO/Mal/234 and can be obtained on request from the Division of Malaria Eradication, World Health Organization, Geneva.

[2] For bovid feeds considerable variety of nomenclature has been used in the past (ox & buffalo, etc.). All these feeds are now grouped together as bovids unless requested otherwise. An interesting observation which highlights the difficulty of serological interpretation of some tests was made by Hurlbut & Weitz (1956). During a study carried out in the Nile Delta it was originally assumed that anti-ox serum would give cross-reactions with the Egyptian variety of Indian water buffalo, but when serum of water buffalo was available it was found that it reacted only at a low titre with anti-ox. It seems likely that most of the bloods grouped under " mammalian unidentified " were from water buffalo.

FIG. 2

PUNCH CARD USED BY WORLD HEALTH ORGANIZATION FOR RECORDING RESULTS OF PRECIPITIN TESTS



LEGEND

Spraying status

? = unknown
N = not sprayed
1 = DDT
2 = dieldrin, BHC
3 = other insecticides

Resting place

H = human dwelling
A = animal shelter
M = mixed shelter
V = vacant shelter
E = outdoor shelter
C = other

The status of spraying of the locality searched has been unknown until recently. Obviously, this would be most valuable information in comparing data for the same species in different localities or years of collection. Also, more emphasis must be placed in future on testing sufficient numbers of many lesser-known vector species.

The total number of precipitin tests carried out during the period under review amounted to 56 377, of which 93.9% gave positive results. (This is the highest number of consolidated precipitin test results ever recorded.) Fifty-one species of *Anopheles* were investigated. Among the positive tests, in 22% the resting-places were not recorded, not stated or did not belong to one of the usual categories.

Negative results were obtained in 6.1% of all the tests. This relatively high proportion of negatives is due to the fact that a number of tests were requested for human blood only or for one or two animal groups. Thus, all the tests negative for man or for a given animal group were reported as " negative " even though a detailed investigation might have revealed the identity of the host. For 1959 the proportion of negative results decreased to 2.6%.

The " human blood ratio " as used here expresses the percentage of human blood in relation to all the tests found positive. Unless this is well understood the term may be misleading. This can be illustrated by the example of two batches sent with a request for testing for the presence of human blood only. One

batch contained 319 smears from *A. pseudopunctipennis* with 293 positives (for man), the other 988 smears of *A. triannulatus* with only 12 positives (for man). For both species the human blood ratio was therefore 100%. It is obvious that this statement might be misinterpreted, especially for *A. triannulatus* in which 99.5% of the tests were negative and presumably positive for animal hosts for which no tests were performed. Consequently, the latter figure has not been included in any of the tables.

Over-all results of the precipitin tests are shown in Tables 1 and 2, which are self-explanatory.

The following species proved to give high human blood ratios, more than 75% of the positives containing human blood: *A. barbirostris*, *A. gambiae*, *A. funestus*, *A. leucosphyrus leucosphyrus*, *A. hancocki* (!), *A. moucheti*, *A. nili*, *A. sundaicus* and *A. wellcomei*.

A very low human blood ratio (less than 5%) was observed in the following *Anopheles*: *aconitus*, *annularis*, *cinereus*, *demeilloni*, *fluviatilis*, *hyrcanus sinensis*, *implexus*, *kochi*, *maculatus*, *maculipalpis*, *maculipennis*, *oswaldoi*, *pretoriensis*, *punctimacula*, *rivulorum*, *rufipes*, *subpictus malayensis*, *superpictus*, *triannulatus davisi* and *vagus*.

The proportions of blood meals from the various hosts, given in Table 2, show that by far the most preferred hosts seemed to be bovids, with horse or donkey a close second, sheep or goat third, and then, to a much lesser extent, dog or cat, pig, and bird. A few specimens with camel or monkey blood were recorded.

The over-all ratio of multiple feeds combining positive results for man and animals was about 0.5%; multiple feeds composed of various animal bloods amounted to about 0.4% of the total number of positive tests.[1]

Tables 3 and 4 show in detail the numbers of positive tests and the human blood ratios in *A. funestus* in relation to the countries concerned and site of capture.

An interesting observation can be made in regard to *A. funestus* in Southern Rhodesia in contrast to nine other countries where *A. funestus* proved to have a high human blood ratio. Only twenty-one out of 571 tests from Southern Rhodesia were positive for human blood. The details of this series of tests will be more clearly understood from Table 5. The reasons for this low figure are not understood, but it is likely that the results are due to great taxonomic difficulties in identifying the *Anopheles* of the *demeilloni-funestus* group in Southern Rhodesia. *A.* "*funestus*" can be distinguished from *A. demeilloni* with certainty only in the larval stage; there is some evidence that *A. leesoni* and *A. confusus* are the dominant members of the *A. funestus* group in Southern Rhodesia and in a part of Tanganyika (Muirhead-Thomson—personal communication).[1]

Table 6, which gives details of *A. funestus* collected from two areas of Tanganyika, is of interest since it shows the local differences between the results of two similar investigations. The data are taken from the report on the Taveta-Pare malaria scheme (East African Institute of Malaria, 1960).

The number of positive precipitin tests on *A. gambiae* and the relevant human blood ratio are shown in Table 7. The results obtained with 3034 specimens of *A. gambiae* tested in 1959 gave an over-all human blood ratio of 85.6%. It is interesting that the proportion of *A. gambiae* feeding on cattle amounted in Somaliland Protectorate, Southern Rhodesia and Zanzibar to 11%, 21.8% and 22.1% respectively.

The results of an investigation carried out in the course of the Western Sokoto Pilot Project in northern Nigeria revealed that in collections from human dwellings over 95% (out of 468) of *A. gambiae* were positive for human blood; in animal shelters (mainly horse stables) 79% out of 195 were found with horse blood in their stomach; on the other hand, when mosquitos were collected in mixed dwellings, such as Fulani huts, 72% of *A. gambiae* (out of 96 collected) were positive to man, 24% to horses or cattle and 4% were mixed feeds. In collections from outdoor shelters the results varied considerably in relation to the distance of the site from human dwellings but out of 138 specimens

[1] The problem of multiple feeds is of interest though of doubtful practical importance, as pointed out by Wharton (1953). Multiple feeds on different members of the same animal species might easily occur during the same gonotrophic cycle but, obviously, they would not show up in the precipitin tests. On the other hand, should a mosquito be able to feed fully on two different hosts within 48 hours, the chance of discovering a "mixed feed" by precipitin tests is slender, since the blood is usually digested in 36 hours. In most of the precipitin tests the proportion of mixed feeds does not exceed 5%. Nevertheless, there might be exceptional cases such as that of *A. aquasalis* in Trinidad, where, according to Senior White (1952), the ratio of mixed double and triple feeds on man, ox, horse and dog was unusually high and reached 7% of all positive tests. Recently, the results of precipitin tests on *A. gambiae* in Tanganyika showed that the number of multiple feeds of this mosquito might be as high as 3.8% (Smith & Weitz, 1959).

[1] In 1959, forty-six *A. demeilloni* were submitted from Southern Rhodesia; all were collected out of doors and yielded no positive tests for human blood.

TABLE 1. CONSOLIDATED TABLE OF PRECIPITIN TESTS AND HUMAN BLOOD RATIO BY RESTING-

Anopheline species	Total			Analysis by					
				Human habitations		Animal sheds		Mixed (human and animal habitations)	
	Total blood-meals	Total giving positive reaction	Human blood ratio	Human blood ratio	Human blood ratio	Total giving positive reaction	Human blood ratio	Total giving positive reaction	Human blood ratio
A. aconitus	431 2 883 24	407 2 515 23	14.9 3.9 87.0	407 575 15	14.9 1.9 80.0	541	0.5	34	0
A. albimanus	1 446	1 433	9.3	535	14.7	498	0	37	0
A. albitarsis	164	148	23.6	35	51.5			89	14.6
A. annularis	510	508	0.6			297	0	12	8.3
A. barbirostris *A. barbirostris* [c]	235 269 2 207	234 267 2 181	93.1 12.7 59.5	32 2 177	100 [b] 59.6	22	0	6	0
A. cinereus	152	137	0						
A. coustani group	118	109	25.7	18	11.1	34	5.9		
A. darlingi	116	114	46.5	5	40.0			109	46.7
A. demeilloni	96	96	3.1						
A. flavicosta	100	73	15.0			9	77.7	7	0
A. fluviatilis	75	58	3.4	1	100	56	1.8		
A. funestus	206 120 490 2 272 2 037	196 119 471 2 001 1 975	75.0 84.8 92.7 47.8 83.7	48 45 111 887 1 456	43.7 93.3 92.7 89.8 97.0	14 10 2 140 35	28.0 70.0 100 14.3 14.3	 23 128 73	 39.0 29.0 83.6
A. gambiae	340 2 586 706 5 885 3 633	308 2 398 634 5 469 3 561	83.1 87.1 92.5 79.3 77.0	172 643 107 3 821 3 034	96.5 94.0 65.0 93.7 85.6	44 25 764 95	22.7 68.0 17.1 21.0	 353 27	 57.2 85.0
A. hancocki	46	45	98.0	4	75.0				
A. hyrcanus nigerrimus	145	145	7.6			1	0		
A. hyrcanus sinensis	177	172	1.2					49	0
A. implexus	88	57	3.5						

ON 51 ANOPHELINE SPECIES, SHOWING POSITIVE RESULTS

PLACES AND YEARS OF COLLECTION

resting-places						Years of collection	Remarks (Countries refer to the place of origin of the specimens tested)
Vacant shelters		Outdoor (incl. some vacant shelters, 1955-58)		Unspecified or collected otherwise			
Total giving positive reaction	Human blood ratio	Total giving positive reaction	Human blood ratio	Total giving positive reaction	Human blood ratio		
		352 8	15.8 100	1 013	3.0	1955 1956-58 1959	Indonesia
288	19.1	9	0	66 [a]	0	1958-59	See Table 8; Colombia, Ecuador, Mexico, Peru. [a] 35 collected on outer walls of unsprayed house
		24	16.7			1959	Bolivia, Colombia, Paraguay
		1	0	198	1.0	1957-58	Indonesia
		4	100	234 207	93.1 1.0	1955-56 1957-58 1955-57	Indonesia, Sarawak [b] Unsprayed huts [c] Information from J. Yong, Sarawak
				137	0	1956	Saudi Arabia
		36	11.1	21	95.2	1955-59	Egypt, French Cameroons, Ghana, Nigeria. S. Rhodesia, Tanganyika, Voltaic Republic, Zanzibar
						1959	Bolivia, Colombia
		46 [d]	0	50 [e]	6.0	1956, 1959	[d] S. Rhodesia (1959), [e] Belgian Congo (1956)
		34	11.8	23	0	1958-59	Voltaic Republic
1	0					1955, 1958	Iran, Iraq, Saudi Arabia
40	85.0	6 1 637 371	100 100 12.0 38.0	134 58 334 209	91.0 79.3 96.4 12.4	1955 1956 1957 1958 1959	See Tables 3, 4, 5 and 6. French Cameroons, Ghana, Liberia, Nigeria, S. Rhodesia, Tanganyika, Uganda, Voltaic Republic, Zanzibar
10	70.0	1 125 393	100 32.0 22.9	91 1 730 527 406 2	86.8 84.7 98.1 94.5 100	1955 1956 1957 1958 1959	See Table 7. Belgian Congo, Ethiopia, French Cameroons, Ghana, Liberia, Mauritius, Nigeria, Saudi Arabia, Somalia, Somaliland Prot., S. Rhodesia, Sudan, Tanganyika, Uganda, Voltaic Republic, Zanzibar
				41	100	1955, 1959	French Cameroons, Liberia
				144	7.6	1957, 1959	Indonesia (Sumatra)
				123	1.6	1957, 1959	Indonesia, South Korea, Taiwan
				57	3.5	1958	Voltaic Republic

TABLE 1. CONSOLIDATED TABLE OF PRECIPITIN TESTS AND HUMAN BLOOD RATIO BY RESTING-

Anopheline species	Total			Analysis by					
				Human habitations		Animal sheds		Mixed (human and animal habitations)	
	Total blood-meals	Total giving positive reaction	Human blood ratio	Total giving positive reaction	Human blood ratio	Total giving positive reaction	Human blood ratio	Total giving positive reaction	Human blood ratio
A kochi	414	414	0			23	0	6	0
A. labranchiae	600	578	19.5	306	35.4	27	3.7		
A. leucosphyrus balabacensis	2 110 476	2 079 432	58.3 13.8						
A. l. leucosphyrus *A. l. leucosphyrus* f	221 1 346	220 1 342	98.6 94.2	1 335	94.3				
A. leucosphyrus group	257	255	43.1	176	42.6	79	45.5		
A. maculatus	274	270	0.4			2	0	256	0.4
A. maculipalpis	167	166	0			156	0		
A. maculipennis	368 878	324 835	2.1 1.3	90 24	3.3 0	234 809	1.7 1.6		
A. marshalli	47 122 85	42 118 78	85.7 27.1 2.6	22	4.5	22			
A. minimus flavirostris	157	140	47.1						
A. minimus minimus	186	129	7.7						
A. moucheti	131 24	125 23	97.5 0	2	0	1	0		
A. multicolor	311	278	6.1	99	7.6	62	3.2	63	12.6
A. nili	268 159	227 151	90.3 78.8	24	91.6	21	90.4	154	89.6
A. oswaldoi	48	48	2.1					48	2.1
A. pharoensis	308 941	294 924	59.8 48.7	27 368	92.5 80.0	107 146	8.4 9.6	81	97.5
A. pretoriensis	163	156	0.6	1	0	12	0		
A. pseudopuncti-pennis	1 643 1 754	1 470 1 719	54.2 16.3	1 403 933	54.0 16.3	65 349	60.0 0	114	62.0

ON 51 ANOPHELINE SPECIES, SHOWING POSITIVE RESULTS

PLACES AND YEARS OF COLLECTION (continued)

resting-places						Years of collection	Remarks (Countries refer to the place of origin of the specimens tested)
Vacant shelters		Outdoor (incl. some vacant shelters, 1955-58)		Unspecified or collected otherwise			
Total giving positive reaction	Human blood ratio	Total giving positive reaction	Human blood ratio	Total giving positive reaction	Human blood ratio		
		3	0	382	0	1957-1958	Cambodia. Indonesia
245	2.4					1959	Morocco
		1 618	58.2	461 432	60.0 13.8	1956 1957	North Borneo
		6 7	50.0 57.0	214	100	1955-57 1955-57	Sarawak [f] Information from J. Yong, Sarawak
						1958-59	Burma, Cambodia, Indonesia (Borneo)
		1	0	11	0	1957-1958	Cambodia, Indonesia, Taiwan
		10	0			1958-59	Ghana, S. Rhodesia, Zanzibar
				2	0	1955 1959	Greece, Iran, Iraq, Portugal
		35	2.8	42 118	85.7 27.1	1955 1956 1958-59	Tanganyika (1955) Belgian Congo (1956) S. Rhodesia, Uganda, Zanzibar (1958-59)
		140	47.1			1957	Indonesia, Philippines
		52	0	77	13.0	1957-58	Cambodia, Taiwan
		1	0	125 19	97.5 0	1955 1957	French Cameroons (1955) Nigeria (1957)
				54	0	1955, 1957, 1959	Iran, Saudi Arabia, Tunisia
		24 143	91.6 82.0	4 8	100 25.0	1958 1959	French Cameroons, Ghana, Voltaic Republic
						1959	Colombia
13	7.7	19 316	5.2 19.6	141	100	1955, 1956, 1958, 1959	Belgian Congo, Ethiopia, Ghana, Nigeria (1955-58), Egypt, French Cameroons, Uganda (1959)
1	0	142	0.7			1958-59	French Cameroons, Ghana, S. Rhodesia
148	12.8	2	50.0	175 [g]	22.2	1958 1959	See Table 9; Bolivia, Colombia, Mexico, Peru. [g] 58 (1.7% human blood ratio), corral roof; 117 (32.5% human blood ratio), bedroom roof

TABLE 1. CONSOLIDATED TABLE OF PRECIPITIN TESTS AND HUMAN BLOOD RATIO BY RESTING-

Anopheline species	Total			Analysis by					
				Human habitations		Animal sheds		Mixed (human and animal habitations)	
	Total blood-meals	Total giving positive reaction	Human blood ratio	Total giving positive reaction	Human blood ratio	Total giving positive reaction	Human blood ratio	Total giving positive reaction	Human blood ratio
A. pulcherrimus	71	44	13.6	44	13.6				
	122	105	6.7			105	6.7		
	343	299	3.6	29	17.2	267	1.8		
A. punctimacula	132	131	0						
A. rivulorum	335	329	0.9	15	0	255	0.8		
A. rufipes	176	161	26.0	8	100	17	11.7	84	22.6
	326	387	2.3	59	5.1	31	0	5	40.0
A. sacharovi	249	212	30.6			32	0		
	81	75	24.0	6	33.3				
	3 941	3 805	4.2	628	12.2	3 119	2.5		
	2 276	2 250	5.4	599	9.3	1 615	3.8	6	16.7
A. sergenti	300	274	14.6			148	0	126	31.7
	352	335	0	6	0	328	0		
A. squamosus	74	69	5.8	3	33.3	20	0		
A. stephensi	52	43	4.6	39	5.1	4	0		
	540	426	5.4	70	22.8	322	1.5		
A. subpictus malayensis	383	296	1.7	296	1.7				
	175	115	0						
	477	474	0.6	2	0	2	0	25	4.0
A. subpictus subpictus	71	71	2.8						
	430	427	28.5						
A. subpictus group	16	11	26.0	11	26.0				
A. sundaicus	578	539	72.9	454	72.4				
	461	460	80.4						
	67	65	87.5	49	94.0	4	25.0		
A. superpictus	734	547	2.0	47	8.5	486	1.4		
	175	138	13.7						
	458	384	4.1	12	25.0	372	2.9		
	358	335	4.2	3	0	21	0		
A. tesselatus	80	79	7.6	1	0	14	0	5	0
A. triannulatus davisi	87	86	2.3					86	2.3
A. umbrosus	85	82	67.1	19	79.0	9	0		
A. vagus	882	865	0.3	13	7.7	244	0	43	0
A. wellcomei	375	325	98.4			2	0		

ON 51 ANOPHELINE SPECIES, SHOWING POSITIVE RESULTS

PLACES AND YEARS OF COLLECTION (concluded)

resting-places						Years of collection	Remarks (Countries refer to the place of origin of the specimens tested)
Vacant shelters		Outdoor (incl. some vacant shelters, 1955-58)		Unspecified or collected otherwise			
Total giving positive reaction	Human blood ratio	Total giving positive reaction	Human blood ratio	Total giving positive reaction	Human blood ratio		
		1	100	2	0	1955 1957 1958	Iran, Iraq, Saudi Arabia
				131	0	1959	Ecuador
		59	1.7			1958-59	Zanzibar
		13 214	53.8 0.5	39 78	15.4 3.8	1955-57 1958-59	French Cameroons, Ghana, Nigeria S. Rhodesia, Voltaic Republic
				180 69 58 30 [h]	34.4 21.1 8.6 10.0	1955-56 1957 1958 1959	Afghanistan, Greece, Iraq, Syria [h] Traps
1	0					1957-58 1959	Saudi Arabia (1957-58) Morocco, Tunisia (1959)
		45	4.4	1	100	1955, 1958, 1959	Ghana, Nigeria, S. Rhodesia, Voltaic Republic, Zanzibar
				34	5.8	1955 1957-58	Iran (1955) Iraq, Saudi Arabia (1957-58)
				115 439	0 0.5	1955 1956 1957-58	Indonesia
				71 427	2.8 28.5	1956 1957	Indonesia
						1959	Indonesia (Java)
				85 460 12	75.2 80.4 83.0	1955-56 1957-58 1959	Indonesia
		14	0	138 311	13.7 4.5	1955 1956 1957-58 1959	Afghanistan, Greece, Iran, Iraq, Saudi Arabia, Syria
		5	0	54	11.1	1955-58	Indonesia, Sarawak
						1959	Colombia
		54	74.0			1958-59	Indonesia, Sarawak
		7	0	558	0.3	1957-59	Cambodia, Indonesia
		4	25.0	319	100	1955	French Cameroons, Nigeria

TABLE 2

PROPORTION OF POSITIVE PRECIPITIN TESTS BY HOSTS IN 51 ANOPHELINE SPECIES

Anopheline species	Years of collection	Total number giving positive reactions	Human blood ratio	Percentage of positives containing animal blood of									Remarks
				Ox or bovid	Horse or donkey	Sheep or goat	Dog	Pig	Bird	Mixed animals	Other	Unidentified mammal	
A. aconitus	1955 1956-58 1959	407 2 515 23	14.9 3.9 87.0 [a]	84.7 95.9 4.3	0.2 0.9	0.01				0.2		8.7	[a] 4.2% (1) mixed human and animal
A. albimanus	1959	1 433	9.3 [b]	10.3	3.5	7.4	30.0 [c]	31.0 [d]	1.3	0.4	0.1	6.5	[b] 0.7% (11) mixed human and animal [c] Mainly from Mexico [d] Mainly from Peru
A. albitarsis	1959	148	23.6	4.7	18.9		23.0	8.8	7.4			12.8	
A. annularis	1957-58	508	0.6	99.2	0.2								
A. barbirostris	1955-56 1957-58 1955-57 [e]	234 267 2 281	93.1 12.7 59.7 [f]	87.3			5.9 28.3	0.9 9.7		 0.6			[e] Information from J. Yong, Sarawak [f] 0.2% (5) mixed human and animal
A. cinereus	1956	137		78.7	17.0						4.4 [g]		[g] Camel
A. coustani group	1955, 1958, 1959	109	10.7	44.5	34.0	2.1						8.5	
A. darlingi	1959	114	45.5				49.0				4.5 [h]		[h] Cat
A. demeilloni	1956	96	3.1	95.0		2.0							
A. flavicosta	1958-59	73	15.0	68.4		4.1	4.1		6.8			1.4	
A. fluviatilis	1955, 1958	58	3.4	58.0	14.0	6.5	1.7		6.5	10.0			
A. funestus	1955 1956 1957 1958 1959	196 119 471 2 001 1 975	75.0 84.8 92.7 47.8 83.7	10.7 12.6 6.7 44.6 10.8	14.3 2.5 0.2 0.1 0.9	 2.8	 0.2 1.8 0.5	 0.2	 0.2 1.8 0.3	 0.05	 3.5	 3.5	
A. gambiae	1955 1956 1957 1958 1959	308 2 398 634 5 469 3 561	83.1 87.1 92.5 79.3 77.0	4.9 10.0 6.1 17.7 13.8	10.7 0.5 0.09 0.7	1.3 0.2 0.3 0.2 1.0	 0.05 0.3 0.7 0.3	 0.5 	 0.3 0.3 0.04 0.2	 0.3 0.5 1.7	 1.1 [i] 0.04 [j] 3.0 [k]	 3.7	[i] 0.7% monkey, 0.4% camel [j] 0.02 % camel [k] Mainly camel

A. hancocki	1955, 1959	45	98.0					2.0					
A.hyrcanus nigerrimus	1957, 1959	145	7.6	90.5		1.4						0.7	
A. hyrcanus sinensis	1957	172	1.2	94.8		0.6		0.6				2.9	
A. implexus	1958	57	3.5	1.7				91.3		3.5			
A. kochi	1957-58	414	0	99.5	0.5								
A. labranchiae	1959	578	19.5	41.5	19.3	0.3	2.3					16.8	
A. leucosphyrus balabacensis	1956 1957	2 079 432	58.3 13.8	34.7 74.0		0.05	5.7 6.7	1.1 4.8	0.3 0.5				
A. leucosphyrus leucosphyrus	1955-57 1955-57[1]	220 1 342	98.6 94.2		0.5		1.0 4.6	0.6	0.4				[1] Information from J. Yong, Sarawak
A. leucosphyrus group	1958-59	255	43.1	37.3			0.8	7.7	2.3	1.1		7.8	
A. maculatus	1957-58	270	0.4	99.2			0.4						
A. maculipalpis	1958-59	166	0	94.5	2.4	0.6	1.2					1.2	
A. maculipennis	1955 1959	324 835	2.1 1.5	81.0 63.5	9.3 0.5	0.3 0.9	0.6 0.1	23.8	2.2 0.2	4.4 0.2		9.3	
A. marshalli	1955 1956 1958-59	42 118 78	85.7 27.1 2.6	4.2 71.8 87.1		9.5 0.9 5.1	2.6				1.3	1.3	
A. minimus minimus	1957-58	129	7.7	90.0				1.5	0.8				
A. minimus flavirostris	1957	140	47.1	48.6				4.3					
A. moucheti	1955 1957	125 23	97.5 0	43.5	43.5	8.7	2.5			4.3			
A. multicolor	1955-57,1959	278	6.1	51.0	25.2				0.4	5.5	4.0	2.9	
A. nili	1958 1959	227 151	90.3 78.8	3.9 2.0	0.9	0.5	3.9 0.7	0.7		0.5		17.9	
A. oswaldoi	1959	48	2.1	37.5	12.5			35.5		2.1		10.8	

TABLE 2

PROPORTION OF POSITIVE PRECIPITIN TESTS BY HOSTS IN 51 ANOPHELINE SPECIES (concluded)

Anopheline species	Years of collection	Total number giving positive reactions	Human blood ratio	Percentage of positives containing animal blood of									Remarks
				Ox or bovid	Horse or donkey	Sheep or goat	Dog	Pig	Bird	Mixed animals	Other	Unidentified mammal	
A. pharoensis	1955-58 1959	294 924	59.8 48.7 [m]	2.9 37.0	31.0 2.7	5.1	1.5			0.3	6.8 [n]	2.7	[m] 0.5% (5) mixed human and animal [n] Mainly camel
A. pretoriensis	1958-59	156	0.6	93.6	2.6	0.6	1.3					1.3	
A. pseudopunctipennis	1958 1959	1 470 1 719	54.2 16.3 [o]	23.0 35.7	0.7 18	4.5 1.6	15.7 7.4	1.4 9.2	0.3 1.5	0.4 0.2		9.2	[o] 0.5% (8) mixed human and animal
A. pulcherrimus	1955 1957-58	44 404	13.6 4.4	82.0 62.0	2.2 30.0	2.2					3.0		
A. punctimacula	1959	131	0	72.5	22.8							4.6	
A. rivulorum	1958-59	329	0.9	95.5	0.9		0.9					1.5	
A. rufipes	1955-57 1958-59	161 387	26.0 2.3	67.7 83.0	1.9 4.9	5.0 3.7	1.8	0.5				3.9	
A. sacharovi	1955-56 1957 1958 1959	212 75 3 805 2 250	30.6 24.0 4.2 5.4 [p]	60.0 50.7 45.5 54.5	3.3 25.3 26.4 21.0	3.8 8.6 6.7	 2.2 1.7	 11.3 11.4	 1.0 0.6	2.4 0.5 0.3		1.8	[p] 0.2% (5) mixed human and anima
A. sergenti	1957-58 1959	274 335	14.6 0	70.0 69.0	11.3 14.3	0.7	1.2	2.3		3.3 1.5		10.5	

A. squamosus	1955-59	69	5.8	46.4	47.7								
A. stephensi	1955 1957-58	43 426	4.6 5.4	70.0 40.5	18.6 52.6	4.2	0.2		2.2	1.0			
A. subpictus malayensis	1955 1956 1957-58	296 115 474	1.7 0 0.6	95.8 100.0 99.4	1.0				0.7	0.7			
A. subpictus subpictus	1956 1957	71 427	2.8 28.5	95.8 71.2	1.4	0.2							
A. subpictus group	1959	11	26.0	74.0									
A. sundaicus	1955-56 1957-58 1959	539 460 65	72.9 80.4 87.5	25.7 19.6 6.2		0.3		0.3	0.2	0.5		6.2	
A. superpictus	1955 1956 1957-58 1959	547 138 384 335	2.0 13.7 4.1 4.2	71.5 78.5 41.2 52.0	12.8 5.1 12.4 11.6	7.9 2.2 33.4 0.5	0.5	4.5	0.2	5.3 0.5	3.7 *q*	32.5	*q* Mainly camel
A. tessellatus	1955-58	79	7.6	63.2			6.3	21.5	1.3				
A.triannulatus davisi	1959	86	2.3		77.0					2.3		16.5	
A. umbrosus	1958-59	82	67.0				14.6		7.3		11.0		
A. vagus	1957-59	865	0.3	99.5		0.2				0.2			
A. wellcomei	1955	325	98.4	0.3	1.5								

TABLE 3

NUMBER OF POSITIVE PRECIPITIN TESTS AND HUMAN BLOOD RATIO FOR *A. FUNESTUS* BY COUNTRY OF ORIGIN AND YEAR OF COLLECTION

Country	1955		1956		1957		1958		1959		Total	
	Total pos. tests	Human blood ratio	Total pos. tests	Human blood ratio	Total pos. tests	Human blood ratio	Total pos. tests	Human blood ratio	Total pos. tests	Human blood ratio	Total pos. tests	Human blood ratio
Southern Rhodesia							528	0.2[a]	43	46.4[b]	571	3.7
Voltaic Republic					416	92.0	939	53.0	327	36.6	1 682	59.3
French Cameroons									220	78.6	220	78.6
Belgian Congo			54	79.6							54	79.6
Nigeria	64	39.0	65	89.2	55	98.1			47	91.5	231	82.4
Zanzibar							480	85.2	410	93.0	890	88.3
Tanganyika	115	88.7									115	88.7
Ghana							54	94.4	34	94.1	88	94.2
Liberia	17	100							846	98.5	863	98.6
Uganda									48	100	48	100

[a] One found in indoor resting-place.
[b] 20 found in human habitations, of which 19 were collected in unsprayed villages and only one in a sprayed village.

TABLE 4

NUMBER OF POSITIVE PRECIPITIN TESTS AND HUMAN BLOOD RATIO FOR *A. FUNESTUS*, BY COUNTRIES AND RESTING-PLACES

Country	Human habitations		Animal sheds		Mixed (human and animal) habitations		Uninhabited (or outdoors, etc.)		Unspecified		Total	
	Total pos. tests	Human blood ratio	Total pos. tests	Human blood ratio	Total pos. tests	Human blood ratio	Total pos. tests	Human blood ratio	Total pos. tests	Human blood ratio	Total pos. tests	Human blood ratio
Southern Rhodesia	103	19.4[a]	52	0			416	0.2			571	3.7
Voltaic Republic	451	95.1	18	1.1	211	45.9	453	24.5	549	63.7	1 682	59.3
French Cameroons	149	90.0	22	22.7	13	34.5	36	72.2			220	78.6
Belgian Congo									54	79.6	54	79.6
Northern Nigeria	145	78.6	30	53.3			56	89.1			231	82.4
Zanzibar	786	96.4	79	18.9			25	52.0			890	88.3
Tanganyika									115	88.7	115	88.7
Ghana	59	94.7					29	93.0			88	94.2
Liberia	806	99.3					40	85.0	17	100	863	98.6
Uganda	48	100									48	100
Total	2 547	93.0	201	18.9	224	48.1	1 055	23.8	735	70.2	4 762	69.0

[a] The 19.4 % human blood ratio refers to 20 specimens, of which 19 were collected in unsprayed villages and only one in a sprayed village.

TABLE 5

PRECIPITIN TESTS ON *A. FUNESTUS* FROM SOUTHERN RHODESIA, 1958-59

Hosts	Number of tests giving positive results [a]		
	Human habitations	Animal sheds	Out of doors
Man	20 (19.4 %)	—	1 (0.2 %)
Bovid	63 (61.1 %)	38 (73 %)	378 (91 %)
Goat and sheep	5 (10.7 %)	12 (23 %)	23 (5 %)
Cat and dog	7 (6.8 %)	—	10 (2.5 %)
Horse	—	—	1 (0.2 %)
Mixed	—	—	3 (0.7 %)
Unidentified mammal	2 (1.9 %)	2 (4 %)	—

[a] Human blood ratios are given in parentheses.

TABLE 6

PRECIPITIN TESTS ON *A. FUNESTUS* COLLECTED FROM HOUSES IN THE TAVETA-PARE PROJECT IN TANGANYIKA [a]

Area	Site	Number tested	Percentage positive for:	
			Man	Cattle
Taveta	Forest	682	86	12
	Forest edge	176	83	11
	Riverine area	425	78	15
Pare	Hillfoot area	134	95	3
	Swampland	664	90	7

[a] After East African Institute of Malaria (1960).

TABLE 7

NUMBER OF POSITIVE PRECIPITIN TESTS AND HUMAN BLOOD RATIO FOR *A. GAMBIAE* BY COUNTRY OF ORIGIN AND YEAR OF COLLECTION

Country	1955		1956		1957		1958		1959		Total	
	Total pos. tests	Human blood ratio	Total pos. tests	Human blood ratio	Total pos. tests	Human blood ratio	Total pos. tests	Human blood ratio	Total pos. tests	Human blood ratio	Total pos. tests	Human blood ratio
Mauritius							606 [a]	18.4			606	18.4
Ghana							50 [b]	46.0	23	74.0	73	54.7
Southern Rhodesia							136 [c]	37.8	436	69.8	572	62.3
Somaliland Protectorate							24 [d]	37.5	801	68.3	825	67.5
French Cameroons	19	100	10	100					261	72.0	290	74.6
Zanzibar							782	79.4	1 123	72.7	1 905	75.0
Nigeria	217	81.5	87	82.7					17	88.2	321	81.4
Ethiopia					89	89.8	865	81.6			954	82.3
Tanganyika	69	82.6									69	82.6
Somalia			1 840	84.7	176	96.5					2 016	85.5
Voltaic Republic					369	91.3	2 772	93.4	39	74.3	3 180	92.9
Uganda									605	95.4	605	94.5
Belgian Congo			461	96.7							461	96.7
Saudi Arabia							99	96.9			99	96.9
Sudan							138	100	98	100	236	100
Liberia	3	100							158	100	161	100

[a] 80 % from animal sheds; 20 % from human habitations with nearly 100 % positive results in man.

[b] 59 % from outdoor resting-places with no positive results for human blood.

[c] 40 % from human habitations with 85 % positive for man, rest from animal sheds or outdoors with less positive for human blood.

[d] All indoor resting-places, mostly mixed habitations.

collected 66 were positive to man, 27% had horse blood and 6% other animal blood.[1]

The French team working in the Bobo-Dioulasso area of the Voltaic Republic found that in 417 *A. gambiae* collected from human dwellings the mean human blood ratio was 85% (Office de la Recherche Scientifique et Technique Outre-Mer, 1959).

In the Taveta-Pare area of Tanganyika a study carried out by the East African Institute of Malaria (1960) showed that in house collections in the forest, where man and cattle share the house, human blood was the preferred source of food of *A. gambiae* (human blood ratio 70%); in hillfoot villages where cattle are rare nearly all *A. gambiae* fed on man (human blood ratio 86%); in swampland villages, where cattle are common, the numbers of mosquitos feeding on man and on animals were the same (41% and 51% respectively). On the other hand, in *A. gambiae* collected in outdoor shelters only 13% were found with human blood and 74% fed on cattle.

It is also of interest that the human blood ratio of *A. gambiae* in Mauritius is low. This confirms the doubts expressed by Dowling (1953) on the vectorial importance of this species in Mauritius and raises an interesting biological problem.

There were some striking differences in the human blood ratio of *A. albimanus* and *A. pseudopunctipennis* in some countries of the American Region. While the over-all human blood ratio of 807 *A. albimanus* from Peru was 16.2%, that for 532 *A. albimanus* from Mexico was 0.6%. This difference was due to the different sites of capture, however, and to the availability of cattle as an alternative host. The discrepancies in the human blood ratio of the *A. pseudopunctipennis* complex in Peru, Bolivia and Colombia are shown in Table 8.

TABLE 8

PRECIPITIN TESTS IN *A. PSEUDOPUNCTIPENNIS* IN THREE COUNTRIES OF AMERICAN REGION

Country	Total		Human habitations		Predominant animal species as host
	Total pos. tests	Human blood ratio	Total pos. tests	Human blood ratio	
Peru	1 249	6.5 %	611 [a]	6.4 %	bovid (48.1 %), horse (24.8 %)
Bolivia	356	37.2 %	322 [a]	34.8 %	dog (13.4 %), pig (10 %)
Colombia	114	61.4 %	114 [a]	61.4 %	dog (27.7 %)

[a] Mixed human and animal habitations.

In Table 9 the available (though still imperfect) knowledge of the vectorial importance of 39 species of anophelines is compared with their human blood ratio. Generally, the data on the human blood ratio do not show much disagreement with the known vectorial importance of the species under review. In comparing these two factors one has to bear in mind that the part played by a species in malaria transmission depends not only on its human blood ratio but also on its numerical prevalence and other conditions. This may apply in particular to *A. flavicosta*, *A. hancocki* and *A. nili*, in which the human blood ratio is rather high; the number of tests performed on these species is small, because of their relative scarcity. According to the available knowledge of their vectorial importance they are classified as secondary vectors. The results with *A. pharoensis* are interesting as this species yielded relatively high human blood ratios in countries for which it was not listed as an important vector.[2]

A. marshalli is listed as not known to be a vector; however, out of 160 positive tests performed from unspecified resting-places the human blood ratio was 41%.

The results of the precipitin tests from *A. subpictus malayensis* and *A. subpictus subpictus* in Indonesia, with human blood ratios of 0.9% and 24% respectively, call for further investigation as the two species are considered either unknown or unimportant vectors in Indonesia.

The difference between the results obtained in this study for *A. fluviatilis* and *A. minimus minimus* and the results given by Senior White (1947), who investigated the " anthropophilic indices " of those *Anopheles* in East Central India, is of interest. In Senior White's series the human blood ratios of two species were considerably higher:

	Senior White (1947)	*Present study*
A. fluviatilis	44.1	3.4
A. minimus minimus	75.5	7.7

[1] Bruce-Chwatt, L. J. (1955) First Annual Report on the Western Sokoto Pilot Project; Bruce-Chwatt, L. J., Archibald, H. M. & Haworth, J. (1956, 1957) Second and Third Annual Reports on the Western Sokoto Pilot Project (mimeographed documents).

[2] In a recent study of the bionomics of mosquitos of the Nile Delta, Hurlbut & Weitz (1956) found that in *A. pharoensis* caught in bait traps out of 77% blood-fed, 97% gave positive results for human blood.

In view of the differences in numbers of specimens and in the method of collection the value of these comparative figures is, however, limited.

Table 10 summarizes the information given in Table 1 and shows the species composition of three groups of *Anopheles* divided arbitrarily into those with human blood ratios of below 10%, between 11% and 50%, and above 50%.

Table 11 contains some information on the human blood ratio of some rare species or those for which the number of precipitin tests was too small to be included in the general table.

There is little doubt that the important problem of the change of behaviour (including biting habits) of malaria vectors following the use of residual insecticides generally and DDT in particular deserves much attention. Muirhead-Thomson (personal communication, 1960) has attempted to show the difference in the biting habits of the *A. funestus* group collected out of doors in Southern Rhodesia in two groups of localities, one untreated and one treated with BHC. His results suggest that in BHC-treated areas there may be an increased amount of outdoor biting of man by the *A. funestus* group. Nonetheless the interpretation should be cautious since that group consisted of a mixture of *A. funestus funestus*, *A. leesoni*, *A. confusus* and *A. demeilloni* in unequal proportions in each area.

Two malaria eradication pilot projects in tropical Africa recently produced a series of interesting data on the problem of changes of behaviour of *A. gambiae* or *A. funestus* in areas where residual spraying was carried out using DDT and dieldrin respectively.

In the Bobo-Dioulasso area of the pilot project in the Voltaic Republic (the former Haute-Volta province of French West Africa) Hamon and his collaborators (1958) found an obvious difference in the human blood ratio of *A. gambiae* and particularly *A. funestus* collected in DDT-treated villages and in untreated villages. Their figures covering the year 1958 are as shown in Table 12.

The wide differences in human blood ratios found in *A. funestus* might be used as supporting evidence of the irritant effect of DDT deposits on the behaviour of this species, by driving them out of human habitations and compelling them to feed on domestic animals either outdoors or in cattle sheds. Unfortunately the findings are rendered inconclusive because of the different sampling techniques used in the two localities. In the untreated villages adult mosquitos were collected from houses. In the treated villages, adults were difficult to find inside the houses, and the collections of engorged mosquitos were made almost entirely in artificial outdoor shelters.

The results of a comparative investigation of precipitin testing before and after dieldrin spraying in the Taveta-Pare project in Tanganyika are interesting. Their details, compiled from two tables in the recent final report on that project (East African Institute of Malaria, 1960), are shown in Table 13. The figures suggest that there was no substantial change in blood preferences of *A. gambiae* as a result of house spraying with dieldrin. This result is in keeping with present knowledge of the relative absence of irritant action by dieldrin as compared with DDT.

Interesting data on the difference in the human blood ratio of *A. leucosphyrus leucosphyrus* and *A. barbirostris* between some areas of Sarawak treated either with dieldrin or with DDT were quoted by Colbourne, Huehne & Lachance (1959) and are shown in Table 14.

The differences between the first two areas may be due to the use of mosquito nets at Kampong Pichin and to the penning of pigs at Kampong Mentong Merau. But the difference between the third area and the first two cannot be explained in such a way and is probably due to the irritant effect of DDT driving the vectors outside houses and increasing their frequency of feeding on domestic animals out of doors.

The possibility of a permanent change of feeding habits of malaria vectors as a result of long-term residual spraying has been envisaged by Gabaldon (1953) and by Muirhead-Thomson [1] but never substantiated. It is therefore of interest that this possibility may be supported by the case of *A. sacharovi* in Greece according to Belios and Hadjinicolaou (personal communication, 1959).

Precipitin test results obtained in the era well before the introduction of DDT are compared with test results recorded during 1958, i.e., after 14 years of insecticide application, in Table 15.

In this case the uniformity of sampling methods (confined to habitations, i.e., stables and bedrooms) used before and after the long period of DDT application makes a direct comparison more feasible and the results correspondingly more significant. It is possible that a permanent change of feeding habits of *A. sacharovi* occurred in Greece, and the problem deserves a thorough examination.

[1] See the article on page 721 of this issue.

TABLE 9

COMPARISON OF KNOWN VECTORIAL IMPORTANCE AND HUMAN BLOOD RATIO OF ANOPHELINE SPECIES TESTED DURING 1955-1959

Anopheline species	Country of origin of specimens tested	Available knowledge of vectorial importance [a]	Human blood ratio [b] in specimens from						Predominant animal species as host
			Human habitations	Animal sheds	Mixed (human and animal habitations)	Vacant shelters	External (outdoors)	Collected otherwise or unspecified	
A. aconitus	Indonesia	Important in Indonesia; of secondary importance in Malaya and Indo-China	8.4 % (997)	0.5 % (541)			15.8 % (352)	3.0 % (1 013)	Bovid
A. albimanus [c]	Colombia, Ecuador, Mexico, Peru	One of the most important vectors in Central and South America	14.7 % (535)	0 (498)	0 (37)	19.1 % (288)	0 (9)	0 (66)	Dog(Mexico) Pig (Peru)
A. albitarsis	Bolivia, Colombia, Paraguay	Unimportant	51.5 % (35)		14.6 % (89)		16.7 % (24)		1. Dog 2. Horse
A. annularis	Indonesia	Important in parts of India		0 (297)	8.3 % (12)		0 (1)	1 % (198)	Bovid
A. barbirostris	Indonesia, Sarawak [d]	Of secondary importance in Indonesia, Malaya and Sarawak	100 % (32)	0 (22)	0 (6)			49.7 % (441)	Bovid
A. darlingi	Bolivia, Colombia	One of the most important vectors in South America	40 % (5)		46.7 % (109)				Dog
A. flavicosta	Voltaic Republic	Unknown; too rare for vectorial importance		77.7 % (9)	0 (7)		11.8 % (34)	0 (23)	Bovid
A. fluviatilis	Iran, Iraq, Saudi Arabia	Important vector mainly in India	100 % (1)	1.8 % (56)			0 (1)		Bovid
A. funestus [e]	French Cameroons, Ghana, Liberia, Nigeria, S. Rhodesia, Tanganyika, Uganda, Voltaic Republic, Zanzibar	One of the most important vectors of tropical Africa	93.0 % (2 547)	18.9 % (201)	48.1 % (224)		23.8 % (1 055)	70.2 % (735)	1. Bovid 2. Horse
A. gambiae [f]	Belgian Congo, Ethiopia, French Cameroons, Ghana, Liberia, Mauritius, Nigeria, Saudi Arabia, Somalia, Somaliland Protectorate, S. Rhodesia, Sudan, Tanganyika, Uganda, Voltaic Republic, Zanzibar	One of the most important vectors of tropical Africa and the Arabian Peninsula	90.3 % (7 777)	19.2 % (928)	59.3 % (380)	70 % (10)	25.2 % (519)	100 % (2)	1. Bovid 2. Horse

A. hancocki	French Cameroons, Liberia	Secondary vector in Central Africa	75 % (4)					100 % (41)	
A. hyrcanus nigerrimus	Indonesia	Of secondary importance in Indonesia and Malaya		0 (1)				7.6 % (144)	Bovid
A. hyrcanus sinensis	Indonesia, China (Taiwan), South Korea	Important in China and southern part of Japan			0 (49)			1.6 % (123)	Bovid
A. implexus	Voltaic Republic	Of no importance in Central and West Africa						3.5 % (57)	Pig
A. labranchiae	Morocco	Important vector in Italy and islands; secondary in Spain and North Africa	35.4 % (306)	3.7 % (27)		2.4 % (245)			Bovid
A. leucosphyrus balabacensis	North Borneo	Important in Borneo; less important in Burma and Indo-China					58.2 % (1 618)	37.7 % (893)	Bovid
A. leucosphyrus leucosphyrus	Sarawak [g]	Important in Indonesia and some parts of India (Assam), Burma and Sarawak					50 % (6)	100 % (214)	
A. leucosphyrus group	Burma, Cambodia, Indonesia	According to the subspecies	42.6 % (176)	45.5 % (79)					Bovid
A. maculatus	Cambodia, China (Taiwan), Indonesia	Important in Indonesia and Malaya		0 (2)	0.4 % (256)		0 (1)	0 (11)	Bovid
A. maculipennis malayensis	Greece, Iran, Iraq, Portugal	Important in Caucasus area and North Iran	2.6 % (114)	1.6 % (1 043)					Bovid
A. marshalli	Belgian Congo, S. Rhodesia, Tanganyika, Uganda, Zanzibar	No vectorial importance	4.5 % (22)	0 % (22)			2.8 % (35)	41.2 % (160)	Bovid
A. minimus flavirostris	Indonesia, Philippines	Important mainly in Philippines, less in Indonesia (Bali, Borneo, Java)					47.1 % (140)		Bovid

[a] References: Russell, Rozeboom & Stone (1943); de Meillon (1947); Boyd (1949); Russell (1952); Horsfall (1955); National Society of India for Malaria and other Mosquito-borne Diseases (1957). Many of these sources refer to " Indo-China " and it has not been possible to determine which of the present States (Cambodia, Laos or Viet Nam) is meant in every case. The old term has therefore been retained.

[b] The numbers of tests giving positive results are shown in parentheses.

[c] See Table 8.

[d] Information from J. Yong, Sarawak: human blood ratio from collections in human habitations was 58.8 % out of 1295 tests performed and from outdoor collections was 100 % out of 4 tests performed.

[e] See Tables 3, 4, 5 and 6.

[f] See Table 7.

[g] Information from J. Yong, Sarawak: human blood ratio from collections in human habitations was 94.3 % out of 1335 tests performed and from outdoor collections was 57.1 % out of 7 tests performed.

TABLE 9

COMPARISON OF KNOWN VECTORIAL IMPORTANCE AND HUMAN BLOOD RATIO OF ANOPHELINE SPECIES TESTED DURING 1955-1959

(concluded)

Anopheline species	Country of origin of specimens tested	Available knowledge of vectorial importance [a]	Human blood ratio [b] in specimens from						Predominant animal species as host
			Human habitations	Animal sheds	Mixed (human and animal habitations)	Vacant shelters	External (outdoors)	Collected otherwise or unspecified	
A. minimus minimus	Cambodia, China (Taiwan)	Very important vector in North India, Burma, Indo-China, Thailand, South China and Taiwan					0 (52)	13 % (77)	Bovid
A. moucheti	French Cameroons, Nigeria	Some vectorial importance in parts of Central Africa	0 (2)	0 (1)			0 (1)	97.5 % (125)	Bovid and horse & donkey equally
A. multicolor	Iran, Saudi Arabia, Tunisia	Possible vector in the Middle East and North Africa	7.6 % (99)	3.2 % (62)	12.6 % (63)			0 % (54)	1. Bovid 2. Horse & donkey
A. nili	French Cameroons, Ghana, Voltaic Republic	Important in North, West and Central Africa when prevalent	91.6 % (24)	90.4 % (21)	89.6 % (154)		83.7 % (166)	50 % (12)	1. Bovid 2. Dog
A. pharoensis	Belgian Congo, Egypt, Ethiopia French Cameroons, Ghana, Nigeria, Uganda	Important in Upper Egypt and Sudan, elsewhere of secondary importance	81.0 % (395)	9.1 % (253)	97.5 % (81)	7.7 % (13)	18.8 % (335)	100 % (141)	1. Bovid 2. Horse & donkey
A. pseudopunctipennis [h]	Bolivia, Colombia, Mexico, Peru	Important in Central and South America	38.4 % (2 336)	9.4 % (414)	62.0 % (114)	12.8 % (148)	50.0 % (2)	22.2 % (175)	1. Bovid 2. Dog
A. pulcherrimus	Iran, Iraq, Saudi Arabia	Important in Iraq and Caucasus area, doubtful vector in India	15 % (73)	3.2 % (372)			100 % (1)	0 (2)	1. Bovid 2. Horse & donkey
A. ruflpes	French Cameroons, Ghana, Nigeria, S. Rhodesia, Voltaic Republic	Doubtful vector except for the *ingrami* form in Belgian Congo	16.4 % (67)	4.3 % (48)	23.6 % (89)		3.5 % (227)	7.7 % (117)	Bovid

A. sacharovi	Afghanistan, Greece, Iraq, Syria	Important in the Balkans and part of Middle East	11.0 % (1 233)	2.9 % (4 766)	16.7 % (6)			25.4 % (337)	1. Bovid 2. Horse & donkey
A. sergenti	Morocco, Saudi Arabia, Tunisia	Important in parts of Middle East and Egypt	0 (6)	0 (328)	31.7 % (126)				Bovid
A. stephensi	Iran, Iraq, Saudi Arabia	Important in parts of India, Burma and Persian Gulf	14.6 % (109)	1.5 % (326)				5.8 % (34)	1. Horse & donkey 2. Bovid
A. subpictus malayensis	Indonesia	Unimportant	1.7 % (298)	0 (2)	4.0 % (25)			1.0 % (544)	Bovid
A. subpictus subpictus	Indonesia	Secondary importance in parts of Indonesia and Viet Nam						24 % (498)	Bovid
A. sundaicus	Indonesia	Very important vector in Eastern India, Indo-China, Indonesia, Thailand; secondary vector in Burma and Malaya	74.5 % (503)	25 % (4)				79.5 % (557)	Bovid
A. superpictus	Afghanistan, Greece, Iran, Iraq, Saudi Arabia, Syria	Important in West Pakistan, Iraq, South Europe and Cyprus	11.3 % (62)	2.3 % (879)			0 (14)	73.6 % (449)	1. Bovid 2. Sheep & goat
A. umbrosus	Indonesia, Sarawak	Unimportant	79.0 % (19)	0 (9)			74.0 % (54)		Sheep & goat
A. vagus	Cambodia, Indonesia	Secondary vector in India, Indonesia and Viet-Nam	7.7 % (13)	0 (244)	0 (43)		0 (7)	0.3 % (553)	Bovid
A. wellcomei	French Cameroons, Nigeria	Unknown		0 (2)			25 % (4)	100 % (319)	

[a] References: Russell, Rozeboom & Stone (1943); de Meillon (1947); Boyd (1949); Russell (1952); Horsfall (1955); National Society of India for Malaria and other Mosquito-borne Diseases (1957). Many of these sources refer to "Indo-China" and it has not been possible to determine which of the present States (Cambodia, Laos or Viet Nam) is meant in every case. The old term has therefore been retained.

[b] The numbers of tests giving positive results are shown in parentheses.

[h] See Table 8.

TABLE 10
CLASSIFICATION OF 51 SPECIES OF ANOPHELINES ACCORDING TO THE HUMAN BLOOD RATIO

1. Species with a human blood ratio less than 10 %

A. aconitus, A. albimanus, A. annularis, A. cinereus, A. demeilloni, A. fluviatilis, A. hyrcanus nigerrimus, A. hyrcanus sinensis, A. implexus, A. kochi, A. maculatus, A. maculipalpis, A. maculipennis maculipennis, A. minimus minimus, A. multicolor, A. oswaldoi, A. pretoriensis, A. pulcherrimus, A. punctimacula, A. rivulorum, A. ruflpes, A. sacharovi, A. sergenti, A. squamosus, A. stephensi, A. subpictus malayensis, A. superpictus, A. tesselatus, A. triannulatus davisi, A. vagus.

2. Species with a human blood ratio between 10 % and 50 %

A. albitarsis, A. coustani (group), *A. darlingi, A. flavicosta, A. labranchiae, A. marshalli, A. minimus flavirostris, A. pseudopunctipennis, A. subpictus subpictus.*

3. Species with a human blood ratio higher than 50 %

A. barbirostris, A. funestus, A. gambiae, A. hancocki, A. leucosphyrus balabacensis, A. leucosphyrus leucosphyrus, A. moucheti, A. nili, A. pharoensis, A. sundaicus, A. umbrosus, A. wellcomei.

The present account of the large-scale study of feeding habits of a number of important malaria vectors indicates only the main lines of the results obtained from this collaborative work. Important details of each individual investigation should be assessed and reported in the light of local conditions. It is hoped that many research workers, who availed themselves of the facilities of the precipitin test service sponsored by the World Health Organization, will describe the way in which this method was of assistance in the course of malaria eradication programmes in the countries concerned.

TABLE 11
LIST OF *ANOPHELES* SPECIES TESTED DURING 1955-59 BUT EXCLUDED FROM THE TABLES

Serial No.	Species	Number tested	Human blood ratio	Known vectorial importance
1	*A. argyritarsis*	2	100 %	Unimportant
2	*A. bradleyi*	11	0	Unknown
3	*A. brohieri* [a]	7	16.7 %	Unknown
4	*A. christyi*	15	0	Unimportant
5	*A. claviger* [b]	9		Important in Palestine
6	*A. d'thali*	1	0	Suspected
7	*A. freetown-ensis*	1	0	None
8	*A. garnhami*	5	0	None
9	*A. hispaniola*	9	0	Important in Algeria and Canary Islands
10	*A. hyrcanus*	21	0	
11	*A. kingi*	38	0	Unknown
12	*A. longipalpis*	2		
13	*A. marteri*	2	0	None
14	*A. matto-grossensis*	5	20 %	Unknown
15	*A. neomaculi-palpus*	3	33.3 %	Unknown
16	*A. pampanai*	4	0	Unknown
17	*A. philippi-nensis*	12	0	Important in Bengal (India)
18	*A. rangeli*	22	4.5 %	Unknown
19	*A. schüffneri*	1	0	Unknown
20	*A. sineroides*	6	0	Unknown
21	*A. smithi rageaui* [a]	71	33.3 %	Unknown
22	*A. strodei*	3	100 %	Unknown
23	*A. triannulatus* (unspec.)	2	50 %	None

[a] Six positive tests. [b] No positive tests.

TABLE 12. PRECIPITIN TESTS ON *A. GAMBIAE* AND *A. FUNESTUS* IN THE PILOT PROJECT OF BOBO-DIOULASSO (VOLTAIC REPUBLIC) [a]

Species	Area	Number tested	Positive for human blood	Positive for ox blood	Human blood ratio
A. gambiae	Untreated villages	2 514	2 343	163	93%
	DDT-treated villages	97	74	7	76%
A. funestus	Untreated villages	271	264	7	97%
	DDT-treated villages	560	80	207	14%

[a] After Hamon et al. (1958).

TABLE 13

PRECIPITIN TESTS ON *A. GAMBIAE* IN THE TAVETA-PARE PROJECT (TANGANYIKA) BEFORE AND AFTER DIELDRIN SPRAYING[a]

Area	Site	Before spraying			After spraying with dieldrin		
		Number tested	% positive		Number tested	% positive	
			Man	Cattle		Man	Cattle
Taveta	Forest	795	74	14	463	81	12
(collection	Forest edge	105	80	19	235	71	18
from houses)	Riverine area	459	62	28	235	64	16
	Dry area	62	69	19	62	69	15
Pare	Hillfoot	1 084	86	4	433	86	8
(collection	Swampland	2 109	41	51	3 194	42	50
from houses)	Swampland (House eaves)	115	4	91	45	20	62
Pare	Box traps	1 763	15	71	479	20	53
(collection out of doors)	Vegetation	281	0	86	273	0.4	93

[a] After East African Institute of Malaria (1960).

TABLE 14

HOST BLOOD RATIO IN *A. LEUCOSPHYRUS LEUCOSPHYRUS* AND *A. BARBIROSTRIS* COLLECTED INDOORS IN THREE AREAS OF SARAWAK[a]

Area	Insecticide	Species and numbers of *Anopheles*		Human blood ratio	Dog blood ratio	Pig blood ratio	Others
Kampong Mentong Merau	Dieldrin	*A. l. leucosphyrus*	141	78.8	17.7	0.7	2.1
		A. barbirostris	249	80.7	12.8	0.4	5.2
Kampong Pichin	Dieldrin	*A. l. leucosphyrus*	1 022	97.3	1.3	0.9	0.2
		A. barbirostris	1 834	54.8	31.7	12.5	0.3
4th Division	DDT	*A. l. leucosphyrus*	37	13.5	24.3	56.7	0
		A. barbirostris	346	27.4	36.4	31.7	3.7

[a] After Colbourne, Huehne & Lachance (1959).

TABLE 15

PRECIPITIN TESTS ON *A. SACHAROVI* IN GREECE IN RELATION TO DDT SPRAYING CAMPAIGN

	Total		Animal sheds		Human habitations (bedrooms)	
	Number tested	Human blood ratio	Number tested	Human blood ratio	Number tested	Human blood ratio
1932-34 [a]	6 835	38.7 %	2 855	7.5 %	3 980	61.3 %
1958, after 14 years, DDT application [b]	3 805	4.0 %		2.5 %		12.2 %

[a] Barber & Rice, 1935. [b] Belios & Hadjinicolaou—personal communication.

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RÉSUMÉ

Le succès des campagnes d'éradication exige la mise en œuvre de toutes les méthodes permettant de mieux connaître la biologie et le comportement des moustiques vecteurs. L'épreuve des précipitines, qui fait l'objet de cet article, est l'une d'elles. Grâce à ce test, appliqué dès 1920 à des études biologiques sur les moustiques, l'origine, humaine ou animale, d'un repas de sang peut être précisée au moyen de sérums hautement spécifiques. Il est ainsi possible de déterminer les préférences des anophèles pour tel ou tel hôte, et indirectement l'importance relative des diverses espèces dans la transmission du paludisme.

Dès 1955, l'OMS en collaboration avec le Lister Institute of Preventive Medicine, Elstree, Angleterre, a mis à la disposition des chercheurs et des travailleurs sur le terrain dans les diverses parties du monde, un centre où sont effectués les tests de précipitines. Dans cet article sont analysés les résultats de plus de 56 000 tests effectués durant 5 ans sur 51 espèces d'anophèles, ce qui représente une documentation unique en son genre par sa variété et son ampleur.

L'« indice proportionnel de sang humain » exprime le pourcentage d'échantillons de sang humain par rapport à l'ensemble des tests positifs. Chez certaines espèces, telles *A. gambiae*, *A. funestus* et *A. sundaicus* ce pourcentage dépasse 75. Dans d'autres, il ne dépasse pas 5-10%. Les hôtes animaux préférés sont les bovidés, suivis de près par les chevaux et les ânes, puis par les ovidés, et enfin loin derrière eux, les chats, porcs et oiseaux. Les particularités de diverses espèces d'anophèles dans le choix de leurs hôtes sont décrites. Le test des précipitines a permis aussi de confirmer le changement de comportement de certains anophèles, à la suite de pulvérisations de DDT, plus irritant que la dieldrine.

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