

## Prevalence of mixed blood meals and double feeding in a malaria vector (*Anopheles sacharovi* Favre)

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*A study was undertaken in a Greek village during 1970 to determine the degree of mixed feeding in a population of Anopheles sacharovi Favre. Exhaustive precipitin testing of 1 025 bloodmeals from 5 sites representing 3 different biotopes revealed that 91 (8.9%) of the 1 021 positive meals contained blood from 2 serologically distinct hosts. In a routine survey in 1971, when the testing was not as exhaustive, mixed meals were detected in only 2 (0.1%) of 1 798 smears tested. In the 1970 study, no mixed meals were found in pit-shelters, suggesting that a mosquito interrupted while feeding out of doors tends to move to an indoor biotope to complete its meal. A portion of the multiple meals—i.e., those completed on the same host species—could not be detected by the precipitin test. The frequencies of these “cryptic” multiple meals were calculated for the three main biotopes studied. The human blood index derived from these tests suggests that, in the absence of insecticidal spraying for 10 years, the host selection pattern of the mosquito had reverted to that found before the malaria eradication programme in Greece commenced.*

The blood precipitin test (Bull & King, 1923; Weitz, 1956) has been used extensively to study the feeding patterns of Anopheline mosquitos, and three global reviews of these patterns have been published (World Health Organization & Lister Institute, 1960; Garrett-Jones, 1964; Bruce-Chwatt et al., 1966). However, little information is available on the frequency with which the mosquitos in a population obtain a single complete meal from two or more hosts as a result of interruptions during feeding.

More recently, the World Health Organization (1972) has again drawn attention to the importance of a detailed knowledge of the patterns of contact between a vector and its hosts for understanding the epidemiology of any vector-borne disease. The epidemiological interest of these patterns prompted the authors, in collaboration with the World Health Organization, to study them by comprehensive precipitin testing in a vector known to have catholic feeding habits. *Anopheles sacharovi* Favre was the

vector chosen for the study, which was carried out in the village of Thermopylae, situated on the Lamia coastal plain of eastern Greece and well known for its therapeutic springs.

No precise terminology has been developed so far to describe blood meals taken from more than one host. With a view to rectifying this omission and clarifying the concepts of “host selection” and “host preference” we have adopted the following definitions, which we believe might be applicable to most blood-sucking insects.

*Simple meal.* A blood meal resulting from a single feed. It may be complete or, as a result of interruption, incomplete.

*Multiple meal.* Any blood meal resulting from two or more feeds the last of which has been taken before the first feed has been digested sufficiently to prevent identification of its origin.

*Unmixed meal.* A blood meal in which only one type of blood is recognized in the laboratory. It may be a simple meal or a multiple meal that is cryptic, i.e., composed of parts that cannot be distinguished in the laboratory.

*Part-meal.* Any component, whether patent or cryptic, that contributes to a multiple meal.

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*Host preference.* (Modified from World Health Organization, 1963, p. 100.) The choice of a particular vertebrate host as a food source, rather than other species equally available. *Note:* Host preference can be determined experimentally by means of animal baits or olfactometers (see, for example, van Thiel & Bevere, 1939; Hamon et al., 1964; Reid, 1961; Senior-White, 1952). Since it is an intrinsic physiological characteristic of the insect, host preference does not take into account host availability or host irritability, and can best be measured in captive samples. Its estimation in nature would involve the measurement of other indices representing these variable environmental factors (World Health Organization, 1972).

*Host selection.* (Used in the title of a paper by Bruce-Chwatt et al., 1966, but not defined.) A pattern of feeding in nature, as shown by the relative frequency of blood of different types observed in specific blood meal samples from a mosquito population at a defined place (a locality or biotope) and period. Patterns of host selection can be quantified by tests on wild-caught mosquitos. The term "forage ratio", defined by Hess et al. (1968), is an attempt to quantify host selection with a view to determining host preference.

#### METHODS

##### *Collection of specimens*

A total of 1 025 engorged *A. sacharovi* were caught in the morning by hand and tested for the presence of mixed blood meals. Most of them were caught during June and July 1970, but 14 were collected in February and 32 in May 1970. The fresh blood meals were smeared on filter paper by the standard technique approved by WHO.

Three main collecting sites were used: two cattle shelters, one (S2) situated in the village and the other (S1) 500 m from the nearest inhabited house. A third site (H1) comprised houses in the village, situated near several animal shelters. The numbers of resting blood-fed mosquitos collected at these sites were: 333 (S1); 377 (S2); and 260 (H1). The remainder were taken in artificial pit-shelters (P1 and P2) dug near cattle shelters S1 and S2 (10 were collected at P1 and 45 at P2).

##### *Potential hosts and their availability*

As recently emphasised by the World Health Organization (1972), the feeding habits of most vector populations depend on (among other factors)

the relative numbers of different vertebrate hosts and the availability of each. A knowledge of these factors is desirable in order to interpret any serological analysis of wild-caught samples. At Thermopylae, according to J. Hadjinicolaou (personal communication, 1972) the resident human population is about 350. This may be increased in the summer to about 1 200 by the influx of visitors who come to take therapeutic baths. However, the accessibility of these persons to the hungry *A. sacharovi* is believed to be kept at a low level by the general use of screens in bedrooms. Most of the residents enjoy similar protection—at any rate at the height of the mosquito season.

In the village there are approximately 85 cattle; 75 Equidae (horses, mules, and donkeys); 3 100 sheep and goats; and 20 pigs. In addition, chickens, rabbits, cats, and dogs are kept in and around the village. The Equidae are normally kept in the village itself, whereas most of the sheep and goats are kept on the outskirts in special shelters (although some are kept in the village). The pigs are kept in sties within the village boundary or in the yards of houses.

##### *Testing of specimens*

The blood meal smears, dried on filter paper (Weitz, 1963), were received at Imperial College Field Station for identification of their host sources (a source, in this context, meaning a species or any wider phylogenetic category). Each blood meal was eluted with 0.5 ml of saline and tested by the precipitin ring test for the presence of human blood and the blood of Bovidae, Equidae, dogs, pigs, birds, or rabbits. Meals giving a strong bovid reaction were subsequently tested for sheep/goat blood (these two hosts cannot be differentiated by the precipitin test). Since no wild bovinds were present in the study area, it can be concluded that the meals taken on Bovidae but not on sheep or goats were derived from the domestic ox. The bird blood detected in meals presumably was taken mainly from domestic fowls. Meals that failed to react to any of the antisera mentioned were tested against a general mammalian antiserum and, if that test was positive, against cat and rodent antisera. Some of the "mammalian" reactions were strong, suggesting that the meal originated from a mammal not mentioned above; but other, weaker, reactions to this antiserum could have denoted an older meal taken on any mammal.

During 1971 a further 1 798 blood smears of *A. sacharovi* were collected at Thermopylae and tested

by the precipitin test to determine their hosts, the meals being discarded once a single host had been identified.<sup>1</sup> The feeds were collected between July and September and comprised 217 smears from S1, 320 from S2, and 1 261 from H1. The sampling and routine testing in 1971 could thus serve as a control of the frequency of mixed meals revealed by the special testing procedure applied in 1970 but mostly passing unnoticed in the following year.

#### RESULTS

The results of the blood meal analysis on the samples collected in 1970 from the 5 collecting sites at Thermopylae are set out in Tables 1 and 2. In a total of 1 025 smears from *A. sacharovi*, all but 4 gave a positive reaction; 91 proved to be of serologically mixed origin. All the meals collected from P1 and P2 were unmixed, as were 87.5% of those collected at other sites. The most important hosts of *A. sacharovi* collected at S1 and S2 were sheep (or goats) and Equidae, whereas, in the H1 sample, the blood of man and pigs was the most prevalent.<sup>2</sup> Feeds consisting of equid blood predominated in both pit shelters.

Not more than 2 components were found in any mixed blood meal (see Tables 1 and 2). However, mixtures containing 3 different types of blood have been reported in anophelids by Senior-White (1952) and by WHO (unpublished data); Thus it is possible that some smears from Thermopylae represented a patent multiple meal superimposed on a cryptic one, or *vice versa*. The commonest source among the components identified in mixed meals from the 2 cattle shelters was sheep/goat. Unexpectedly, the next most important source, identified in 20 of the 68 mixed meals, was found to be birds. Pig's blood was the commonest component in the mixed meals collected in houses. The equal frequency of human and sheep/goat components in the mixed meals in this

Table 1. Precipitin test analysis, by collecting site, of blood meal smears from *A. sacharovi* sampled at Thermopylae, Greece, 1970

Host species or group	Numbers of positive smears from 5 collecting sites <sup>a</sup>					Total
	S1	S2	H1	P1	P2	
sheep/goat	222	132	18	3	10	385
pig	13	49	69	1	8	140
horse <sup>b</sup>	42	60	17	5	15	139
man	1	4	100	—	1	106
bird	16	31	9	—	2	58
dog	8	16	11	1	5	41
cow	3	23	11	—	2	39
rabbit	1	6	1	—	—	8
unidentified bovid	3	2	—	—	—	5
rodent	1	—	—	—	—	1
cat	—	1	—	—	—	1
mammal <sup>c</sup>	—	4	1	—	—	5
mammal <sup>d</sup>	1	—	—	—	1	2
sheep/goat & bird	12	11	3	—	—	26
sheep/goat & horse	1	9	1	—	—	11
cow & pig	1	8	1	—	—	10
sheep/goat & pig	1	2	5	—	—	8
cow & bird	1	4	—	—	—	5
man & pig	—	—	5	—	—	5
cow & horse	2	2	—	—	—	4
man & sheep/goat	1	2	1	—	—	4
pig & bird	—	2	1	—	—	3
man & cow	—	—	3	—	—	3
horse & unidentified bovid	1	1	1	—	—	3
pig & unidentified bovid	—	2	—	—	—	2
sheep/goat & rabbit	1	1	—	—	—	2
horse & pig	—	1	—	—	—	1
man & horse	—	1	—	—	—	1
horse & bird	—	—	1	—	—	1
man & dog	—	—	1	—	—	1
unidentified bovid & dog	—	1	—	—	—	1
negative	1	2	—	—	1	4
total positive smears	332	375	260	10	44	1 021
mixed meals	21	47	23	0	0	91
percentage mixed	6.3	12.5	8.8	0	0	8.9

<sup>1</sup> Occasionally, if a mixed feed was suspected because reactions to the first host identified were weak whereas the meal looked strong, the meal was subjected to further testing for other hosts. This explains the detection of mixed meals in 2 (0.1%) of the 1 798 smears tested in the routine survey in 1971.

<sup>2</sup> Most houses in Thermopylae are fitted with window screens against mosquitos, which therefore proved difficult to collect in large numbers. In fact, the majority of the blood-fed females from this biotope were found in a single room in which the screens were in poor condition and which overlooked a yard where pigs were kept. For this reason, the sample from houses was probably unrepresentative in that it contained a lower proportion of human blood (and possibly more mixed meals) than might have been expected in typical houses of the village.

<sup>a</sup> S1 and S2 are animal shelters; H1, houses; P1 and P2 are pit shelters dug near S1 and S2, respectively.

<sup>b</sup> Includes donkeys and mules.

<sup>c</sup> A blood meal that gave a strong reaction to a general mammal antiserum, but failed to react with any of the specific antisera used.

<sup>d</sup> A blood meal that gave a very weak reaction to the general mammal antiserum. These feeds could be derived from any mammals in the area.

Table 2. Composition of mixed blood meals

Host source	Components of mixed meals, by collecting site			Total	Proportion (%) of total meals	Total positive reactions to source <sup>a</sup>
	S1	S2	H1			
sheep/goat	16	25	10	51	56.0	436
bird	13	17	5	35	38.5	93
pig	2	15	12	29	31.9	169
cow	4	14	4	22	24.2	61
equid	4	14	3	21	23.1	160
man	1	3	10	14	15.4	120
unidentified bovid <sup>b</sup>	1	4	1	6	6.6	11
rabbit	1	1	0	2	2.2	10
dog	0	1	1	2	2.2	43
others <sup>c</sup>	0	0	0	0	0	9
reactions	42	94	46	182	200.1	1 112
meals	21	47	23	91	100	1 021

<sup>a</sup> The number of positive reactions to each host is the sum of the mixed and the unmixed meals containing its blood. The total for all sources is the whole positive sample (1 021) plus the number of mixed meals (91).

<sup>b</sup> At every collecting site, the source was more often sheep/goat than cow. Most of the unidentified bovid reactions were therefore probably from the former source and the true proportion of mixed meals containing sheep/goat blood may thus have approached 62.6%.

<sup>c</sup> Our method excludes from detection any mixture (except one containing avian blood) in a smear reacting only to the general mammalian antiserum, i.e., 7 of these 9 specimens.

biotope contrasts with their great disparity (100 against 18) in the unmixed samples.

Table 2 shows the host sources of mixed meals listed in descending order of frequency. It is apparent that these frequencies are uncorrelated with the total positive reactions shown in the last column. This represents the ratio of patent part-meals to the total positive reactions (part-meals plus whole meals) for the given host source. This ratio was highest for birds and cows—sources from which the total numbers of meals were rather small.

The positive precipitin reactions were graded by eye as strong or weak according to the density of the precipitates. Only 20% of the mixed meals gave a weak reaction for one or another component. This suggests that most interrupted meals were completed the same night. A mixture composed of one part from an unidentified mammal group and another from an unidentified group would be classed as "unmixed". The figures in Table 1 suggest that the samples in-

cluded no such mixtures, or too few to represent an important error in the calculations in Table 2. The same cannot be said of cryptic multiple meals—those made up of 2 or more feeds from the same (serological) source.

The human blood index (HBI) of mosquitos sampled from houses should be considered separately from the HBI of those collected at the other 4 sites. Human blood was found in 42.2% of the 237 unmixed meals and in 43.5% of the 23 mixed meals collected inside houses. The overall proportion containing human blood in this biotope was 110/260—an HBI of 0.423. Among the 693 unmixed meals from the other biotopes, 0.87% contained human blood, the proportion among the 68 mixed meals being 5.9%. In these biotopes the HBI was 0.013 (i.e., 10/761). The unweighted mean of the HBI values from the collecting sites with and without human hosts was 0.218. This mean was considered by Garrett-Jones (1964) as being often the best available evaluation of the HBI of a mosquito population whose blood-fed females rest freely in biotopes of both classes.

The results of the routine precipitin testing of 1 798 blood meals collected from the same vector population in 1971 are set out in Table 3. Their most striking feature, in the light of the fuller analysis of the 1970 samples as described, is the fact that only 2 mixed meals (0.1%) were detected by the routine procedure. Human blood was found in 61.2% of the meals taken in houses and in 0.9% of those from 2 stables. Thus the HBI, given by the unweighted mean of those proportions, was 0.310.

## DISCUSSION

### *Problems of sampling and testing*

The interpretation of the results tabulated in this paper calls for some caution in view of the wide divergences in the prevalence of mixed blood meals observed in various mosquitos by other workers, and the inferences drawn from them. Thus Senior-White (1952) found that, of 867 positive smears from *A. aquasalis* collected at outdoor resting sites in Trinidad, 12% contained mixed meals; 89 of the 104 mixed feeds contained 2 detectable components; 14 had 3 components; and 1 had 4 components (man, goat, horse, and dog).

Shemanchuk et al. (1963) collected mosquitos in irrigated areas of southern Alberta and, using 8 different antisera, detected 6% of mixed meals. Most of the species tested were Culicinae. In that study, the

Table 3. Routine precipitin test analysis, by collecting site, of blood meal smears from *A. sacharovi* sampled at Thermopylae, Greece, 1971 <sup>a</sup>

Host species or group	Numbers of positive smears from 3 collecting sites			
	S1	S2	H1	Total
sheep/goat	134	208	137	479
pig	7	46	133	186
horse	60	13	73	146
man	1	3	773	777
bird	2	20	41	63
dog	12	13	57	82
cow	—	10	25	35
rabbit	—	1	2	3
unidentified bovid	1	—	4	5
cat	—	—	2	2
mammal <sup>b</sup>	—	2	13	15
mammal <sup>c</sup>	—	3	—	3
unidentified bovid & horse	—	—	1	1
man and sheep/goat	—	1	—	1
total positive smears	217	320	1 261	1 798
mixed meals	—	1	1	2
% mixed meals	—	0.3	0.1	0.1

<sup>a</sup> These samples were not exhaustively tested for the presence of mixed feeds.

<sup>b</sup> See Table 1, footnote c.

<sup>c</sup> See Table 1, footnote d.

low figure was attributed to a low rate of interruption of feeding on the main host or hosts, whereas greater ability to disturb the feeding mosquito was ascribed to rodents—a host source contributing to about half of the mixed feeds recorded. The inference may be correct in this case. However, most of the multiple meals taken are likely to be cryptic, not mixed, in any biotope or locality where one favoured host outnumbers all the others.

Edman & Downe (1964) analysed the blood meals taken by 13 culicine and one anopheline species of which samples were collected in light traps in Kansas City. By means of 8 different antisera they were able to detect proportions of mixed meals ranging, according to the species, between 9.7% and 61.8%.

In considering reports of this type, it is evidently important to relate them to the biotopic distribution of the blood-fed females of each species (or at least

to that of the samples collected), as well as to the distribution and prevalence of the various hosts represented.

Extensive data analysed by Tempelis (1970) refer to more than 60 000 blood meals taken by mosquitos of over 40 species from the nearctic and neotropical regions. In only 2 populations—*Culex quinquefasciatus* in Hawaii and *C. tarsalis* in Texas—did he find an incidence of mixed feeding greater than 0.1%. In both cases, the incidence was about 1%. Tempelis concluded that “Multiple feedings do not appear to be a common phenomenon among mosquitos”. In our terminology, this statement refers to all multiple meals. It does not seem to allow sufficiently for the prevalence, under certain conditions, of cryptic multiple meals obtained by feeding twice on the same host species.

#### *Epidemiological implications of multiple meals*

In an arthropod vector of a disease affecting man, the taking of cryptic multiple meals may be expected to increase the man-biting habit, and thereby the vectorial capacity of the population (Garrett-Jones & Grab, 1964). The chances of acquiring and of transmitting the disease agent would both be improved in consequence.

An epidemiological question that remains to be investigated is whether malarial infection of the mosquito is more likely to result from parasites ingested with the first part-meal or with the second. Downe (1965) showed in the laboratory that the time taken by *Aedes aegypti* (L) to digest a part-meal from man was increased, even exceeding 48 hours in some female mosquitos allowed to complete the blood meal between 2 and 12 hours after the original feed. Such a delay could favour infection. On the other hand, the earlier part-meal would become surrounded by the later one, and migrating ookinetes in the former might face increased difficulties in reaching the wall of the gut. One would expect the obstacles to be more severe with a mixed blood meal, in which the successful parasite would need to traverse a layer of disparate blood that is foreign to it.

#### *Multiple meals: mixed and cryptic*

The tests carried out on 1025 smears collected at Thermopylae during 1970 were designed to demonstrate mixed meals (Table 1). However, in the majority of tests instigated by the World Health Organization since 1955, this has not been a primary object in the routine analysis of anopheline blood meal smears (World Health Organization & Lister Insti-

tute, 1960; Bruce-Chwatt et al., 1966). Bruce-Chwatt et al. (1966) recorded smears from 9 259 *A. sacharovi* originating from Afghanistan, Greece, Iraq, and Syria, and under 1% were recognized as being of mixed origin.

There is little doubt that the low index obtained in 1971, and the earlier indices quoted by Bruce-Chwatt et al. (1966), resulted from the procedure normally followed, i.e., discarding the sample once a positive reaction is obtained. The findings from the exhaustive tests demonstrate a much higher proportion (8.9%) of mixed meals in *A. sacharovi* in Greece, and suggest that this may apply in other parts of its range.

Nor is this the whole story, since our observations revealed only the serologically patent fraction of the multiple meals; another portion—the cryptic fraction—has not so far been determined by direct observation. But it is hoped to develop a test method by which this fraction also may be determined. The total absence of mixed meals in the sample of 54 smears from pit-shelters may be compared with an expected finding of about 5 such meals in view of

the results from the animal sheds nearby. This difference is significant ( $P < 0.05$ ). Subject to further sampling in this biotope, it is possible that only mosquitos that obtained a complete meal outdoors sought an outdoor resting place after gorging, whereas others, whose meal outdoors was interrupted, tended to move indoors before seeking further blood. Following such movement, the host species that provided the first part-meal might no longer have been readily available and, when a second part-meal was taken, a mixed meal would usually have resulted.

Presumably interruption of feeding is as probable indoors as outdoors, but in the former case the part-fed mosquito is perhaps more likely to complete its meal in the same feeding-place. A high proportion of these multiple meals will be cryptic, especially in a biotope containing predominantly one host, e.g., a bedroom or hen-house. Since the cryptic multiple meals are those of most epidemiological interest, we have computed in Tables 4 and 5 the expected proportions of such meals at the 3 col-

Table 4. Manner of computing an index of the proportion of multiple meals (including cryptic meals) in a sample (data from houses)

Data	Host species or group						total
	derivation	man	pig	sheep/goat	horse	other	
(1) Number of unmixed meals	—	100	69	18	17	33	237
(2) Probability of feeding on host:							
1 in 1 feed (p)	pos./total	0.422	0.291	0.076	0.072	0.139	1.0
0 in 1 feed (q)	1—p	0.578	0.709	0.924	0.928	0.861	4.0
2 in 2 feeds	p <sup>2</sup>	0.178	0.085	0.006	0.005	0.019	5.0
0 in 2 feeds	q <sup>2</sup>	0.334	0.500	0.853	0.861	0.742	
1 in 2 feeds	2 pq	0.488	0.415	0.141	0.134	0.239	
(3) Ratio of probabilities, cryptic: patent	p/2q	0.365	0.205	0.043	0.037	0.080	—
(4) Observed contributions to mixed meals	—	10	12	10	3	11	46
(5) Expected number of cryptic multiple meals <sup>a</sup>	(4) × (3)	3.65	2.46	0.43	0.11	0.88	7.53
(6) Inferred number of all multiple meals	(4) + (5)	13.65	14.46	10.43	3.11	11.88	30.53 <sup>b</sup>
(7) Ratio of all multiple meals to all positive smears	(6)/[(1)+(4)]	0.12	0.18	0.37	0.16	0.27	0.12 <sup>b</sup>

<sup>a</sup> The figures in parentheses under "derivation" refer to the numbers down the left margin.

<sup>b</sup> In calculating the total number of multiple meals, the sum of the contributions to mixed meals must be halved. Likewise, for the ratio of multiple meals to positive smears:  $30.53/(237+23) = 0.117$  (11.7%).

lecting sites in Thermopylae. For this purpose, it has to be assumed that the conditions governing host selection (as distinct from host preference) are such that it is random—i.e., the same alternative hosts are available in a given biotope when the first and second part-meals are taken.

Table 4 shows the method of computation, using as an example the findings from houses (H1). From the analysis of unmixed meals 5 series of probabilities are calculated, covering all the possible numbers of contacts between a feeding mosquito and a

given host in obtaining a simple or a 2-part meal. From these probabilities a ratio is derived, representing the expected relative frequency of cryptic and patent multiple meals. The number of patent multiple meals being known, the expected number of cryptic meals is derived from it by applying this ratio. Finally, the sum of the patent and the expected cryptic multiple meals—i.e., the inferred total number of multiple meals—is expressed as a proportion of all the smears found to contain blood from the given host.

Table 5. Computed proportions of multiple meals, according to host and collecting site<sup>a</sup>

Data	Host species or group							total <sup>b</sup>
	man	sheep/goat	cow	horse	pig	bird	other	
<i>collecting site S1</i>								
Contributions to mixed meals	*	16	*	4	*	13	9	42
Expected number of cryptic multiple meals		16.5		0.29		0.29	0.77	17.85
Inferred total of multiple meals <sup>c</sup>		32.5		4.29		13.29	9.77	38.85
Total of positive smears		238		46		29	59	332
Proportion of multiple meals (%)	*	13.7	*	9.3	*	45.8	16.6	11.7
<i>collecting site S2</i>								
Contributions to mixed meals	*	25	14	14	15	17	9	94
Expected number of cryptic multiple meals		10.60	0.53	1.60	1.29	0.90	0.50	15.42
Inferred total of multiple meals <sup>c</sup>		35.60	14.53	15.60	16.29	17.90	9.50	62.42
Total of positive smears		157	37	74	64	48	42	375
Proportion of multiple meals (%)	*	22.6	39.3	21.1	25.4	37.3	22.6	16.6
<i>collecting site H1</i>								
Contributions to mixed meals	10	10	*	3	12	*	11	46
Expected number of cryptic multiple meals	3.65	0.43		0.11	2.46		0.88	7.53
Inferred total of multiple meals <sup>c</sup>	13.65	10.43		3.11	14.46		11.88	30.53
Total of positive smears	110	28		20	81		44	260
Proportion of multiple meals (%)	12.4	37.3	*	15.6	17.8	*	27.0	11.7

<sup>a</sup> An asterisk denotes that the number is small and has therefore been included among "other".

<sup>b</sup> Total number of positive blood meals tested for each biotope, not the sum of the total numbers of positive smears shown in the columns under "Host species or group", since these include mixed meals.

<sup>c</sup> Sum of the two preceding lines, except in the final column, where the total number of mixed meals is equal to half the number of contributions recorded. For example, for collecting site S1 the total number of inferred multiple meals is  $42/2 + 17.85 = 38.85$ .

In Table 5 the computations described above are summarized for sites S1, S2, and H1. The highest proportion of multiple meals (16.6%) occurred at S2—the site where the variety of hosts was widest and the greatest numbers were present. It is instructive to compare sites S1 and H1, in each of which an index of 11.7% multiple meals was inferred. This would not have been evident from the mixed meals alone, since in S1 these numbered only 6.3%, whereas in H1 they accounted for 8.8% (see Table 1). At S1 a single host (sheep/goat) was represented in over 2/3 of the total sample, whereas the main host in the houses, i.e., man, contributed to less than half the sample from that biotope. This explains why, in S1 but not in H1, the inferred number of cryptic multiple meals on the leading host was greater than the observed number of its contributions to mixed meals.

#### *Correction of the HBI by multiple-meal analysis*

The results showed an HBI of 110/260 (42.3%) in the sample from site H1. However, the computations given in Table 4 imply that the 260 smears may have represented just over 290 meals and part-meals (i.e.,  $237 + 7.53 + 46$ ) taken by the sample shortly before collection. Allowing for all the multiple meals, 114 ( $100 + 3.65 + 10$ ) of these feeds, i.e., 39.0%, are estimated to have been obtained from man. Although this is slightly below the HBI, it is worth noting that, in a more strongly anthropophilic vector, the expected proportion of feeds on man would be substantially higher than the proportion of meals containing human blood, supposing that about 10% of meals are interrupted and completed later. Analogous estimates may of course be made for the proportion of feeds taken from any given host in any biotope.

Certain hosts, such as birds and cows (Table 2) are more strongly represented in the mixed than in the unmixed meals. One explanation could be that these are the hosts that react with the most irritability when bitten. Edman & Kale (1971) have demonstrated that, in the case of *Cinconiiform* birds, host behaviour is important in determining the blood sources of mosquitos. However, the high values recorded could equally be due to a combination of two factors: the relative scarcity of these hosts at the particular collecting sites used and a tendency of the part-fed mosquito to move from one site to another before completing its meal. Samples taken from cow-sheds or hen-houses might have given a different result. It should be rewarding to analyse blood meal samples drawn from a wider variety of classified biotopes

that are freely used as feeding and resting sites by a vector population.

#### *Decline and recovery of the HBI in A. sacharovi*

It was considered by the World Health Organization & Lister Institute (1960) that the reduction in the HBI of *A. sacharovi* recorded in Greece after 14 years of residual house-spraying was the only evidence of a permanent change of feeding habits of malaria vectors as a result of long-term residual spraying (as envisaged by Gabaldón (1954) and by Muirhead-Thomson<sup>1</sup> but never substantiated) and that the problem deserved a thorough examination. The implication was that insecticide selection pressure might modify the genetic constitution of a vector population with consequent changes in its inherited host preference. The proof of this would require laboratory experiments on captive mosquitos, designed to eliminate the environmental variables (see definitions, pp. 605-606). Failing such experiments, it is worth examining the available field observations relating to *A. sacharovi* in Greece (Table 6).

It is immediately apparent from the table that a dramatic change took place in the vector's host-selection pattern some time between 1934 and 1958, followed by a reversion towards the original pattern between 1964 and 1968. The question is: does the evidence suggest that the selection of animal rather than human hosts in the DDT and post-DDT years was due merely to direct reactions by many blood-sucking females to their changed environment, overriding their innate host-preference; or does it rather support the hypothesis that the vector's inherited host-preference itself underwent a change by reason of the sustained insecticide selection pressure? The first view would offer an extrinsic, ecological explanation; the second, an explanation that is intrinsic, i.e., genetic and physiological.

We believe that only the latter explanation is consistent with the evidence, and this for two reasons: first, that the HBI remained at low levels for about 5 years after DDT was last applied to the houses in Lamia, i.e., 1959, according to Hadjinicolaou & Betzios, 1973); and, secondly, that the reduction of the percentage positive for human blood was most marked in the samples collected inside houses. Here it should be noted that the HBI was calculated in each case by means of Garrett-Jones's (1964) formula, which gives equal weight to both components irrespective of the relative densities of resting mosquitos found in each biotope. Thus the reduction in

<sup>1</sup> Unpublished observations, 1960.



Table 6. Field observations relating to the human blood index of *A. sacharovi* in Greece

Source	Area and year	Spraying status <sup>a</sup>	% with human blood		HBI <sup>b</sup>
			houses	other biotopes	
Barber & Rice (1935)	Macedonia, 1932-34	PS	61.3 (3 980)	7.5 (2 855)	0.344
Belios & Hadjinicolaou, 1959 (quoted by World Health Organization & Lister Institute, 1960) <sup>c</sup>	Greece, 1958	UT	12.2 (564)	2.5 (3 241)	0.074
Bruce-Chwatt et al. (1966)	Greece, 1959-64	UT	8.7 (357)	3.6 (1 095)	0.062
Bruce-Chwatt et al. (1966) <sup>d</sup>	Greece, 1959-64	US/FS	8.2 (305)	6.1 (921)	0.072
Hadjinicolaou & Betzios (1973)	Lamia, 1968	FS	61.5 (304)	2.9 (411)	0.322
Boreham & Garrett-Jones (this paper)	Lamia, 1970	FS	42.3 (260)	1.2 (759)	0.218
Boreham & Garrett-Jones (this paper)	Lamia, 1971	FS	61.2 (1 261)	0.9 (534)	0.310

<sup>a</sup> PS = prespray; UT = under spray treatment; US = unsprayed; FS = formerly sprayed.

<sup>b</sup> The HBI is expressed throughout as the unweighted mean of the values in the two biotopic components (see Garrett-Jones, 1964).

<sup>c</sup> A total of 3 805 positive smears was given. The numbers from each biotope were calculated by the authors of the present paper.

<sup>d</sup> The samples were mostly from formerly sprayed localities, and include the smaller samples listed by Garrett-Jones (1964).

the index cannot be attributed to ecological influences such as DDT-irritation leading to exodus after feeding—the more so as any considerable exodus by females that had fed on man would tend to increase the HBI component in the other biotopes where they might go to rest, leaving unaffected the component derived from those females that remained in the dwellings.

From the observations in Greece (Table 6) we infer that, in the presence of residual DDT there was increased zoophily in the host-preference of *A.*

*sacharovi* (though this is still unconfirmed by laboratory tests). However, if this was a heritable change, it was not permanent, since it gradually reverted after the DDT residues had disappeared. Whereas this interpretation means that we do not share the view that *A. sacharovi* remained highly anthropophilic throughout the DDT era, we concur with Hadjinicolaou & Betzios (1973) in estimating that it now has approximately the same feeding habits and the same potential efficiency as a malaria vector as it did in the 1930s.

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## RÉSUMÉ

PRÉVALENCE DES REPAS DE SANG MIXTES ET DE L'ALIMENTATION SUR DEUX HÔTES  
CHEZ UN VECTEUR DU PALUDISME, *ANOPHELES SACHAROWI* FAVRE

En 1970, un échantillon de 1025 repas de sang d'*Anopheles sacharovi* provenant de cinq biotopes dans un village de Grèce a fait l'objet d'une analyse approfondie par la réaction des précipitines afin de déceler les repas mixtes. Sur les 1021 spécimens positifs, 91 (8,9%) contenaient du sang de 2 hôtes sérologiquement distincts. Lors d'une enquête similaire mais moins poussée, en 1971, des repas de sang mixtes n'ont été décelés que dans 2 (0,1%) des 1798 spécimens étudiés. Dans quatre cas sur cinq, les repas de sang mixtes identifiés dans le 1<sup>er</sup> échantillon donnaient deux fortes réactions des précipitines, indiquant qu'un repas interrompu avait été complété au cours de la même nuit.

Dans les repas de sang provenant de deux étables, les hôtes les plus fréquemment identifiés ont été les moutons et les chèvres avec 55,4% de positivité des spécimens contenant un seul type de sang et 60,4% de positivité des spécimens mixtes. Dans deux endroits situés à proximité de ces étables, tous les repas d'*A. sacharovi* contenaient un seul type de sang, en majeure partie d'équidés (37%). L'homme était l'hôte le plus commun (42,5%) dans les repas de sang d'un seul type récoltés dans les habitations,

mais venait après le porc (52%) dans les spécimens mixtes.

L'indice d'anthropophilie calculé lors de l'enquête de 1970 s'établissait à 0,218; il atteignait 0,310 en 1971. Il semble d'après ces chiffres qu'après plusieurs années d'abandon des insecticides les préférences trophiques du vecteur soient du même type que celui existant avant la campagne d'éradication en Grèce.

L'absence de repas de sang mixtes chez les moustiques capturés à l'extérieur des habitations amène à conclure que les femelles dont le repas a été interrompu à l'extérieur pénètrent dans les habitations avant de le compléter, alors que celles qui ont eu leur repas interrompu à l'intérieur des habitations n'en sortent pas, ou rarement.

La proportion des repas multiples, avérés ou occultes (achevés sur un même hôte après une interruption), est de 11,7% du total des spécimens dans la 1<sup>re</sup> étable, de 16,6% dans la 2<sup>e</sup> et de 11,7% dans les habitations. Sur les 260 spécimens positifs recueillis à l'intérieur des habitations, 42,3% contenaient du sang humain. La proportion des repas prélevés sur l'homme est estimée à 39,0%.

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