

Jack N. Humbal
1941

PROCEEDINGS AND PAPERS
OF THE
TWELFTH ANNUAL CONFERENCE
OF THE
CALIFORNIA MOSQUITO CONTROL ASSOCIATION

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Richard F. Peters, Secretary-Treasurer,
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The Twelfth Annual Conference of the California Mosquito Control Association was called to order by President Ernest Campbell, Superintendent of the Contra Costa County Mosquito Abatement District, at 9:15 A.M., December 15th, 1941 at Agriculture Hall, University of California, Berkeley.

President Campbell: The Twelfth Annual Conference of Mosquito Abatement Officials is now in session

In the past we have not always had an opening address by our president and the wish for this has been expressed. Our program has always been full and today's is no exception, with important discussions, so it is not my intention to take up much of our time.

However, I would like to say a few words. This is one of our most important conferences. It is our first two-day session. In a short period of time we have been plunged from a condition that demanded alleviation of unemployment, to a condition wherein man power is at a premium. Now more than ever the practice of our profession should be guided by an intelligent understanding. This intelligent understanding by a pooling of knowledge is the ultimate aim of these sessions.

In normal times we work towards better living and human welfare. Today we have another immediate objective, national welfare. Our ideal of a human destiny, wherein peace on earth is founded on good will towards men and is not just an interlude between conflicts, is overshadowed for the moment.

Forces that serve human welfare serve the national welfare. So it is in our work. We are performing a function that has become more important. We must discharge this function with efficient dispatch. More than ever we cease to be important as individuals but as parts of a whole we achieve significance. As a group we are significant only as we fit into a larger picture.

And so as we open our Twelfth Annual Conference; already dedicated to human welfare, let us dedicate it also to our national welfare.

In the name of the Conference as well as personally, I especially want to welcome those who are here for the first time. We always welcome visitors and new members. I want to express my thanks and appreciation to all who are participating in any way, to thank Miss Prefontaine for her work in taking and transcribing the Proceedings; to express sincere appreciation to the University Faculty which makes these Conferences what they are. Sid Doumes has made a big contribution, too. We will miss him this year. The money

collected after the previous conference, for the publishing of the Proceedings, amounted to \$147.95. The expenditures were \$109.40, leaving a balance of \$38.55. The cost of publication next year will be a little higher, which will use up that balance we now have in the treasury. I would like to suggest and move that we continue on next year and assess the various districts on the same basis.

Gray: I will second this notion.

Passed by unanimous vote.

Campbell: Before going further, I shall appoint a Nominating Committee consisting of three past-Presidents -- Emerick, Hayes and Butler. This Committee will please report this afternoon.

Is there any other business which should be brought up at this time?

Robinson: Some of us have been interested in getting pictures made of the most common types of mosquitoes. A Mr. Oliver, Jr., was supposed to be here today with data on this. May I ask if he is here now and if we may hear from him?

Campbell: Mr. Oliver has not come in yet. Probably he will be here later.

We will now have a symposium on equine encephalomyelitis. Professor Herms will introduce the discussion.

Herms: I would like first of all to extend a welcome to those here today on behalf of the University of California and particularly the Division of Entomology and Parasitology. I am glad to see so many here. It means something to have you here for you have come out under circumstances which might make you feel you want to stay at home. It indicates that you realize the importance of the work in which we are engaged.

There has been some advance made since our last meeting. We are dealing now perhaps in a broader sense with the subject of the encephalitides. Heretofore we have adhered to equine encephalitis but things have happened this past year which indicate that mosquitoes may have something to do with several similar diseases. Today we are to hear from Miss Beatrice F. Hoyitt of the Hooper Foundation who is doing a fine piece of work. She has a paper for us on "The Relationship of the St. Louis and Western Equine Viruses of Encephalitis to Man and Animals in California". She has contributed greatly to our knowledge of these diseases.

"RELATIONSHIP OF THE
ST. LOUIS AND THE WESTERN EQUINE VIRUSES
OF ENCEPHALITIS
TO MAN AND ANIMALS IN CALIFORNIA"

by

Beatrice F. Howitt
The Hooper Foundation for Medical Research,
University of California Medical School,
San Francisco.

I - Human Cases of Encephalitis

When in 1930 the virus of western equine encephalomyelitis was first isolated in California (1), about 6000 horses were affected with a mortality of 49 percent. It was then considered primarily an equine disease and became of economic importance for several years, only to subside later, especially after the introduction of the chick embryo vaccine. Cases still continue to occur, however, in varying numbers, indicating its endemicity.

Reports of sporadic cases of human encephalitis had been made to the California State Department of Health for a number of years but they had not been accredited to any one etiological agent. In the summer of 1937, however, there was a marked increase in the reported cases of acute human encephalitis; 102 for the State with 40 deaths or 39.1 percent case fatality rate. Fresno County was the main focus of the outbreak, where 28 cases occurred in July and August with 13 deaths (42.8% case fatality). No virus was recovered from 3 fatal cases at that time. Serum from convalescent patients was tested for neutralizing antibodies against the virus of St. Louis encephalitis and 16, or 55.1 percent, were positive. Wynns and Hawley (2) of the State Department of Health considered the clinical picture as being similar to that given by the St. Louis disease. There were many cases among the older age groups and not many residual effects, except among those whose sera failed to neutralize this virus.

The incidence declined in the autumn but re-appeared again the following summer of 1938, not only in the Fresno districts but throughout the Central Valley from Kern County to Marysville. Sera were again collected through the cooperation of the different State and County Health Departments. Many of them again neutralized the St. Louis strain.

However, in August, 1938, brain material from a child dying of acute encephalitis at the Fresno County Hospital (3) yielded a virus, which instead of being that of the

St. Louis strain, proved to be similar to the active agent of the western equine encephalomyelitis and corresponded to the virus previously isolated from the horses in the San Joaquin Valley in 1930. Although in 1932 Meyer (4) had suspected 3 human cases of having the equine disease, yet no virus had been recovered, so that it was not until 1938 that the real significance of these observations was proven. At the same time the eastern strain of equine encephalomyelitis was recovered from human cases in Massachusetts (5). This disease had now become not only an agricultural but also a public health problem.

Sera were collected from convalescent encephalitic patients, and by means of the serum neutralization test it was shown that many cases of the human western equine disease occurred throughout the areas already endemic for the horses each year. Howitt (6) reported 32 (37.8%) positive sera out of 86 tested during 1937 to 1938. Since then blood samples have been received every year from acute cases of neurotropic virus disease, including poliomyelitis, throughout the central and northern part of the State.

Sera were not only positive each year for the western equine virus but also for the St. Louis strain as well, although in a lower percentage. It was likewise observed that while some persons showed antibodies only for the equine or only for the St. Louis strain, a third group of sera neutralized both these viruses as shown in the table 1 and chart 1.

During the past three years a total of 498 sera were tested, representing various types of neurotropic virus diseases including poliomyelitis. Of these 213 (42.7%) were positive to the western equine virus, 55 (11.5%) of 475 were positive only to the St. Louis strain, while 62 (13%) neutralized both of the viruses. The percentages did not vary markedly each year, although there were more of the human cases of the equine disease in 1940 than during the other periods. The sera neutralizing the St. Louis strain were always comparatively few in number but have been increasing slightly in the past two years as shown in the table. The counties mainly affected by both types of encephalitis have been those of Kern, Tulare and Fresno in the San Joaquin Valley, and Yolo, Sacramento and Yuba-Sutter in the Sacramento Valley with varying reports from the other valley districts.

Sera were likewise tested from acute cases of encephalitis around the San Francisco Bay Region. Of a total of 144 for the 3 years, all were negative to the western equine virus and only one (0.6%) neutralized the St. Louis strain. There were 3 tests positive to the latter, however, from several coastal counties.

Cases of acute encephalitis markedly declined during 1941, particularly in the northern part of the State, where only 11 were reported with 4 positive to the equine virus, 2 for the St. Louis alone and 3 for both strains. While the mortality rate for encephalitis has decreased for the Central Valley since 1938, yet the western equine virus has been isolated from brain material of three fatalities, 2 in Kern County in 1940 and 1 in Fresno during 1941. So far the St. Louis virus has not yet been recovered in 1941.

Seasonal Occurrence

A definite seasonal occurrence for encephalitis has been noticed each year, beginning with an occasional sporadic case in April or May and rising steadily in incidence to a peak in July or August or maybe in September as in 1941 for the equine disease (Chart 2). The delayed appearance this year might be attributed to the heavy late rains which may have prevented the early presence of many insects.

Geographical Distribution

The human equine disease is found each year in certain definite localities of California. It not only reappears in the same counties but in the same towns or rural districts, close to the centers of the agricultural population. Quite often these places are associated with low, swampy lands near irrigation canals or pools of stagnant water. Map I shows the distribution of cases during the 3 years that had positive neutralization tests for the western equine virus, for the St. Louis alone and for both strains. The same localities are implicated each year, although the number of cases has varied. The majority of people have been in rural areas, usually on small farms or places of about one acre on which chickens and other domestic fowl, cows or other animals may be present. Although the affected homes may be free of such animals, they are found somewhere in the neighborhood. Everywhere in the endemic areas mosquitoes are present some time during the spring or summer. It is also of interest that the individuals showing antibodies to the St. Louis virus likewise reside in the same regions where the equine disease is prevalent among man or horses. The similarity of distribution is striking, as may be seen in Map 2 showing the incidence of encephalitis for Fresno County in 1941.

Age and Sex Distribution

It is significant that for each year more males than females have been affected by both the encephalitic diseases. Over twice as many males as females (176.82) showed antibodies for the two encephalitic strains. If the

data are combined for the 3 years and the ages are subdivided, even then the males predominate, although the difference in sex is less noticeable among the younger children through the 12th year. During the earlier period of life the chances of exposure to an insect vector are apparently more evenly distributed. On the other hand owing to the more exposed outdoor life of the men in these agricultural regions, males over 15 years of age seem to run a greater risk of becoming infected than females. This is also true for the similar age period in regard to exposure to the St. Louis virus.

All ages varying from 16 days to 74 years are affected by the western equine strain, with the greatest numbers in the group 0-10 years. In Kern County alone for 1938, 1939 and 1940 (7) the highest proportion of cases was under 10 years of age, with many infants under one year. In California the ages most affected by the St. Louis virus lay between 15 and 30, although the range was from 1 to 66 years. One 6 months old baby showed antibodies only to the St. Louis strain, while 7 under one year were positive for both the two viruses.

Neutralization Tests on Sera of Non-Encephalitic Individuals

The results of determining what percentage of the normal population in an endemic area may have neutralizing antibodies against the western equine virus has been reported by Buss and Howitt (7). It was found that of 82 sera from normal people in Kern County, only 6 (7.3%) were positive, while 97 (86.6%) of 112 sera from acute cases of encephalitis in the same regions reacted to this strain. Only 5 (6.0%) of 82 poliomyelitic patients showed antibodies. Thus a normal expectancy of 6.6 percent positive neutralization tests for the western equine virus is probably correct for a comparable group of non-encephalitic individuals in an endemic area.

The results, however, are not so conclusive in regard to the value of the test against the St. Louis strain, since many people in the general population may be affected in an endemic region. Of 95 sera from poliomyelitic patients or normal contacts in the two valleys, 15 (15.7%) were positive for the St. Louis virus, or an expectancy of over twice as many for this disease among the normal population in the same area as for the equine strain. The percentage of positive tests in the California districts was about one half that given by Nelson (8) for the Yakima Valley, Washington, where the incidence for the St. Louis virus is higher. It would appear therefore that the presence of antibodies for the latter strain may not be as etiological-ly significant in the diagnosis as their presence for the western equine virus in a similar area.

II - Results of the Study with Wild and Domestic Animals

In an earlier study (9) it has been found that many domestic fowl from areas in California endemic for the western equine virus were more resistant to inoculation of this strain than were birds from supposedly non-endemic regions. However, there were also resistant fowl from among the latter group, even among a small percentage that were without antibodies in the blood stream.

Because of finding these neutralizing substances in a small number of fowl sera mainly from endemic districts, and because a relationship had been previously noticed between the presence of antibodies to the equine strain and that of the St. Louis virus in human sera, a fresh study was undertaken to determine if a similar relationship existed in the sera of different mammals and various types of domestic fowl. Material was collected from representative endemic and supposedly non-endemic areas in the State, often from small farms or homes where either human or equine cases of encephalitis had recently been reported.

I. Neutralization Tests on the Sera of Mammals

The sera of 60 domestic mammals from endemic, and 36 from supposedly non-endemic areas, were tested against the western equine and the St. Louis viruses as shown in Table 2. 95.5 percent of the former group were positive to the equine strain, while 77.7 percent reacted to both viruses. Of those from the non-endemic regions 55.5 percent were positive to the equine, and only 2.7 percent when combined with the St. Louis type. One horse showed antibodies only to the latter virus. Sera from unvaccinated horses were obtained from endemic Yolo, Fresno and Kern Counties and from Santa Clara, San Mateo, Sonoma, Napa and Mendocino along the coast where encephalomyelitis is rarely reported among horses. It is of significance that an unusual number of acute equine cases was reported for San Mateo in 1941. All sera tested had antibodies against the equine virus. No human cases, however, were known. The horses tested in Mendocino County had not been ill but 4 or 40 percent were positive to the equine strain and one to the St. Louis. Nine of the 35 unvaccinated horses from non-endemic regions were acutely ill. If they are excluded, then 10 or only 40.9 percent of the normal animals showed antibodies to the equine virus as compared to 57.5 percent inclusive of the former group. The percentage of positive tests against the St. Louis virus was low from these regions.

Neutralizing substances against the western equine strain were found in the blood of 12 cows while 2 were positive to the St. Louis virus. Four of these were range cows

from the foothills of Madera County and 8 were milk cows from ranches in Kern County where horses had encephalomyelitis earlier in the season. The 3 sheep tested were from a mountain area in Kern County that was considered to be non-endemic.

Not only was blood obtained from domestic animals but from 101 wild rodents trapped alive from valley districts, and from 1 fox and 28 mice and rats from the non-endemic area around the Calaveras Dam or in Marin County. The sera of the latter group were entirely negative but weakly positive tests were obtained on sera taken in Kern County near localities where sick horses had been reported. Blood was obtained from 4 species of mice, 3 of rats and 2 of squirrels. The sera from all rats and squirrels were tested separately, but those of 2 or 3 mice were pooled on account of the small amount obtainable from individual animals. Nine or 8.9% of the rodents showed weak antibodies to the western equine virus, and 4 or 6.7% to the St. Louis strain. The kangaroo rats were mainly affected, 4 out of 22 positive to the equine and 2 out of 18 to the St. Louis strain.

Table 2 gives the total numbers of sera tested from both wild and domestic mammals, showing that of 161 samples from endemic areas, 65 (40.3%) were positive for the western equine strain, 5 (4.2%) out of 119 for the St. Louis alone, and 37 (31.0%) for both viruses, while from non-endemic regions of 65 sera 20 (30.7%) reacted against the equine strain, one (1.7%) against the St. Louis alone, and one (1.7%) against both types.

2. Neutralization Tests on Fowl and Bird Sera

In tabulating results of neutralization tests on sera of domestic fowl, a greater difference was noted between the figures obtained for the valley areas and those for the coastal counties than was observed for the horses. More horses in supposedly non-endemic regions appeared to have contact with the encephalitic viruses than did the fowl. However, the latter were tested from only a few localities, San Mateo, Sonoma and Mendocino Counties. Of the 30 or 40 chicks hatched in the laboratory all were negative to both viruses. Only 5 out of 78 fowl (5.8%) were positive to the western equine strain and none for the St. Louis, of those taken from coastal areas, while 70 of 161 sera (43.4%) from endemic regions neutralized the equine, 8 (5.2%) of 153 the St. Louis and 30 (13%) both types, as shown in table 3.

3 - Geographical and Seasonal Occurrence

Blood from endemic areas was taken from chickens, turkeys, ducks, pigeons, peacocks, pheasants, 1 Canada goose and 1 mourning dove as well as from 6 English sparrows, but

only from chickens and pigeons along the coast. The 5 positive chicken sera from the latter area were obtained near Petaluma in Sonoma County.

In Kern County alone 49 chickens were bled through the courtesy of Dr. Norman Twissellmann of the Kern County Health Department and 28 (59.1%) of the sera protected against the equine strain and 13 (26%) against both the viruses.

At Davis, Yolo County, the chickens tested were kept largely in houses and only used a small run-way outside. Only 4 of 27 or 14.8 percent had sera positive for the equine strain, and 3 or 11.1 percent for the St. Louis alone, while the blood from 8 of 13 turkeys (61.5%) that roamed in a large enclosure were positive to the equine virus. The latter birds were undoubtedly more exposed to mosquito bites and subject to inapparent infection than were the chickens, although both fowl were not far distant from each other.

The chickens tested were of varied breeds, more of the large colored types in the valleys than on the coast where the white leghorns predominate. With exception of the chicks raised in the laboratory, all those tested were from 1 to 2 years of age and had lived through at least one summer, so could have been exposed to insect bites. The other fowl bled were also in the older groups and the majority were normal healthy birds. Sick chickens were obtained from 3 places where illness had been reported but no virus was recovered from any tissues examined, although antibodies against the equine virus were found in the sera of 5 in one place in Kern County. In fact no virus was obtained from the tissues of 7 chickens killed in 17 days to 3 months after previous inoculations of both strains.

However, it has been found experimentally that not only the western equine virus but also the St. Louis strain may be in the blood stream of young chicks in 6, 18, 24 and 30 hours after either intracerebral or subcutaneous injection. They might thus serve as potential reservoirs if exposed to a biting insect during that period. The virus disappears later and usually the bird survives without symptoms, particularly with the St. Louis strain (10).

Map 3 shows the areas from which material was obtained from the fowl, and the relative proportions of positive tests for each type of encephalitis as compared with the distribution of human and horse cases. The information in respect to the horses was compiled from the reports kindly supplied by Dr. C. U. Duckworth of the State Department of Agriculture.

No human cases of western equine encephalomyelitis have as yet been reported from the north-central coastal counties unless imported from the Central Valley, although a few individuals with disturbances of the central nervous system have shown antibodies for the St. Louis virus. However, cases in horses have been diagnosed, some with confirmation by the neutralization test. These have been mainly from San Mateo County.

During the past two years many cases of encephalomyelitis in horses have been reported to the State Department of Agriculture from areas shown on the map in the southern part of the State. These cases, however, were usually diagnosed on clinical evidence unconfirmed by laboratory findings, except in a few instances when the western virus was recovered from the brains of 2 horses in Los Angeles County and one in Orange County in 1940, and from 2 others in Los Angeles in 1941. Apparently the conditions are favorable for the occurrence of encephalomyelitis in these regions. As far as known the human population has not been affected, although in 1941 antibodies for the equine strain were found in the serum of one encephalitic case in Orange County.

Chart 3 gives the incidence for the disease among horses for 1940 and 1941 as compiled from Dr. Duckworth's reports. The cases have been divided into 3 groups, those from the South, the Central Valley, and the North-Central coastal counties and are recorded by monthly incidence. The total number of cases for each month are also designated. The highest incidence has been in July or August for both years with a marked reduction in numbers for 1941. The highest incidence has been in the south where the human disease has not been recognized. Does this indicate a probable outbreak in man during the next few years? The cases along the northerly coastal counties have always been low in number, never over 6 for any one month, while those in the Central Valley usually run higher except for 1941. Perhaps this decline was owing to negligence in reporting.

Because of the topographical differences in many of the California counties, often extending from the valleys into the high mountains or the desert country, the findings in certain districts need not apply to the whole county. Conditions appertaining to the low valley regions are not similar to those found in the higher altitudes, and the exact locality in a county should be considered before deciding as to the probable presence of encephalitis.

For this reason the chicken sera from a mountain ranch in Ladera County should be considered as from a non-endemic region, as likewise the 3 sheep and 4 turkeys from the higher country in Kern County. All were negative to

both viruses, although one horse from the latter place had antibodies for the equine strain. While cases of encephalomyelitis have not been recorded from the Mojave Desert, yet 5 (55.5%) of 9 horse sera reacted to the equine virus. Differences in antibody incidence was also noticed in testing chicken sera from within the city limits of Bakersfield; only 4 (33.3%) of 12 birds were positive to the equine strain, while 24 or 64.8 percent of 37 sera from endemic ranches outside showed antibodies.

4 - Tests for Virus in Animal Tissues

After the wild rodents were exsanguinated, the brain, liver and spleen were removed and tested for the presence of virus by intracerebral inoculation of white mice. The liver and spleen of each animal were pooled but the brain kept separately. The material from 100 wild rodents, including 4 species of mice, 2 of rats and 2 of squirrels proved avirulent except for 2 house mice (Mus musculus) from Fresno County. These were obtained from two different rural localities and both had the virus of lymphocytic choriomeningitis in brain and organs, as confirmed by neutralization and cross-immunity tests.

III - Discussion

From the clinical histories and from the results of many neutralization tests made each year, it seems evident that the virus of western equine encephalomyelitis has become endemic in California, probably for a longer period than can be estimated. Although the disease has never reached the epidemic proportions reported by Leake (11) this year for North Dakota and the central northern states, yet the constant reoccurrence each year in the same localities renders a burden on the community and leads to a condition of disquiet in the rural population during the summer season. It is true that cases recover without residuals, yet on the other hand all stages of the disease may be seen, hence the physicians are becoming cognizant that permanent defects to the central nervous system may be expected.

In 1937 when cases of encephalitis first became of importance in California, evidence seemed to indicate the virus of St. Louis encephalitis as the active agent. Over 50 percent of encephalitic patients tested had neutralizing antibodies for this strain, but no virus was found (12). After recovery of the human western equine strain in 1938 and when the serum neutralization tests indicated its widespread incidence in these regions, the latter disease took precedence and the significance of the St. Louis encephalitis was correspondingly minimized. A combination of circumstances at the time of first reporting suggested that the antibodies for the St. Louis virus, especially if together with those for the equine, had been acquired previously

(6). Many of the sera were from people in the older age group who could have had an earlier infection. Many of the individuals were migratory laborers and it was suggested that they had brought in the disease to the state or had acquired it through contact with their neighbors. From experimental evidence and data collected since that period, it seems that the previous impression of introduction by the migrants, largely prompted by testing only human sera, should now be corrected.

The finding of antibodies for the St. Louis virus in sera of horses, domestic fowl and wild mammals, together with their presence in the blood of infants under one year of age, furnishes evidence that this virus has been endemic in the state for some time, although it has not as yet been recovered from human or animal tissues. It is probable, therefore, that one is dealing with two types of encephalitis in the same community, due to two different viruses. As suggested by Harmon (8) and by Harmon and Howitt (13) for the cases in the Yakima Valley, Washington, and as will be brought out further by Harmon and his associates (14) in their study on the mosquitoes in that region, it is quite possible that a dual infection may often occur. This would explain the presence of antibodies to both viruses in one individual.

Experimentally both the equine and the St. Louis strains are immunologically and serologically distinct, but by producing a mixed infection in monkeys (15) it has been shown that a highly virulent western equine virus may take precedence, inducing the symptoms and the lethal effect. The equine strain may be recovered post-mortem even though the St. Louis virus may be found in the blood during the first 48 hours after inoculation. Antibodies may be only weakly positive for the latter while those for the equine are promptly evident. It is experimentally possible, therefore, to have neutralizing substances in the blood due to a simultaneous infection with the two separate viruses.

The views in regard to the different animals as reservoir hosts for both the equine and the St. Louis viruses have been much extended during the past few years, as shown by the recovery of the eastern strain from pigeons and from pheasants in nature and of the western type by Cox and his associates (16) from a prairie hen and a deer. While many mammals and, except for chickens, most of the birds inoculated have proven susceptible to the equine viruses, yet only a few animals, man, mice, monkeys irregularly, hamsters and the embryonic chick have succumbed to the St. Louis strain.

It was interesting, therefore, to find unexpectedly that horse sera showed antibodies to this virus and later to realize that the sera of other mammals and many fowl likewise behaved in the same manner. Further confirmation of this finding was obtained in the report by Cox and the group in

Montana (17) who were able also to infect horses intracerebrally with the St. Louis strain (18). Hammon and his associates have likewise reported on similar results in antibody formation (19). Although the early work with the St. Louis virus discouraged the connection of horses as a possible host, yet the recent observations indicate that horses may be as vulnerable to this virus as to the so-called "equine" strains. The early failures were probably due to the natural immunity of many of the animals in the endemic community under investigation. Since Cox et al (18) has shown that the St. Louis disease manifests symptoms in horses similar to those of equine encephalomyelitis, there is also a possibility of confusing the two forms in nature.

Similar conditions seem to exist in respect to infection of fowl with the St. Louis virus, although only a low percentage among those tested in California showed positive sera, usually in endemic areas and in association with the equine antibodies.

Older chickens have been found relatively resistant to intracerebral inoculation of the western equine virus regardless of whether or not antibodies are present, although the virus may be in the blood for several days after injection. Chickens of similar age are likewise usually resistant to infection with the St. Louis strain. Usually young chicks may survive without symptoms, although circulating virus may be found in the blood 30 hours after injection. They may occasionally succumb, however. It was found that 4 out of 25 (16%) chicks and one English sparrow died after injecting the St. Louis virus, another recovered after showing symptoms, while 6 out of 9 (66.6%) baby chicks were susceptible to the western equine strain. Antibodies for the latter develop promptly after injection but those for the St. Louis strain are slower to appear, increasing after subsequent inoculations.

Young chicks, therefore, could be infected with either strain by an insect vector early in the spring, have circulating virus for a short period, and an absence of clinical symptoms. Experimentally this is possible with either virus, although the likelihood of recovery is greater for the birds given the St. Louis than the equine type. Because antibodies for the former strain are not so readily developed, it would probably take repeated attacks of infected insects to produce detectable amounts of such substances.

IV - Summary

It has been found that of 496 human sera cases of acute neurotropic virus disease in the Central Valley of California, 213 or 42.7 percent neutralized the virus of western equine encephalomyelitis, 55 or 11.5 percent of 475

sera the St. Louis strain, and 62 or 13 percent neutralized both viruses.

On the other hand, of 144 sera tested from the San Francisco Bay Region all were negative to the equine virus and only one (0.6%) was weakly positive to the St. Louis. Three sera from other coastal counties reacted to the latter virus alone, however.

There were over twice as many males as females among the total number of encephalitic cases, although when the ages were subdivided, no sex distinction was noticeable in the youngest age groups infected with the equine disease.

All ages from under one year to 74 were affected, but the majority of infected cases occurred under 10 years for the equine disease, and in the years 13 to 30 for the St. Louis.

Both types of encephalitis were found mainly in the rural, agricultural sections throughout the large Central California Valley, with a definite seasonal occurrence for both diseases that rises to a peak in July or August or even into September.

A normal expectancy of 6.6 percent positive neutralization tests for the western equine virus was found among the normal population in an endemic area as compared to 15.7 percent for the St. Louis strain in the same environment.

In testing the animals it was found that of 60 domestic mammals (horses, cows and rabbits) in endemic regions of the Central Valley, 56 (93.3%) neutralized the western equine virus, one (1.6%) the St. Louis alone, and 37 (61.6%) both strains; while of 101 sera from wild rodents in similar localities only 9 (8.9%) were positive to the western equine and 4 (6.7%) out of 119 to the St. Louis virus.

In one endemic county alone the horse sera were 100 percent positive to the equine and 90.4 percent to both the latter and the St. Louis strains.

In the supposedly non-endemic regions the sera of 20 (55.5%) of 36 domestic mammals (mainly horses) were positive for the equine virus, one for the St. Louis alone and one (2.7%) for both strains, while 28 wild rodents (rats, mice and squirrels) and one fox were entirely negative to both viruses.

The fowl sera from the Central Valley showed that 70 (43.4%) out of 161 birds (chickens, turkeys, ducks, pheasants, pigeons and peacocks) neutralized the equine virus, 8 (5.2%) the St. Louis alone, and 30 (18%) both strains. In the supposedly non-endemic regions along the coastal counties, of

86 fowl tested only 5 (5.8%) were positive for the equine and none for the St. Louis.

Neither of these viruses was recovered from the tissues of 98 wild rodents, although the virus of lymphocytic choriomeningitis was isolated from the brains of 2 mice (Mus musculus) from Fresno County. Neither was virus found in the tissues of 7 fowl killed in 17 days to 3 months after previous inoculation of both the St. Louis and the western equine strains.

From the results of the neutralization tests it may be said in general that in the endemic areas of California more domestic fowl and mammals have antibodies against these two viruses than similar animals from supposedly non-endemic regions, and that the percentage of positive results was higher among the domestic animals tested than among the wild rodents.

Antibodies against the western equine virus have been found more often in horses than in the other animals, both from endemic and from supposedly nonendemic localities.

From accumulated epidemiological, clinical, and laboratory evidence it is apparent that the virus of western equine encephalomyelitis has become established for man and animals in the Central Valley of California and that the St. Louis strain is likewise closely associated. From the finding of antibodies in the sera of fowl and mammals and in children under one year of age, it is evident that the St. Louis virus has been likewise in California for some time and not imported from the midwest as formerly proposed.

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Table 1.
Results of Serum Neutralization Tests for 1939, 1940 and 1941

Year	Western Equine Virus		St. Louis virus alone		Both W. Eq. & St. Louis	
	Number Tested	Number and % Positive	Number Tested	Number and % Positive	Number Tested	Number and % Positive
1939	208	75 (36.0%)	206	15 (7.2%)	206	17 (8.2%)
1940	161	90 (55.9%)	140	15 (10.7%)	140	23 (16.4%)
1941	129	48 (37.2%)	129	25 (19.3%)	129	22 (17.0%)
Totals	498	213 (42.7%)	475	55 (11.5%)	475	62 (13.0%)

Table 2. Results of Neutralization Tests on Mammals

Mammal	Endemic Areas				Supposedly Non-endemic Areas			
	Number Tested	Positive W. Equine	Positive St. Louis alone	Pos. W. Equine & St. Louis	Number Tested	Positive W. Equine	Positive St. Louis alone	Pos. W. Equine & St. Louis
Wild mice and rats	St.L. 57 W.Eq. 99	9 (9.0)	4 (7.0)	0	St.L. 22 W.Eq. 28	0	0	0
<i>Citellus beecheyi</i> ground squirrel	1	0	0	0				
<i>Citellus richsoni</i> antelope squirrel	1	0	0	0				
Fox					1	0	0	0
Totals	St.L. 59 W.Eq. 101	9 (8.9)	4 (6.7)	0	St.L. 23 W.Eq. 39	0	0	0
Sheep					3	0	0	0
Rabbits	2	1 (50)	0	1 (50)				
Cows	13	12 (92.3)	1 (7.6)	1 (7.6)				
Horses in vaccinated	45	43 (95.5)	0	35 (77.7)	33	20 (60.6)	1 (3.0)	1 (3.0)
Totals	60	56 (93.3)	1 (1.6)	37 (61.6)	36	20 (55.5)	1 (2.7)	1 (2.7)
Total of all Mammals	St.L. 119 W.Eq. 161	65 (40.3)	5 (4.2)	37 (31.0)	St.L. 57 W.Eq. 65	20 (30.7)	1 (1.7)	1 (1.7)

Table 3. Results of Neutralization Tests on Sera of Fowl and Birds

Birds	Endemic Areas				Supposedly Non-endemic Areas			
	Number Tested	Positive W. Equine	Positive St. Louis alone	Positive to both viruses	Number Tested	Positive W. Equine	Positive St. Louis alone	Positive to both viruses
Chickens	St.L. 99 W.Eq. 104	39 (37.4)	7 (7.0)	17 (17.1)	St.L. 82 W.Eq. 74	5 (6.7)	0	77 (93.0) 69 (94.0)
Ducks	8	2 (25.0)	1 (12.5)	1 (12.5)				
Turkeys	19	12 (63.1)	0	1 (5.2)				
Pheasants	8	5 (62.5)	0	2 (25.0)				
Peacocks	2	2 (100)	0	2 (100)				
Mourning Doves	1	1 (100)	0	0				
Canada goose	1	1 (100)	0	1 (100)				
Pigeons	12	8 (66.6)	0	6 (50.0)	4	0	0	0
English Sparrows	St.L. 3 W.Eq. 6	0	0	0				
Totals	St.L. 153 W.Eq. 161	79 (43.4)	8 (5.2)	30 (13.0)	86	5 (5.8)	0	77 (89.5) 69 (88.5)

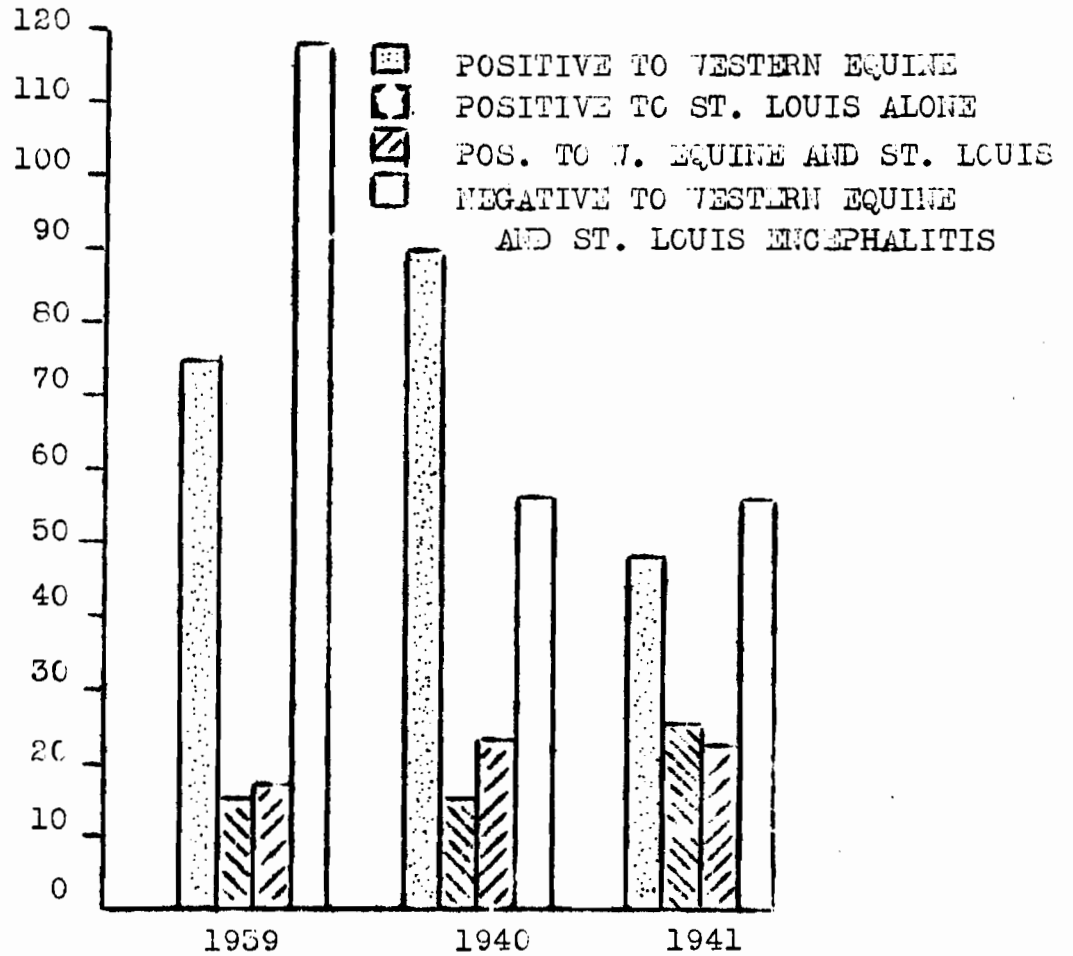


CHART I

COMPARATIVE INCIDENCE OF WESTERN EQUINE AND ST. LOUIS ENCEPHALITIS IN THE CENTRAL VALLEYS OF CALIFORNIA FOR 1939, 1940 AND 1941 AS DETERMINED BY THE SERUM NEUTRALIZATION TEST.

SEASONAL DISTRIBUTION OF
WESTERN EQUINE AND ST. LOUIS
ENCEPHALITIS IN CALIFORNIA

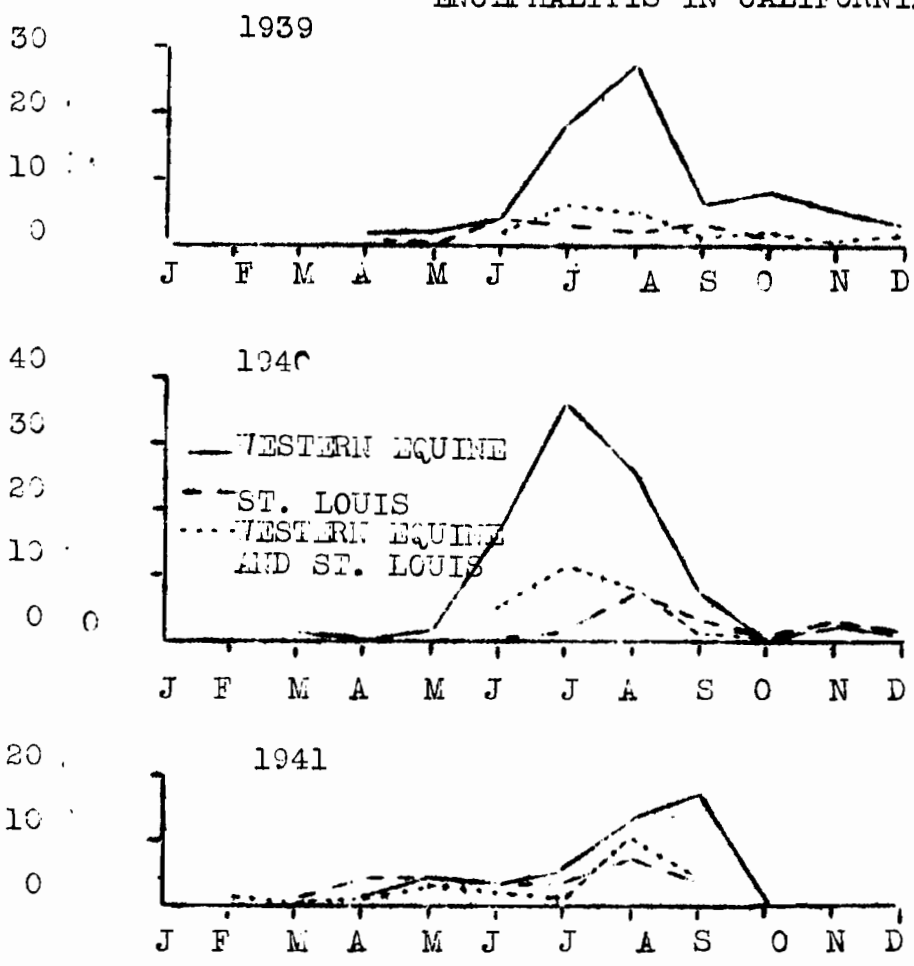


CHART 2

SEASONAL INCIDENCE OF WESTERN EQUINE AND ST. LOUIS ENCEPHALITIS
IN THE CENTRAL VALLEY
OF CALIFORNIA FOR 1939, 1940 and 1941

INCIDENCE OF EQUINE ENCEPHALOMYELITIS
IN HORSES THROUGHOUT CALIFORNIA

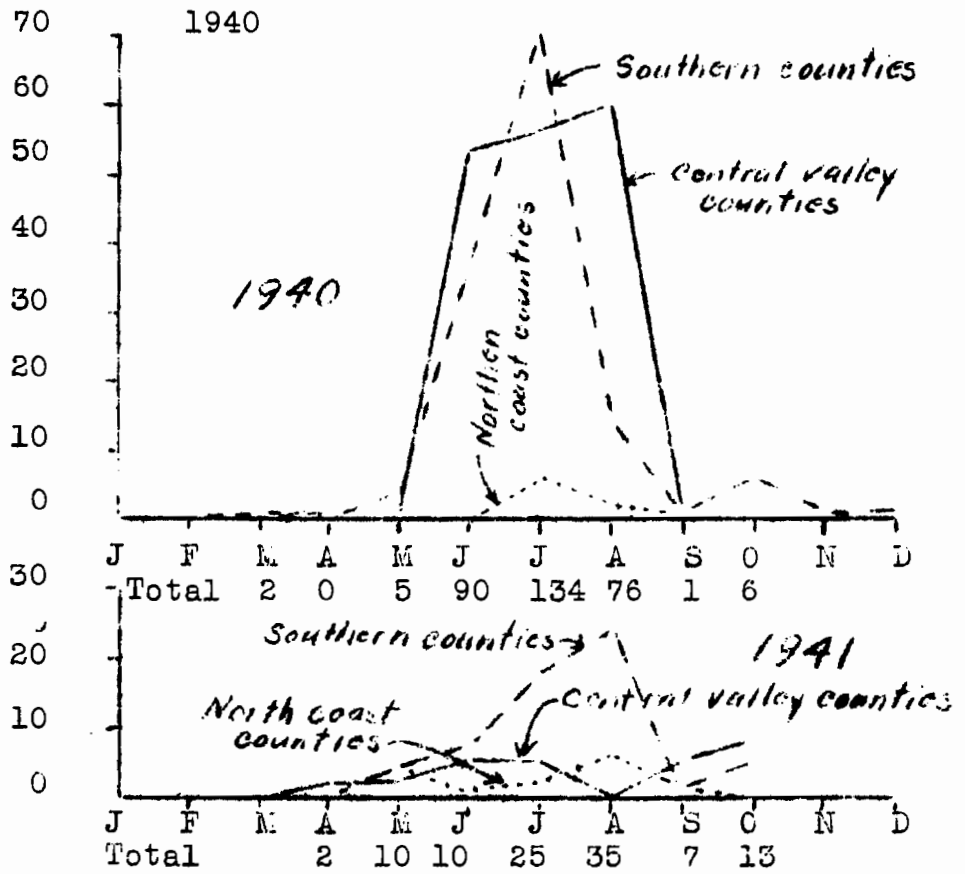


CHART 3

SEASONAL INCIDENCE OF ENCEPHALOMYELITIS IN HORSES
THROUGHOUT CALIFORNIA IN 1940

Miss Howitt: I am indebted to a great many people for the material in my paper--a number of individuals and to several County Health Departments.

(Miss Howitt then illustrated her paper with lantern slides).

Herns: That was a very interesting presentation. Thank you.

May we now hear from Bill Reeves on "Newer Developments in Knowledge of Insect Hosts and Vectors of Western Equine and St. Louis Encephalitis"? I think most of you know Bill Reeves. He is from the University here.

NEWER DEVELOPMENTS IN KNOWLEDGE OF INSECT HOSTS AND VECTORS
OF WESTERN EQUINE AND ST. LOUIS ENCEPHALITIS

by

William C. Reeves, Division of Entomology and Parasitology,
University of California.

Last year at this conference an entire morning was devoted to a symposium on equine encephalomyelitis, including several papers on the common knowledge of insect vectors. Dr. Merrill (1940) summarized most of what was known of mosquitoes and ticks as experimental vectors of eastern and western equine encephalomyelitis. Subsequent remarks by Herns and Reeves brought out the pioneer work of Herns, Wheeler and Herns (1934), and the later work in this Division by Herns and Reeves on transmission of Western equine virus by mosquitoes. Other late papers brought to the attention of the conference were by Davis (1940), summarizing the entire field of experimental mosquito borne Eastern and Western equine encephalomyelitis; and the work of Kitzelman and Grundman (1940) on the Western virus with the conenosed bug Triatoma sanguisuga. Dr. Aitken (1940) presented a paper on "The relationship of the distribution of cases of equine encephalomyelitis (human and equine) and mosquitoes in California", correlating the two in so far as was possible. At that time he expressed some skepticism about the sole importance of the genus Aedes.

Another virus disease of considerable interest to us, one which Miss Howitt has already discussed and Dr. Hammon will further discuss, is St. Louis encephalitis. There have been two schools of thought concerning the probable mode of transmission of this infection; one favors mosquito transmission, Lumsden (1933), Casey and Brown (1938); and the other favors such means as respiratory infection. Japanese workers, Mitamura et al (1937), have reported successful transmission of St. Louis encephalitis virus by Culex pipiens var. pallens. Various American workers, Fulton and his associates (1940), however, have reported negative results in their attempts at transmission.

During the past week two experiments with St. Louis virus were completed in a cooperative project between the laboratories of the Division of Entomology and Parasitology and Hooper Foundation of the University of California. It is now possible for Dr. Hammon, Mr. Izumi and myself to report successful transmission of St. Louis virus to normal, non-immune pigeons by Culex pipiens mosquitoes. The mosquitoes were infected by feeding on a suspension of virus and defibrinated rabbit blood. After infection the mosquitoes were held in incubators at 23 to 27 degrees centigrade. In experiment one the mosquitoes were allowed to feed on a non-immune pigeon after the following periods of incubation: 3 days, 5 days, 7 days, 9 days, 10 days, 11 days, 12 days, 15 days, 17 days and 18 days. The pigeon was subjected to a total of 111 feedings. Fifteen days after the last mosquito fed the pigeon was bled and the serum tested for development of antibodies to St. Louis virus. The pigeon had developed a high antibody titre, thus demonstrating that transmission of infection had taken place, the virus stimulating antibody production.

In experiment two the same general procedure was followed as for experiment one. The infected mosquitoes were fed on the following days of incubation - 5, 8, 10, 11 and 12. A total of 30 feedings were recorded. A blood sample taken from this bird 14 days after the last feeding period contained a high titre of antibodies.

A normal pigeon which was kept in the same cage as the two experimental birds did not develop any protective antibodies during this period. None of the pigeons showed visible clinical symptoms during the course of the experiment.

On the basis of these two experiments we may report successful transmission of the virus of St. Louis encephalitis by means of Culex pipiens.

A closely related virus infection, Japanese B virus, has been shown (Iitamura et al., 1939) to be carried by Culex pipiens and Culex tritaeniorhynchus. Infected mosquitoes have been collected in nature, and transmission from animal to animal in the laboratory is not difficult.

A summary of experimental work which incriminates the various arthropod species as vectors of these virus infections is given in Table 1. With this knowledge of positive and negative experimental vectors, a brief review of the field surveys which have been made to recover the viruses from insect hosts infected in nature is in order. The first attempt reported by F. C. Bishopp and Carrol Smith was carried out in Maryland in 1933. At that time 935 mosquitoes

including 158 Culex, 548 Anopheles, and 228 Aedes were tested; all resulted negatively. The second attempt was made in 1936 in Montana and reported by Philip and Cox. Inoculations of collected arthropod material all resulted negatively. In 1937 the U. S. Bureau of Animal Industry, and Entomology and Plant Quarantine, made another attempt on the Eastern coast in which mosquitoes, horseflies, stable flies, house flies, and spinose ear ticks were all tested with negative results. In 1924 Gwatkin reported on 527 Dermacentor andersoni from South and Central Alberta, Canada which when inoculated were all negative. Kitzelman and Grundman from Kansas reported in 1940 on the positive results by inoculation and by the bite of the conenosed bug Triatoma sanguisuga collected in nature infected with Western equine encephalomyelitis. This was the first successful isolation of the virus from a naturally infected insect.

TABLE I - RESULTS OF ATTEMPTS TO TRANSMIT WESTERN AND EASTERN EQUINE ENCEPHALOMYELITIS BY VARIOUS BLOODSUCKING ARTHROPODS*

<u>Insect vector</u>	<u>Western equine</u>	<u>Eastern equine</u>	<u>Worker and Date of Report</u>
<u>Aedes aegypti</u>	Yes	Yes	Kelser, 1933
<u>Aedes sollicitans</u>	Yes	Yes	Merrill, Lacaille, TenBroeck 1934
<u>Aedes vexans</u>	Yes	Yes	Kelser, 1937
<u>Aedes taeniorhynchus</u>	Yes	Yes	Kelser, 1937
<u>Aedes sylvestris</u>	Yes	Yes	Merrill, 1940
<u>Aedes dorsalis</u> (Fresh water)	Yes		Herns, 1934
<u>Aedes albopictus</u>	Yes		Simmons, Reynolds, Cornell, 1936
<u>Aedes nigromaculis</u>	Yes		Madsen and Knowlton, 1935
<u>Aedes lateralis</u>	Yes		Reeves, 1940
<u>Aedes cantator</u>		Yes	Merrill, et al, 1934
<u>Aedes atropalpus</u>		Yes	Davis, 1940
<u>Aedes triseriatus</u>		Yes	Davis, 1940

<u>Insect vector</u>	<u>Western equine</u>	<u>Eastern equine</u>	<u>Worker and Date of Report</u>
<u>Aedes dorsalis</u> (salt marsh)	No		Herms, Wheeler, Herms, 1934
<u>Aedes varipalpus</u>	No		Reeves, 1940
<u>Anopheles maculipennis</u>	No		Herms, Wheeler, Herms, 1934
<u>Anopheles quadrimaculatus</u>	No	No	Merrill, et al, 1934
<u>Anopheles punctipennis</u>		No	Davis, 1940
<u>Culex salinarius</u>		No	Davis, 1940
<u>Culex pipiens</u>		No	Merrill, et al, 1934
<u>Mansonia perturbans</u>		No	Davis, 1940
<u>Triatoma sanguisuga</u>	Yes		Kitselman and Grundman, 1940
<u>Dermacentor andersoni</u>	Yes		Syvertson and Berry, 1936
<u>Haematobia serrata</u>	No		Herms, Wheeler, Herms, 1934
<u>Tabanus punctifer</u>	No		Herms, Wheeler, Herms, 1934
<u>Stomoxys calcitrans</u>	No	No	Merrill, et al, 1940

*This is not a complete summary of experimental work, but a list of the work with each arthropod species giving the first result which appeared to be conclusive.

In 1941 three attempts were made to isolate the virus from insects infected in nature. The first was an investigation of an outbreak of Eastern equine encephalomyelitis in horses in the Rio Grande Valley of Texas by the Bureau of Animal Industry. Dr. D. E. Howell, formerly of the Division of Entomology and Parasitology of the University of California and now at Oklahoma A. and M. College, was sent by the Bureau to participate. No detailed reports have been forthcoming hence we must assume that their work was negative. The second

attempt in 1941 was made in North Dakota where the United States Public Health Service investigated a large outbreak in man and horses. Dr. C. B. Philip was entomologist in charge of this work. No published reports have appeared.

Regarding the preceding attempts at field isolation the small size of the samples and the method of handling for virus isolation were probably factors contributing to failure.

The third attempt in 1941, and one which has appeared in a preliminary report by Hammon, Reeves, Brookman, Izumi, and Gjullin (1941), announces the successful recovery of both the Western equine and St. Louis viruses from Culex tarsalis infected in nature. A detailed report will be given of this work. Four persons who are present today, Hammon, Brookman, Izumi and Reeves, were among those concerned with this investigation at Yakima, Washington by the Hooper Foundation for Medical Research of the University of California, in cooperation with the Washington State Health Department and the United States Bureau of Entomology and Plant Quarantine.

The purpose of the entomological approach to the epidemiological survey of Western equine and St. Louis encephalitis at Yakima, Washington was to form a link between the two distinct phases of these diseases, that is, the study of human and equine cases with casual entomological observations, and detailed and intensive laboratory experimentation on possible vectors. As complete a survey as possible was made of the probable insect hosts of the viruses, and samples as large as possible were collected.

One of the primary concepts necessary in this study is that a small percentage of the total insect population of possible hosts or vectors is infected at one time. Consider the contributing factors: virus in the blood stream of vertebrate hosts, in sufficient concentration to infect arthropod vectors, for a period of probably not exceeding 48 to 60 hours; the small number of vertebrate hosts infected at one time; and the chances of an insect host feeding on the animal during its infective period. A study of malaria in highly endemic or epidemic areas often shows that only one in 500 or more Anophelines are infected, and in this disease the vertebrate host may be infective to the vector for a much longer period. One condition favorable to infection of insect hosts is the wide range of possible vertebrate reservoirs, as has been brought out by reports on neutralization test findings in other portions of this symposium.

The results of the Yakima insect survey support these statements and present further possibilities. During

the survey of premises and areas surrounding horse and human cases, word of mouth reports concerning insect density or activity were not accepted. Instead routine collections were made of blood-sucking arthropods by means of light traps, by sweeping, and by hand collections. These were identified under light chloroform anesthesia, sealed in glass vials, frozen on dry ice and shipped to the Hooper Foundation in San Francisco for animal inoculation.

During the period May 15th to September 15th, 15,610 arthropods were shipped for inoculation. There were 12,465 mosquitoes including 4,655 Culex tarsalis, 2,826 Aedes dorsalis*, 1,925 Anopheles maculipennis freeborni, 1,179 Theobaldia inornata, 648 Aedes increpitus, 738 Aedes vexans, 260 Culex pipiens, 106 Aedes cinereus, 55 Theobaldia incidens, 23 Aedes nigromaculis, 39 Anopheles punctipennis, and 10 Aedes lateralis. Other groups tested included 57 horse flies, 478 stable flies, 110 Hippelates, 626 Ceratopogonidae, 81 fleas, 150 mites, 162 ticks, 39 bed bugs, and 1,443 miscellaneous insects.

From this material 254 pools were formed according to species, and eight pools showed virus on inoculation into mice. Five contained Western equine and three contained St. Louis virus; all recoveries were made from one species of mosquito, Culex tarsalis. Here we are faced with an interesting point. Many workers in considering the need for virus recovery from naturally infected insects before insect transmission was proved, failed to foresee the possibility of infected species being taken which might be incapable of further transmission, that is, they were acting as hosts to the virus, not as vectors.

Four species of Aedes mosquitoes that had been previously shown to act as laboratory vectors were included in our collections. Several collections of Aedes dorsalis (light form) were artificially infected in the Yakima Laboratory with Western equine virus and used at the San Francisco Laboratory as checks on the method employed for virus isolation. In all cases virus was readily demonstrated to be present. Culex tarsalis, the mosquito from which eight isolations were made,

*Two forms of Aedes dorsalis were noted and separated in the collections. These are designated as the light form and the dark form. The latter is the typical female Aedes dorsalis with the third longitudinal vein predominately dark scaled. The light form had the third vein with many white scales. In existing systematic keys this form comes out as Aedes campestris, however, on the basis of male terminalia and larval characters this form must be called Aedes dorsalis. In our collections there were 1,599 of the light form and 1,033 of the dark form.

has never been incriminated in the laboratory as a vector of either virus, as a matter of fact it has never been worked with in a satisfactory experiment. Culex pipiens, another species captured at Yakima, has been reported as incapable of transmitting Western equine, and capable of transmitting St. Louis virus. We are now faced with the necessity for incriminating Culex tarsalis as an experimental vector; however, the finding of infected individuals has definitely shown that mosquitoes can obtain an infective meal in nature.

The collection figures showed that Culex tarsalis was by far the predominating mosquito in the area studied. It composed 40.8% of the total mosquitoes tested; the next most common were Aedes dorsalis 23% and Anopheles maculipennis freeborni 14%.

The result of the neutralization tests on vertebrate blood samples, as will be brought out by Dr. Hammon, showed little difference in the incidence of St. Louis and Western equine antibodies, so it is possible the same species of host reservoir may have acted as the source for mosquito infection with both viruses. Of the 4,655 Culex tarsalis tested, five lots were infected with Western equine virus in sufficient concentration to allow recovery, and three with St. Louis virus. This recovery of both viruses certainly proves they were active at the same time in this one area. Complete proof is offered by the fact that one night's trap collection of 126 Culex tarsalis on August 15th was split into two inoculation pools and one pool contained St. Louis virus and the other Western equine virus.

At least one specimen of each 900 Culex tarsalis was infected with Western equine virus and at least one of each 1,552 was infected with St. Louis virus. With this in mind it is possible perhaps that the samples of all but four species of mosquitoes of 12 sampled were too small to make the inclusion of an infected individual probable. This is, of course, presupposing they all had an equal opportunity to become infected, which is unlikely, as will be pointed out later. One of these species, Anopheles maculipennis, has been shown not to be a laboratory vector of Western equine virus under controlled laboratory conditions, although it may harbor the virus for a number of days, and Aedes dorsalis is a very efficient vector. These facts have been demonstrated repeatedly in the laboratory of the Division of Entomology and Parasitology of the University of California and elsewhere. There has been no work with Theobaldia.

The interpretation of these findings and correlation with what is already known of these diseases is of interest. Dermaecentor andersoni and Triatoma sanguisuga may be dismissed from consideration of summer encephalitis cases.

in Yakima, Washington, as the former was rare and the latter was apparently not present. Of the mosquitoes proven to be laboratory vectors of equine encephalomyelitis or St. Louis encephalitis Aedes nigromaculis, Aedes lateralis, and Aedes vexans were not sufficiently common over the entire valley to play an important role, and might have been involved only in local areas where they were found. Culex pipiens was in too small numbers to be of any great importance. Aedes increpitus, Aedes cinereus, Theobaldia incidens, and Anopheles punctipennis may be dismissed for the same reason. This leaves two species to be considered as of probable importance over the entire valley, Aedes dorsalis and Culex tarsalis. The latter was by far the most common and widespread mosquito in the area investigated. It was taken in every locality where traps were run, which includes the environment of all cases which occurred in the Yakima Valley during the period May 15 to September 15, 1941. Generally speaking, it may be associated with domestic habitats.

A comparison of the samples 4,655 Culex tarsalis as against 2,642 specimens of Aedes dorsalis would indicate that the same ratio of infected individuals did not exist. To explain this difference various theories may be advanced. One of the more likely is that of host preference. Aedes mosquitoes are noted for their attacks upon large warm blooded mammals (man and live stock), while Culex mosquitoes in general have been reported as commonly feeding on birds. There is considerable deviation from these habits as Culex tarsalis was observed in Yakima to feed also on man, cattle, and horses, and certainly Aedes mosquitoes occasionally feed on birds. It would seem possible, however, that Culex mosquitoes with their habit of feeding frequently on avian blood would have a better chance of becoming infected, because of the higher percentage of infected birds than mammals as indicated by the neutralization tests, and because of the greater number of avian individuals in an area than mammalian. Culex tarsalis and Anopheles maculipennis freeborni were the two species most commonly found in domestic habitats. This fact is of potential importance, as the neutralization tests show that domestic avian and mammalian species have a much higher percentage with neutralizing antibodies than have wild avian and mammalian species. This would indicate that these two species of mosquitoes in their feeding are more frequently exposed to infected animals. Yet Culex tarsalis was found to be infected and Anopheles maculipennis freeborni was not. Usually a high percentage of both species were freshly engorged at the time of capture. There is no apparent explanation at this time for the presence of infected Culex tarsalis and no infected Anopheles maculipennis freeborni. The preceding possibilities may indicate that the endemic situation with inapparent infections in numerous host species, as shown

by neutralization tests, is disseminated by Culex tarsalis. On the other hand when the infection builds up and sufficient Culex mosquitoes become infected to transfer the infection over to the mammalian hosts, in their occasional feedings on this type of host, the more susceptible mammals may infect the Aedes mosquitoes feeding on them in large numbers. With the infection of these efficient vectors, as demonstrated in the laboratory, an epidemic situation may develop if a sufficient number of susceptible hosts are available. The other possibility is that an occasional Aedes mosquito may feed on infective avian blood and transfer the infection to mammals during subsequent feedings.

Another factor favoring the interrelationship of Culex tarsalis and the endemic situation is its winter hibernation habit. In this manner infected individuals might pass through the winter and reestablish the infection in vertebrate hosts the following spring and summer. Aedes mosquitoes infected with Western equine virus have been shown to remain infected for the remainder of their life, periods over 90 days. In view of our knowledge of the Aedes borne virus infections yellow fever and dengue, such an explanation seems plausible. These diseases die out as soon as cool weather causes a decline in vector activity. The following spring they do not reappear unless new human cases are introduced. The disease is wiped out by disappearance of adult insect vectors. The habit of Aedes mosquitoes passing the winter as eggs, not as adults, is the important point. The possibility of transovarian infection with infected eggs carrying the virus overwinter does not seem likely. Tests by Merrill and Ten Broeck (1935) were all negative with western virus, when they attempted to demonstrate virus in the eggs of infected mosquitoes. The two disease entities with which we are dealing appear to decline in activity as the winter season approaches, although the weather may remain fairly warm. Observations this past summer indicated that in the Yakima Valley the blood-sucking activities of Culex tarsalis declined fairly early around the first of September. Collections of adults showed a noticeable decrease of engorged specimens at this time, to a point where none were to be found by mid-September. Apparently a prehibernation form which did not feed became prevalent. A large population was still present, but a seasonal habit had decreased their feeding activities, cutting off the infection of new susceptibles in humans or horses.

Another important concept regarding the importance of population density of mosquitoes is the fact that the controlling factors of disease transmission may well be the presence or absence of correct environmental conditions for virus development in the mosquito, or correct conditions to allow the mosquito to live longer than the minimum incubation period of the causative agent. The latter factor was shown by Freeborn (1932) to be very important in malaria transmission by Anopheles maculipennis in California.

A summary of our present knowledge concerning Culex tarsalis is important, as we are considering this species a potential disease vector for the first time. It is a North American species common throughout the states west of the Mississippi River, and becomes less common eastward. It has been reported from 17 of the 22 states west of the Mississippi River. In California it is probably the most widespread of all the mosquitoes, and wherever it is found it is usually abundant. It has been collected in 51 of the 57 counties of California. The only ones from which it has not been recorded are Inyo, Kings, Lassen, Nevada, Plumas and Trinity. The absence of records from these few states and counties is probably due to lack of proper collecting.

Culex tarsalis larvae are found in many types of ground pools, favoring those supplied with sunlight. In Yakima, it was collected in permanent ponds, irrigation seepage, barnyard drainage, sewage, roadside ditches, hoof prints, rain barrels, borrow pits, and large irrigation overflow pools on sagebrush lands.

The feeding habits of Culex tarsalis have not been studied extensively. Direct observations made in the Yakima Valley indicate that it feeds on man, horses, mules, cows, mallard ducks, and possibly quail. Other workers have indicated that it feeds on avian blood (Freeborn, 1926) and that it may feed on man (Hearle, 1926).

Adults may be collected in large numbers while resting in shelters such as barns, houses, and under bridges.

Hearle (1926) states that in temperate regions adult females are reported to hibernate in sheltered places, emerging in the spring to commence egg laying.

If this species is proven to be an important vector of Western equine and St. Louis viruses it may be necessary to direct control programs against it. From the above description of breeding habits you may see what difficulties this would involve. Unlike our Anopheline malaria vector in California, with its restricted breeding habits to clear seepage water, control of this species would involve the treatment of many types of water for effective results. It might even prove more advantageous if control programs were directed against adults resting in shelters, although a detailed study of the problem would be necessary before such a recommendation could be made.

Summary:

1. Epidemiological evidence indicates that arthropod vectors play a role in the spread of Western and Eastern equine and St. Louis encephalitis to man and animals.

2. Mosquitoes of the genus Aedes may meet the requirements for transmission of Eastern and Western equine encephalomyelitis virus as there is conclusive experimental evidence that they are capable of taking up, incubating, and then transmitting them to healthy laboratory animals. Culex pipiens may meet the requirements for transmission of St. Louis encephalitis virus as it is capable of experimental transmission.
3. There is evidence of other arthropods being implicated:
 - a. Dermacontor andersoni has been demonstrated as an experimental vector of Western virus and is capable of transferring the infection to its offspring. Its geographical distribution and seasonal incidence limits its importance.
 - b. Triatoma sanguisuga the conenosed bug has been collected in nature infected with the Western virus and has been demonstrated to transmit the infection to experimental animals. The limited distribution of this insect necessitates its dismissal except in local areas where it may be common.
 - c. Culex tarsalis mosquitoes have been collected in nature infected with Western equine and St. Louis viruses. This mosquito is very common in all of Western North America but nothing is known of its ability as a disease vector.
4. The finding in the field of infected Culex tarsalis conclusively demonstrated that mosquitoes may obtain an infective meal in nature.
5. If Culex tarsalis is proven to be capable of acting as a vector, hibernating **females** may carry the infection over winter, answering the perplexing question of winter carry-over of infection in areas where vectors are inactive in the winter, and no active cases occur in vertebrate hosts.
6. There is no reason to attach responsibility to a single species as the vector for any of these three virus infections. Many Aedes species have been demonstrated as laboratory vectors of Eastern and Western equine viruses, and Culex pipiens for St. Louis virus. Now Culex tarsalis has been demonstrated naturally infected with Western equine and St. Louis viruses. Any of these might play a role in the survival and transfer of infection.
7. The seasonal activity of Culex tarsalis as well as Aedes sp. coincides with the outbreak of the disease. In Yakima, Washington it appeared that a cessation of feeding by Culex tarsalis coincided with a cessation of new human and horse cases.

8. Explanation of the irregular local distribution of the disease in man and horses points to either a far flying insect vector, or to a common wide-spread vector which does not commonly feed on hosts which develop clinical infections, that is man and horse.

9. The host preference of certain Culex mosquitoes for avian blood and Aedes species for mammalian blood may be important in the following ways:

- a. Culex species may be responsible for the endemic phase in inapparent avian reservoirs. The high incidence of infections in birds, as shown only by the neutralization tests, could be explained in this way.
- b. Species such as Culex tarsalis in their occasional feedings on mammalian hosts may transfer the infection from avian reservoirs, and Aedes species may then act as efficient vectors to other susceptible mammals.
- c. Aedes species by occasional feedings on birds may pick up the infection and transfer it to mammalian hosts.

10. The domestic habits of Culex tarsalis bring it into contact with domestic avian and mammalian species, a fact which is important as a much higher percentage of this group of animals has neutralizing antibodies to Western and St. Louis viruses than have wild avian and mammalian species.

11. The possibility of other methods of transfer such as respiratory infections have still not been ruled out. If the viruses are present in nasal secretions the additional possibility of mechanical transfer by flies must not be overlooked.

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(Mr. Reeves' paper was illustrated with lantern slides),

Herms: This was a very excellent paper, Bill. You have given us information which is quite illuminating. Dr. Rosenau made the statement in 1912 that poliomyelitis was transmissible by the stable fly and we began to realize then something more as to the importance of our work in regard to various diseases of the nervous system. Our first work on poliomyelitis was done primarily with monkeys, the results being negative. While we do not have the yellow fever mosquito here, it is of interest to note that Dr. Kelser in his

experimental work transmitted horse encephalomyelitis through the yellow fever mosquito. Several years ago when we met here, it was thought that mosquitoes played no part in the transmission of encephalitis and they were not incriminated as vectors of this human sleeping sickness. Dr. Karl F. Meyer and Miss Howitt have been working on this problem of encephalomyelitis for some time and have made many discoveries that have already proven helpful to us in our work and will be increasingly beneficial. From the epidemiological standpoint, it is quite probable that human encephalitis and equine encephalomyelitis may be transmitted by mosquitoes. Equine encephalomyelitis is transmissible to and occurs in humans. Poliomyelitis is suspected to be transmitted by biting insects and possibly by mosquitoes of a type we have abundantly here in the temperate zone.

Reeves: Culex pipiens is suspected to be a possible vector of poliomyelitis. You are all familiar with this reddish or reddish gray mosquito with an unbanded proboscis. You know it and how this mosquito seems to prefer to breed in foul water.

Herms: Dr. W. McD. Hammon of the Hooper Foundation has a paper for us on "Host Animals of Virus Encephalitis". May we have this paper now?

**ANIMAL RESERVOIRS, GENERAL EPIDEMIOLOGICAL SUMMARY,
AND POSSIBLE CONTROL MEASURES FOR THE AMERICAN
SUMMER ENCEPHALITIDES**

by

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In the preceding papers of this symposium on the American summer encephalitides, we have heard discussed data regarding the infection of man and certain possible reservoir hosts, also the present status of knowledge concerning vectors. It is my purpose to add some specific information gained from a relatively broad sampling of the mammalian and avian kingdoms; to present other significant epidemiological findings from the field and laboratory, and to construct several hypotheses on the basis of all the available facts. These epidemiological hypotheses will be tested in the light of certain apparently conflicting observations. Then, if our hypotheses seem sufficiently well proven, the question will be raised as to what we can and should do about control. I hope that many of you, as well as the experts who follow on the program, will discuss this latter aspect.

Survey of Mammals and Birds for
Potential Reservoirs of Virus

Following certain preliminary observations by Miss Howitt and others, and apparently in parallel with some of her more recent work which she has just reviewed, I determined to sample as completely as possible all species of birds and mammals in an endemic area, for the purpose of determining the infection rate to two encephalitic viruses. The Yakima Valley, Washington, a small, rather isolated, irrigated valley where annual outbreaks of summer encephalitis were occurring, lent itself well to these studies. The previous year, assisted by Miss Howitt for the laboratory tests, over 50 cases of encephalitis in man in this area, together with a very limited sampling of wild and domestic animals were studied. We obtained evidence suggesting that the viruses of St. Louis encephalitis, of Western equine encephalomyelitis and of poliomyelitis were present and manifesting seasonal endemicity. For many reasons this area recommended itself to the survey of reservoir hosts and insects, the latter already described by Mr. Reeves.

The collecting of bloods and identification of species was placed in the hands of Mr. John A. Gray, Jr., ornithologist and Dr. F. C. Evans, mammalogist. They were aided by Mr. Edward Norton and Mr. George Downs.

The testing of the sera was done in part by Mr. Ernest Izumi and myself at the Hooper Foundation, and in part by Dr. Howard Lundy of the State College of Washington. All sera were tested by the mouse neutralization test against the two encephalitic viruses. In this test, as modified and standardized for this particular work, the survival or death of mice for each serum and each virus, served to demonstrate the presence or absence of antibody. Protection of the mice, if non-specific or cross reactions can be ruled out, we interpret as evidence of a previous infection at any time during the life of the animal. Since, except in horses, there had been no obvious evidence of an epizootic, any such suspected infection was probably of a mild or entirely inapparent nature. All ages were sampled, but a special sample was taken of the season's young at the end of the summer, to determine the percent infected in that particular year. To have ruled out all possible non-specific "positive s" would have required control samples from all species from non-infected areas. This was not practical at the time, but as shown in Table I over a hundred controls were run from 9 species which appeared to have the highest proportion of positives in the Yakima area. The results, all negative but one, indicate satisfactory specificity. Isolation of both encephalitis viruses

from mosquitoes of the region, further confirmed the implications of the test results. A preliminary report of tests on 162 birds and 153 mammals was published in "Science". Final analysis is still to be made, but for this occasion I have available the results on 284 birds and 282 mammals, a total of 566 bloods. The results do not differ essentially from those reported previously, but they now include more species and larger samples of each. Species samples are still too small for reliable comparisons, but with certain reservations, conclusions may be drawn in regard to certain groups.

In Tables 2 and 3 are presented the results of tests on the sera of 120 domestic birds and 164 wild, representing 45 different species. After several attempted groupings, there appeared to be a very significant difference between those which were domesticated or in captivity, and those which were wild. The latter group, however, included certain species which, as far as their environment was concerned, should be considered as domestic; i.e., robins and rats, and most of these, as will be pointed out later, appear to belong in order of infection rates in the domestic group, but in the tables the more classical definition of domestic has been followed.

Those species represented by 5 or more specimens are tabulated by species in alphabetical order of common names. The species from which only a few samples were obtained, for reasons of space, have been included as a miscellaneous group in each table.

In Table 3 the totals indicate that almost exactly 50 percent of all domestic fowl sampled had at some time been infected with these two encephalitic viruses. In many instances a serum contained antibody to one virus and not the other, but one specimen had antibody to both more frequently than would occur by chance alone. Two possible explanations for this may be offered: (1) Since in some flocks of chickens all had antibody and in others none, it might be concluded that where conditions were suitable for the one virus they were suitable for both, or more specifically, the same insect vector played a role in both. This would appear to be true. (2) The same vector may carry in some instances a double infection--not yet demonstrated--although experimental dual infection of the reservoir host has been. However, the important and more obvious finding is that about 50 percent of chickens, ducks, turkeys, geese, pigeons, etc., become infected.

In this group were 81 fowls which had lived through a previous summer season and of these 55 percent had been infected with the St. Louis virus and 54 percent with the Western equine strain. There were 39 young birds, a few of which were bled relatively early in the season.

Of the young, 34 percent had become infected with the equine and 39 percent with the St. Louis virus. The summer of 1941 appears to have been a relatively poor summer for encephalitis as reflected by the number of human cases, but during this season the evidence is, on the basis of the above figures, that about 35 percent of all domestic fowl became infected - a truly alarming situation with grave potentialities. Fortunately, it would seem that the vector or vectors prefer these fowls to man, as a source of blood meals.

Table 3 shows the results of tests of sera from 164 wild birds. The striking findings here are: (1) that certain species would appear to be almost entirely free from infection, the Brewer and redwing blackbirds and the sparrows - although many of these latter are relatively domestic in habitat; (2) that the robins, quails, pheasants and doves have rates closely comparable to the domestic fowls and these are species representing a high proportion of the bird population living in, or frequenting areas in close proximity to man; (3) that the predatory birds, listed separately in Table 4, have high infection rates; (4) that, taken as a whole, all wild birds sampled show only about a 20 percent infection rate, in contrast to 50 percent among the domestic varieties.

In Tables 5 and 6 are presented the results of tests on 159 domestic mammals and 123 wild mammals, representing 29 species. In Table 5 is listed the domestic group including wild species kept in captivity under a domestic environment. 36 percent neutralized the St. Louis virus and 35 percent the equine. In this group the outstanding findings are: (1) The cat - 18 tested - shows no signs of infection. Laboratory tests indicate that this animal is really immune. Following inoculation no virus can be found in the blood stream and no antibody formation takes place. It is also possible that mosquitoes do not feed on the cat, but on this I have no information. (2) About 46 percent of 106 domestic mammals exposed for at least one previous season had developed antibody, while only about 20 percent of 53 young of the season showed evidence of infection. In both instances this runs just a little below the figures for domestic birds, but high enough to have considerable significance from the reservoir standpoint. (3) It is very striking that practically all horses and mules not vaccinated for the equine virus, and never having developed a clinical infection, have antibodies to both of these viruses. It is obvious that a higher percentage of these than of any other animal become infected. Perhaps this is due to the size of the target they present to the vector, or the predilection of the vector for this species. Numerically, however, on the basis of data now available, the horse would appear to represent an unimportant reservoir in the Yakima area. Recent experiments on horses

inoculated with a Culex strain of St. Louis virus, performed in cooperation with the Department of Veterinary Science, show that following subcutaneous or intracerebral inoculation virus appears in the blood stream at some time between 12 and 36 hours after inoculation. However, no elevation of temperature or symptoms occurred. Cox, Philip and Kilpatrick were able to produce clinical encephalomyelitis in the horse by intracerebral inoculation of a "fixed", mouse adapted, neurotropic, laboratory strain of St. Louis virus. All this is only further evidence that the St. Louis virus should not be set apart from the so called equine viruses either in name or thought, but belongs epidemiologically to the same group. To me, the term equine is objectionable. Geographical designations are confusing and only of historical significance. Certainly some better terminology might be substituted, such as seasonal virus encephalitides, types 1, 2, 3, etc., or insect-borne virus encephalitides. A previous, now outmoded, non-etiological classification of types A and B encephalitis precludes the use of the alphabet. Chronologically, by isolation of the viruses they would be - Type 1 Japanese B, Type 2 Western Equine, Type 3 Eastern Equine, Type 4 St. Louis and Type 5 Russian Spring - summer tick-borne encephalitis. Perhaps the African tick-borne Bwamba virus of Smithburne and Hughes would be Type 6. However, I will not confuse you at present by shifting to this terminology.

Table 6 gives the results of tests on wild mammals. Eight percent had been infected with each of the viruses, in contrast to 35 and 36 percent in the domestic group, again emphasizing the greater importance of the domestic reservoir in this area. There is absolutely no overlapping, however, in the results of the two tests where only one infection has been produced experimentally.

Thirty-one predatory mammals and 25 predatory or carrion eating birds were tested. Unfortunately some were in captivity, and if the whole is broken down into four groups, numbers become very small. However, both in small groups or taken as a whole the percentages of reactors are higher than would be expected. This suggests that gastrointestinal or even respiratory infection might play some small role in this group of flesh eaters, in addition to insect-borne infection.

It is possibly more significant to point out that a number of migratory birds have been infected. They probably do not endanger other areas as a result of infection in Yakima, unless infected early in the season on a stop off for points farther north. But, it raises a question, which will be mentioned again, as to their potential

danger when infected in a warm southern area, just as they leave for other scattered destinations. The lower Rio Grande Valley is such an area. Probably many of these migratory birds will become infected with the Eastern equine virus next spring in that very area.

Now let us carefully consider the exact epidemiological significance of positive findings in the serum neutralization survey. Finding antibody, per se does not incriminate any animal as a reservoir. Any animal to be an important reservoir of virus might be expected to fulfill the following criteria: to be numerically significant, to serve as a suitable host for the vector, to offer to the vector a high titre of circulating virus in the blood stream, and to maintain such over a relatively long period of time. Numerical significance is relatively easy to judge. The fact that any animal shows a high proportion of positives to the neutralization survey indicates that it serves frequently as a host to the vector. In regard to the quantitative blood virus question we have little information. Miss Howitt has done a large share of the preliminary work that we can apply at present. Unfortunately much of the earlier work that was done on host susceptibility was to see if symptoms developed following intracerebral inoculation. This is of no particular application in so far as our problem is concerned. By the criteria of intracerebral inoculation Miss Howitt found most chickens "naturally immune" irrespective of the presence of antibody in the blood. However, following inoculation those fowls were shown to have circulating virus. This is important! I feel strongly, however, that all these tests should be made by the intracutaneous or subcutaneous route. A number of species have now been tested, some to one virus, some to both, but still very few to the St. Louis virus. Mr. Izumi and I have now tested the horse, the sheep, the rabbit, the guinea pig and the cat. All these, in respect to the St. Louis virus, but, not in all cases to the equine, are considered relatively insusceptible. All but the cat we find can serve as reservoir hosts, for virus is found in the blood. Miss Howitt has shown this to be the case for several domestic fowls. Davis and others have obtained similar results with a few birds, domestic and wild, with the eastern equine virus. The quantitative work on these remains to be done.

It will be some time before we can evaluate the reservoir more accurately, for many individuals of each species must be tested, length of time during which virus circulates determined, also the amount of virus present. This will be time consuming and very expensive. All our survey indicates, then, is that certain species have been infected, a few of these have been shown to fulfill the criteria of reservoirs,

and many others are possible reservoirs and deserve further laboratory study for "reservoir efficiency".

I do not consider it either necessary or justifiable to spend thousands of dollars in attempting to isolate the virus from these animals in nature, since more information can be obtained at less expense by the methods we are using. It is probably worth while pointing out that to be certain that results obtained in the laboratory will parallel those in nature, freshly isolated viruses should be used, not brain adapted laboratory strains. Use of the other could lead to serious error in case negative results were obtained. Such discrepancy of results has already been shown between the horse experiments of Cox et al and ours.

Vector preference should be considered in selection of potential vertebrate hosts to be first studied in the laboratory for "reservoir efficiency". Of this, we know little at present - in fact we are still not certain of the vector in one single area of this country, and it may vary from one area to another. However, if we select these vertebrates on the basis of high infection rates as determined by the neutralization test, we will probably not go amiss, for infection is evidence of having been fed upon, with certain possible exceptions, such as in the predatory group.

Perhaps the most important conclusion we can draw from this study is that areas with high concentrations of domestic animals (or probably of almost any animal, for density of population is probably the most important factor), are the danger areas for human infection, providing the virus and vector or vectors are also present. Where human population and animal population densities are both high is where we actually have found human case rates to be the highest in all epidemics on record where such data is available. These areas are in general - the periphery of cities and large towns, the whole of small towns, and rural areas composed of small plot owners or tenants.

Let us now construct and test a few hypotheses on the basis of the facts we have. From the data presented by Mr. Reeves, mosquito transmission appears to be more firmly established than ever, sufficiently so I feel to justify its acceptance as one mode of spread to animals and man. Is it the only method? Evidence now available from many areas where the disease has occurred in both horses and man indicates that the peak of the horse epizootic precedes that of the epidemic in man by several weeks. This could be interpreted as suggesting different vectors. We don't know when the infection occurs in all the other domestic

animals. The possibility of multiple vectors to explain this cannot be excluded and might explain the fact, but we have thus far failed to find that other vector. I would prefer to explain the earlier peak of the horse epidemic on the basis of facts now known. Observations I have made in both Yakima and Texas, indicate that in areas where the equine types of infections appear in severe enough epizootics to involve man, practically 100 percent of the horses on the ranches affected by the epizootic show serological evidences of infection. In areas where annual outbreaks occur, illness is restricted almost entirely to the young animals, probably evidence of mass immunity of the older animals. This gives us evidence to conclude that the peak of the epizootic in horses, and its subsequent rapid decline is determined by exhaustion of the exposed susceptible animals. In other words, there remain too few susceptible animals to permit a continuation of the high incidence. However, many smaller reservoir animals remain susceptible to infection (our survey shows their infection rate to be from 8 to 50 percent only) so the reservoir for mosquito infection is possibly still on the increase and the number of infected mosquitoes still increasing while the horse epizootic is on the decline. At this point, man, less constantly exposed to the vector, and perhaps not so diligently sought by the vector begins to show evidence of the "spill over" of this tremendous although most inapparent, lower animal epizootic. This explanation satisfactorily incorporates and helps to correlate many observations, and obviates the necessity of postulating an additional strictly zoophilic vector.

In addition, another fact needs explanation and could be explained by postulating an entirely different method of spread from man to man - the respiratory route. As you will recall from the tables given, the number of animals infected by both viruses was practically equal in almost every instance, and I believe in all instances where numbers were large enough to be significant. In man, however, an entirely different situation was found. About 65 percent of the normal, resident, human population of the Valley showed neutralizing antibody to the St. Louis virus in the summer of 1941 and practically none to the Western equine virus. Many explanations might be advanced, and one of these is that contact infection by the respiratory route might give rise to a mild type of unrecognized infection in man. Nasal secretions of man suffering from this disease have received too scant attention. Cox and his associates found the virus in the nasal secretions of one horse during the late and terminal phase of an experimental infection. In the mouse, encephalitis can be readily produced by introducing this virus into the nose. We have stimulated antibody response in a horse by intranasal inoculation, but we never found virus in the nasal

washings of our horses inoculated by any route but did find it in the blood. However, none of our experimental horses ever developed clinical encephalitis and it was during clinical manifestations that Cox and associates found it in the nose. Records and Vawter repeatedly found the western equine virus in nasal washings of experimental horses and demonstrated how easily experimental infection occurred by this route, but pointed out carefully that epidemiological observations excluded this mechanism as an important natural route of infection. I would also like to emphasize that the epidemiological data collected by Lumsden and by Casey and Broun in the St. Louis outbreaks make it appear highly improbable that contact infection plays an important role in clinical epidemic St. Louis encephalitis. Our data strongly supports this and we accept on the basis of epidemiological field observations the insect-borne St. Louis infection as being the more important, at least. Another, perhaps better explanation for this discrepancy in antibody findings in man is that the St. Louis virus has been present in the community for many years and the equine virus is a recent invader. In the group of persons living only 3 years in the Yakima Valley, the number of positives to both viruses is equal and negligible. Most of the animal sera tested represent short lived animals and indicate infection contracted during the last 2 or 3 years, a period when both viruses were present in the region. Man, seldom attacked by the vector, probably reflects a cumulated history of many years exposure to the one virus and only recently to the other. I rather prefer this hypothesis, since it again is the simpler, one method of spread rather than two.

Having decided that local spread to man is probably for the most part or entirely, domestic animal to mosquito to man, let us consider spread from one area to another. Some might challenge the view that it does spread, insisting that scattered inapparent foci in animals have long existed and that for reasons unknown, it periodically becomes apparent in horses and man. The possibility of this cannot be denied entirely, but the probability, in many instances, I question. Of one thing I feel quite certain, but cannot prove; that the so-called eastern type of equine infection is spreading westward. It was found in Alabama in 1940 and this summer (1941) was isolated repeatedly in Texas from horses and I found there serological evidence for its etiological role in one human case, and many infections in horses. Although looked for in other western states, it has not been found elsewhere. I predict that without the intervention of man or God it will be found in the near future in areas further west, and probably in the valleys of California.

Assuming, then, that these three viruses are not already established in all places where environmental

conditions make their presence possible, let us postulate the method or methods of spread. These will be outlined briefly. (1) The infected vector, if mosquito, can be readily carried long distances by airplane, automobile, train or boat. (2) The recently infected (bitten) domestic animal may be shipped to another area where a vector can obtain an infective blood meal. Laboratory data proves that this may be from 12 hours to 4 days after the infecting bite in small animals or up to 6 days in the horse. (3) Migratory birds similarly infected and infective may travel freely to new areas. These probably represent the more likely means of distant spread. (4) The flight range of the vector may explain local spread, or spread from adjacent areas.

Now to stimulate discussion, and to help to promote action on the most important issue, let us consider what can be done to control these infections.

Local Control

Professor Herms, Mr. Reeves, and most of you who are present, are more competent to deal with the control of the vector than I. Obviously, in each affected region the vector must first be determined, for economy of effort. Control of breeding for the mosquito, and for man screened barriers, limited hours of exposure and repellants, may all be employed.

Control of the reservoir may, in many instances, be more practical than complete mosquito control. Since proximity of human population to dense domestic animal population appears to be highly important, it is only reasonable where many human beings live to protect them by zoning restrictions, excluding chickens, ducks, cows, horses, goats, etc. Organized communities, when presented with the facts, will do this only as they becomes "encephalitis conscious". Health officials and others should start this educational program where encephalitis is an important problem. Such measures would probably be highly effective in cities and towns in endemic areas, if administratively possible.

Certain animals should be eliminated or reduced in number - pigeons and rats obviously, and theoretically pheasants, quails, doves and robins, and probably others. The question of practicability and degree will require much discussion, and the opposition of the wild-life group may be overwhelming. Nevertheless, there are justifiable reasons why in some instances such action should be taken.

Certain essential domestic animals in towns and on farms might be vaccinated. This would prevent them from

serving as reservoirs. Technics can be developed, but application would be expensive and difficult. Its use, voluntarily, would be highly commendable.

Prevention of Spread to New Areas

This is not just an intrastate problem, but an interstate, national and international one. As it concerns us at present, the Western virus is spread from Mexico through the United States and part of Canada. In the lower Rio Grande Valley, the Eastern virus is present in Mexico and the United States. From this point it stands an excellent chance of spreading. The distribution of the St. Louis virus is extensive, but not well known, due to its manifestation in man alone.

Local mosquito control by reducing local infection of vectors and reservoirs will reduce the possibility of spread by vector or reservoir transportation. Control on one side of the Rio Grande must obviously be accompanied by similar control on the other side. In yellow fever areas and certain malaria zones, efforts are made, apparently fairly effectively, to prevent transportation of mosquitoes. Spraying and inspection are used. In certain areas some such defense lines might be thrown out to check the spread of encephalitis. California already has a rather phenomenal set-up for exclusion of agricultural pests. Is a mosquito barrier worth the cost and would it be effective? Quarantine regulations for shipments of domestic animals is by no means a new administrative control procedure. In this instance, four to six days would be required--depending on the animal--and screened quarters would be obligatory, except in areas where no vectors are found. For horses, screening would present a difficult. Vaccination a few weeks before shipment would make it unnecessary. Vaccination of other animals before shipping would also render quarantine unnecessary.

Infected migratory birds might still carry the infection despite these barriers. Elimination of the birds is out of the question. Prevention of their infection, or reduction in the number of those infected would be the result of satisfactory local control measures, previously described. It is thus apparent that local control is not a matter of purely local interest, and must not be left entirely to the whimsical moods or partisan activity of local government, but in selected instances is of national and international importance and action of a nature fitting such a situation is indicated.

Vaccination and treatment of man I have left to the last, but it is and will be no less controversial. However, the details are possibly of less interest to you. Vaccination for man appears to be available and effective against the

Western and Eastern equine viruses, but none is available at present for the St. Louis disease. Nevertheless, vaccinations are always accompanied by occasional untoward reactions and even deaths. Unless morbidity rates in man in the general population become much higher than they are at present in most areas, the risk of vaccination will be about equal to that of the disease. The cost and inconvenience to the community of mass vaccination would be unjustifiable. Moreover, most of the worst epidemics are unexpected; clinical differentiation of the specific type of infection is impossible and by the time the type has been determined in the laboratory, the time to use a vaccine effectively will have passed. However, it is quite likely that in selected groups, probably on an occupational basis, there will be a place for yearly vaccination for one or more types of viruses. I expect more to be accomplished however, through other methods of control.

We have left, one bright prospect. In these days of chemotherapy there is always the possibility of finding an effective therapeutic agent. If this is found, much of the fear of the disease will vanish and the fatality rate will be greatly reduced. Therapy, therefore, may in certain communities offer the cheapest and most effective means to control this disease as far as man is concerned. At present we have found no such therapeutic agent, and serum therapy from evidence now available, will probably never accomplish much.

The explosive and disasterous outbreak this summer in the area of the Dakotas and surrounding states and in Canada, the appearance of the Eastern virus in Texas in thousands of horses and in man, compel us to face the seriousness of this problem. The potential danger to the thousands of military personnel concentrated in Texas on the Gulf Coast, and in the Rio Grande Valley and other more central areas is, I believe, important. The Surgeon General's office in Washington is showing true concern in this matter. The knowledge acquired during recent investigations gives us the key to do something, and some concrete policies should be formed now and be put into effect before the next warm season. One aspect of this, mosquito control, is your specialty, and I hope you will put your heads together and decide when, how, and how much should be done, and then make the necessary recommendations. The vector is, in many insect-borne diseases, the most logical point of attack. I feel strongly that we should have some expression of opinion from this gathering in respect to the practicability of mosquito control before too much emphasis is placed on the other possible points of attack.

Table 1
Neutralization Tests on Sera of "Control" Animals

	St. Louis			W. Equine		
	Pcs.	Ques.	Neg.	Pos.	Ques.	Neg.
Chicken	0	0	11	0	0	11
Duck, Domestic	1	0	22	0	0	22
Goose, Domestic	0	0	13	0	0	14
Owl, Great Horned	0	0	11	0	0	11
Pheasant	0	0	11	0	0	14
Pigeon, Domestic	0	0	9	0	0	17
Turkey, Domestic	0	0	13	0	0	14
Cow	0	0	12	0	0	12
Goat	0	0	15	0	0	15
Total	1	0	117	0	0	130

Table 2
Neutralization Tests on Sera of Domestic Birds
and Those in Captivity

	St. Louis			W. Equine		
	Pos.	Ques.	Neg.	Pos.	Ques.	Neg.
Chicken	9	0	23	14	3	17
Duck	10	0	10	8	1	12
Goose	14	0	4	9	3	6
Pigeon	7	1	4	5	2	5
Turkey	5	0	8	10	0	6
Miscl. (10 species)	9	0	9	9	0	8
Total	54	1	58	55	9	54

Percent positive
(omitting quest.) 48%

50%

(50)

Table 3
Neutralization Tests on Sera of Wild Birds

	St. Louis		W. Equine				
	Pos.	Ques.	Neg.	Pos.	Ques.	Neg.	
Blackbird, Brewer	0	1	19	0	2	13	
Blackbird, Redwing	0	1	3	0	0	5	
Coot	1	0	2	0	0	6	
Dove, Mourning	3	0	2	0	0	6	
Flicker, Redshafted	1	1	4	1	0	4	
Killdeer	0	0	9	2	0	5	
Owl, Burrowing	3	0	4	2	3	2	
Pheasant, Ringnecked	3	0	6	2	1	6	
Quail, Valley	3	0	7	3	1	7	
Robin, Western	7	0	5	6	0	7	
Sparrow, English	0	1	11	0	2	8	
Miscl. (18 species)	9	0	32	5	1	32	
Total	30	4	104	21	10	101	
Percent positive (omitting quest.)	22%		17%				

Table 4
Neutralization Tests on Sera of
Predatory and Carrion Eating Birds

	St. Louis		W. Equine				
	Pos.	Ques.	Neg.	Pos.	Ques.	Neg.	
Crow	0	0	3	2	1	1	
c Eagle, Golden	0	0	3	2	0	1	
c Eagle, Southern Bald	0	0	1	1	0	0	
Hawk, Sparrow	1	0	2	2	1	2	
c Hawk, Western Red-tail	2	0	0	1	0	0	
Owl, Burrowing	3	0	4	2	3	2	
c Owl, Horned	3	0	0	3	0	0	
Owl, Long-eared	1	0	1	0	0	0	
Raven	1	0	0	1	0	0	
Total	11	0	14	14	5	6	
Percentage Positive (omitting quest.)	44%		70%				

c = captive

Table 5

Neutralization Tests on Sera of Domestic Mammals

	St. Louis			W. Equine		
	Pos.	Ques.	Neg.	Pos.	Ques.	Neg.
Cat	0	1	17	0	3	15
Cow	6	1	16	10	9	14
Dog	3	0	8	3	0	8
Goat	9	1	8	6	1	14
Horse	23	1	3	18 ^{nv}	0 ^{nv}	0 ^{nv}
Mule	2	0	0	2	0	0
Pig	2	0	6	3	1	4
Sheep	2	3	19	2	5	17
Miscl. (4 species)	1	0	7	0	0	8
Total	48	7	84	44	19	80
Percent positive (omitting quest.)	36%			35%		

nv = not vaccinated for western equine infection

Table 6

Neutralization Tests on Sera of Wild Mammals

	St. Louis			W. Equine		
	Pos.	Ques.	Neg.	Pos.	Ques.	Neg.
Chipmunk, Great Basin	0	0	9	1	0	12
Groundsquirrel, Townsend	0	0	12	0	0	12
Mouse, Field	0	2	8	1	7	1
Mouse, Whitefooted	0	0	8	1	0	4
Pocket Gopher	1	2	7	0	1	7
Rabbit, Cottontail	1	0	9	0	0	10
Rabbit, Jack	2	0	12	2	0	12
Rat, Brown	3	0	6	0	0	10
Rat, Black	1	0	4	0	0	5
Weasel	0	1	4	2	0	3
Miscl. (7 species)	0	1	9	1	1	7
Total	8	6	98	8	3	89
Percent positive (omitting quest.)	8%			8%		

(Dr. Hammon's paper was illustrated with lantern slides).

Hermes: That was a very excellent paper, Dr. Hammon, and well given. You gave us some very significant information. There is a great deal in what you have said for us to think about carefully,

I now have the pleasure of asking Dr. J. T. Harrison, U. S. Public Health Service, who is Liason Officer in the 9th Corps Area, U. S. Army, to discuss these papers.

Dr. Harrison: I am very sorry not to have been able to hear the first part of this program since, entirely aside from my personal interest in the subject, several army posts are situated in areas where equine encephalitis has been reported in animals or men. To date, however, no case has appeared in the military personnel, with the exception of one sailor who was apparently infected while on leave in the Bakersfield area. The possibility of the disease appearing in the military personnel is uppermost in the minds of members of the Army Medical Department.

The first sizeable outbreak of encephalitis in man in the United States was apparently the St. Louis epidemic of 1933. Mosquitoes were considered as possible vectors at that time, and careful experiments, both in man and laboratory animals, were done in an effort to transmit the disease. Culex pipiens, Anopheles quadrimaculatus and Aedes aegypti were used, both in biting and injection trials, but with completely negative results. Culex pipiens was by far the most numerous mosquito in the area, breeding in open sewers in enormous numbers. A few Anopheles and Aedes were tried in view of former successful experiments with equine encephalitis, and the ease with which these species may be reared under laboratory conditions.

It would appear that to protect the military from infection, intensive control measures directed against the mosquito must be insisted upon. The area within the post will be handled by the post surgeon, but contiguous territory and nearby recreational areas will require action by local civil authorities. Naturally, any measures carried out in these latter areas will be equally or more beneficial to the civil population.

It has been a pleasure to attend this meeting; the first report of new evidence in the chain of disease transmissions is always thrilling. The discovery of the virus in the insect under natural conditions represents a gratifying advance in our knowledge of the epidemiology of the disease.

Herns: Thank you very much, Dr. Harrison.

Dr. Ellis Sox of the State Department of Public Health, who was to lead us in further discussion, is not here. During these days it is hard for these men to get away. Since he is not here, I will call on members of this group to discuss such phases as they may wish. May we hear from Dr. Morris Stewart, please?

Stewart: I think all of us are very much impressed by these papers, which show the seriousness of the problem and also the work that is being done. This is no longer a highly speculative field but there is more certainty of outlook.

I think possibly some of you may remember when we were discussing malaria in past conferences that I mentioned that there must be a certain number of mosquitoes per capita to maintain an endemic malaria, and that in an area having a low Anopheline population any change in one of the factors, either vectors, susceptibles or infected humans, may cause malaria to increase. In 1934 indications showed evidence of more malaria than usual here in California. It was thought to be at least in part caused by an epidemic in San Joaquin County, which was attributed at least partly to the introduction of a considerable number of infected migrants from the southern states. In this case the balance was upset by the introduction of a considerable number of infected persons, without appreciable change in the vectors.

We now are confronted with a problem of the introduction of new military personnel into areas where malaria is of very low endemicity, but where if the Anophelines only remain static in relative numbers we may have an epidemic due to the upsetting of the existing balance by the introduction of large numbers of susceptibles, most of whom are probably derived from urban areas. Only if active steps are taken to reduce greatly the numbers of vector Anophelines in such cases can outbreaks of malaria be prevented in either the introduced military population or the adjacent civil population. I think that this is a problem which must be faced squarely by both military and civil officials.

As to encephalitis, bird reservoirs are an interesting problem. Most of the bird life of the marshes is protected by the Migratory Bird Treaty. This is the only international bird protection treaty. Both game and non-game birds are considered wards of the Biological Survey. I am familiar with the situation in Texas, having spent a number of years there in migratory bird control work. In view of the treaties protecting migratory birds, we must proceed through diplomatic channels if control of encephalitis should be attempted through the control of migratory bird

reservoirs of infection.

Some of the so-called wild birds are practically domesticated. The robin, for instance, has been mentioned as a possible reservoir. But in Texas, for example, the number of robins is slight. Several seasons may pass without our seeing a robin. Only when we have unusually cold winters will we see them and then only for a short time.

In view of the present emergency, and in the light of this newer knowledge of possible mosquito transmission of encephalitis, we are going to be faced with certain new angles in our control practice, and must evaluate the possibilities and probabilities in the light of economic and efficient measures of mosquito control. In most districts we have conveniently classified certain mosquitoes as of primary importance and we have devoted our attention almost exclusively to those. Now we may be faced with the control of different species, and that means we must re-equip ourselves with the mental elasticity we had when we first went into this work. We must recognize that we may have to make rather extensive changes in control methods to correspond with the particular habits of the various species in question.

Professor Herms for many years has been pointing out to you the vital necessity of knowing in the first place the various species with which you are dealing and their habits, basing our control measures upon that information. We must study and be prepared to deal with species formerly somewhat neglected, which may become of great importance, and particularly in areas where many people are assembled now where perhaps there were only a few a short time ago.

Herms: I don't know anyone I would rather call on for a discussion of control measures as applied to tarsalis or pipiens, particularly the latter, than Harold F. Gray. Let's hear from Mr. Gray at this time.

Gray: So far as the application of control measures is concerned within the various districts, we are reasonably well equipped to proceed provided there is available to us a little more of the wherewithal with which to work, plus the possibility of getting more personnel. With the exception of Mr. Hayes' district and the Alameda County District, most of the districts in the state have rather limited funds, and it would be impossible for them to expand their activities appreciably without additional funds for materials and men. There will be increased cost all along the line for both materials and labor. Contracts we are taking now show that prices are definitely going up on things requisite for our work. You can't do with the

same amount of money the things you have been doing. We have heard about additional Federal funds for mosquito control in California in the vicinity of army and navy establishments. In order to be able to do added work, particularly in the vicinity of military establishments, more funds will be needed, especially for equipment but mainly for personnel. There is a job to be done but we have to have facilities with which to do it. The problem, as I see it, is the extension of our work to additional species which we have not so much concerned ourselves with in the past. In some of our Districts, most of the work is against Anopheles mosquitoes and they haven't done extensive work against pest mosquitoes.

Assuming you can get the funds, you still have the problem of personnel. There is a shortage of good men now and that shortage will increase. The shortage of public health trained engineers is serious. That is one difficulty. But given the funds and equipment and the opportunity to train personnel, I don't see why our districts and the county health departments cannot do this work and do it well, but perhaps on a different basis than that on which we have been working. Our work must be more intensive and directed to species which we have more or less neglected in the past in most areas.

As Dr. Stewart said, when we were younger we were fortunate in having a certain amount of mental elasticity. Some of us, like Professor Herms and myself when we started out in 1910, had little else. Perhaps we have developed a little mental rigidity over the years. This is one of the things on which we want to check ourselves up on these days and reexamine everything we have been thinking and doing.

Herms: I don't believe a change of conditions has a great deal to do with the control of pipiens. It is largely a matter of inspection. As a rule, engineers are not interested in such details as house to house inspection. Harold Gray, in his District, is thoroughly and well equipped to handle the situation, but the reason I ask him "Why?" is that I realize more personnel is necessary and more personnel means more money. The reason is the need for detailed work on tarsalis and pipiens. We have been accustomed, here in California, to handle them on a large scale.

Campbell: Thank you, Professor Herms.

The session then adjourned for lunch, and reconvened at 1:30 p.m. for the afternoon session.

Mr. Richard Peters, Entomologist of the Bureau of Sanitary Engineering, in the absence of Dr. Bertram F. Brown, State Director of Public Health, introduced Professor Stanley B. Freeborn, Assistant Dean of the College of Agriculture, who was asked to speak on "Military Mosquito Control in World War I".

Freeborn: When you get to the point where they put you on the program to reminisce, you are practically on the shelf. That seems to be what is expected of me when you ask for a talk about military mosquito control in the other world war. However, I do welcome the opportunity to talk informally on these programs.

In attempting to get some of the material together for this discussion, it struck me that a striking commentary was the fact that the present day sanitarian is going into the problem of malaria control on exactly the same basis as in the last war. That is, for practically every disease of military importance the sanitarian has a new and effective tool for its control, that was not present in World War I, except in the one case of malaria. For every other disease present today the sanitarian has a first class tool. It might not be out of keeping in connection with these reminiscences, to mention a few of those tools at the command of the present day sanitarian.

The progress of military sanitation from the American standpoint is interesting and I think no more effectively shown than in the statistics of the various wars in which we have engaged. In the Mexican war, there were seven times as many casualties from disease as from other causes. In the Civil War, there were twice as many deaths from sickness as from wounds. In the World War I, it was almost an even break. There were about 58,000 more cases of disease in World War I than from battle casualties. But of course in the case of World War I the scales were tipped somewhat by the appearance of influenza, which was a new problem and made a marked difference in statistics.

Another thing to consider is modern knowledge and attention to nutritional problems. It hadn't occurred to me that the term "vitamin" as applied today was not even known or used during the first World War. The term was introduced in 1912 but not applied as now understood until 1920.

It is beginning to be more and more recognized that a well nourished population is not particularly susceptible to epidemic diseases. As additional work is done on the importance of nutrition, particularly from the vitamin standpoint, we see more and more clearly that it is not the

well nourished population that is susceptible to epidemics. We had some idea of the connection between nutrition and disease resistance before, but we didn't understand it very well. We still may not understand it any too well but, with the attention being placed on nutrition at the present time, we ought to be able to avoid some of the striking epidemic appearances that we have had in previous wars. It may be a blessing in disguise that we will have to take in hand certain nutritional problems through the necessity of war, and military and civilian population will arrive at a better understanding of the importance of proper nutrition and the way to attain it.

Now let us run down through some of the diseases that might prove of great importance.

Yellow fever wasn't of much trouble to us in World War I but it may be in this. The annual report of the International Health Division of the Rockefeller Foundation several years ago had a lot that was interesting and valuable regarding yellow fever vaccine. Even as late as a few months ago it was reported in the British Sudan that there has been developed a new vaccine in which we can put considerable confidence. Yellow fever vaccine is very generally used throughout the Navy at the present time. Men who apparently have no possibility of being in a yellow fever area are being vaccinated nevertheless. Yellow fever is not dependent upon the presence of Aedes aegypti. In certain parts of South America there is yellow fever and no evidence of these mosquitoes. Monkeys may harbor it, being possible reservoirs.

Typhus was the bane of our existence in the previous World War but there again we now have a vaccine. Tetanus is now subject to prevention by a vaccine, and every man in our forces is now protected to a certain degree against the disease.

Influenza was a very serious problem. Certain vaccines have shown some promise but we are finding many different strains of influenza. But with influenza and pneumonia we are interested not so much with vaccines against the disease, but more with the sulpha compounds of various types which are very effective as curative agents. We have a new specific sulpha compound for practically every bacterial disease. So even if vaccines may be somewhat of uncertain value in such diseases, we do have first class new tools which we can use.

In the last war, we had a good many scares with meningitis outbreaks. Now we know from our experience with sulphathiazene that we can handle epidemics with a fatality

rate which will not run over 2%. This has been an enormous improvement.

Gonorrhoea is also now handled quite satisfactorily by treatment with sulpha compounds. We have these new tools at hand with which to handle these diseases. There is also the problem of syphilis, and much progress has been made there too.

But malaria still stands out as the dominant disease in connection with our consideration of troops, and for its control we do not have any really effective new tool. Malaria, in the last war, developed some interesting statistics and observations. For instance, you might develop a camp site at a place that was not known by the authorities to be a place where malaria was prevalent. After the camp was set up, immediately malaria appeared. The answer was the fact that areas considered non-malarial still had Anopheles present in small numbers--not in large enough numbers to cause epidemics until there were more infected persons and more susceptibles. Malaria then developed in places that had previously been considered non-malarial in character. Previously the place had been non-malarial but it was not non-Anopheles.

The bibliography on the Anopheles mosquito is more extensive than that on any other insect. Anopheles mosquitoes are spread all over the world and so are of importance throughout the world.

Intelligent mosquito control depends upon our knowledge of the species involved. By reducing your Anopheles population to a continuously very small number per capita of humans, you can have no malaria epidemic, though you may still have occasional cases of malaria. We must be able to recognize this Anopheles mosquito. The breathing syphon in the larva consists merely of two small flaps in the top of the eighth abdominal segment. If no long breathing tube can be seen, the larva is undoubtedly Anopheles. In the adult, if the palpi are as long or longer than the proboscis, your mosquito must be Anopheles. If the palpi are short, it could not be Anopheles. The wings of our western Anopheles maculipennis are marked with four dark colored spots. There are 164 species of Anopheles.

There are no cases on record where Anopheles were found incapable of transmitting malaria. We have had definite cases of malaria in Wrangell, Alaska. The Anopheles wouldn't be there unless conditions were right for the transmission of malaria. If Anopheles mosquitoes are present in an area, you can't rule out the possibility of malaria transmission. Within a given area, there may be

ecological conditions that will permit their presence without an appreciable malaria incidence, but I don't think we are safe in considering any area certainly non-malarial. If you crowd susceptibles and carriers in, you are playing with dynamite.

It is certain that malaria was present in California before the Gold Rush days but it is not definitely proven how it came here. The records show a great amount of it among the gold miners in 1849-50. The entire town of Oroville is recorded as being sick with it in 1850. People moved many times in pioneer days to escape malaria. Records of military expeditions in northern California very early report the presence of the disease. When it became established among the California Indians is not definitely known, but it was probably not prior to the gold rush of 1849-50, or the Spanish Californians would have undoubtedly acquired the disease during their many excursions and expeditions into the San Joaquin Valley.

As to the matter of the control of malaria during the last war, in our experience on this side of the Atlantic we had two types of problems. One was an urban problem, the other a localized problem, and the two are handled entirely differently. The urban problem meant that there were large numbers of civilians in groups, continuously in contact with the introduced military population, wherever both congregated, as for example, the movies.

In handling what we may call an "urban" malaria control problem, it was necessary to handle the situation as a job of "extra-cantonment" sanitation. The area could be cleared of mosquitoes by the normal methods of ditching and oiling. The army could even go in for concrete lined ditches and do a thorough job of mosquito control exactly as it would be done under normal urban conditions. Under such conditions malaria control for military personnel is only possible as a job of community sanitation, as it is not feasible to entirely or appreciably segregate the armed personnel from the adjacent civilian population.

The other situation is a localized problem where bodies of troops are well isolated from a civilian population, but in the presence of very large numbers of Anophele mosquitoes. Under such conditions you can see why the English and the French health authorities very early in their career became dispensers of quinine. They probably looked at the areas where they had miles and miles of Anopheles breeding, and they decided to let the mosquitoes go, feeling they just couldn't cope with the tremendous mosquito problem with the limited funds and personnel available to them. It

was not that the problem was not recognized or that the value of ditching was not known. But mosquito control seemed to be an impossible job because of its magnitude, and so they did what they considered the next best thing.

I got into one situation in Virginia during the last war where one river came down and joined another. Except for a few hills, the place was as flat as a pancake. Trees were growing out in the water, and there were areas for twenty miles that we couldn't get to except in a boat. There were jungles where you would have to use string to be able to get back. Mosquito control in this area would have cost as much as establishing the port of embarkation. Dikes and gates would be required to keep out the water and dry out the area. It was an enormous engineering project. Preventing the breeding of mosquitoes was out of the question at any reasonable cost, and yet men had to be there. Something had to be done. We took a blood smear index within two miles of camp, and everybody in the area had malaria. It was necessary for some of the men to stay out-of-doors on duty all night. The problem was to protect them. We tried to protect them from mosquitoes that had bitten somebody else. We used quinine prophylaxis. We tried to devise ways and means to get the men to take the quinine, but the men just wouldn't take it. We tried all sorts of things but were none too successful.

Finally we developed this scheme. The men slept inside screened barracks. After they got to bed at night we went around to see that they were tucked in. We had a squad of men that collected all the mosquitoes they could find in the barracks. The mosquitoes would go to the windows. The squad went through with brooms and boxes and collected all the mosquitoes they could find on the windows. They would get a big catch each day in the barracks in spite of everything we could do. There were double screens on the doors.

After this scheme of control was in effect we didn't have a single case of malaria in eight months among the men we had in barracks. Then a group from a wagon company came who were not under our control. They insisted on sleeping with the horses. Within three weeks we had an outbreak of malaria among this group. They were given prophylactic quinine where they would take it. The only thing they lacked that our men had, was that we collected all the mosquitoes we could catch in the barracks. After the first two weeks we even made no effort to protect the men outside at night on guard duty, as we found they could not or would not keep veils and gloves on in the heat and humidity. This was in a place where the history of malaria was 100% endemic.

It was a perfect demonstration of what can be done. That is what they are planning to do on the Burma Road over a 300 mile front at the present time. Fundamentally, it is simply to prevent a mosquito from biting twice, by catching her in quarters and killing her before she has a chance to take the second bite.

I think it boils down to this, that where you have troops in relatively isolated areas with little contact with a civilian population, even though in the presence of a large Anopheline population, protective measures through catching adult mosquitoes in quarters will be quite effective as a prophylactic measure, and is one that can be easily undertaken by the men themselves. Even the men on guard duty out doors at night will be relatively safe from infection, as these out-of-doors mosquitoes will be probably non-infected -- just wild, simple, pure mosquitoes that had not come from any house.

Those of us who have worked with this problem get over-sensitive about being bitten by one mosquito and so getting malaria. I know I have wondered lots of times in some areas. I have thought I might come down with it. However, the chances are several hundred to one that a single bite won't do it. Therefore in a localized situation, you should take every possible step to prevent your personnel being bitten twice by any one mosquito. There you get a fairly decent control.

In the present war we are going to be faced with problems that are going to vary with every single area and every single case. The history of each area is going to be somewhat different from every other area. It will take a whole lot of figuring to meet conditions most effectively. It boils down to the use of good common sense.

In conclusion, we have to face the situation that we haven't a single tool today in malaria control that we didn't have in World War I. Malaria is about the only disease where this predicament is true. So far as malaria is concerned, it is the same old problem. There are interesting days ahead for all of us. We know more about mosquitoes than we did at the time of the other war, but we need to know a great deal more yet. However, by applying intelligently the tools that we have, I think we can lick the problem without too much difficulty.

Peters: Thank you, Professor Freeborn.

We are now to hear from Dr. R. H. Creel who is the District Director of the U. S. Public Health Service.

Dr. Creel has something further for us on this important subject.

Dr. Creel: I am somewhat reluctant to discuss this subject. When I was asked, I couldn't help but feel that it could be much more satisfactorily discussed by Mr. Gillespie or some of his assistants in the State Bureau of Sanitary Engineering. The Public Health Service is like the promoter who agrees to furnish the ocean if someone else will furnish the ships. The Public Health Service will furnish and has been furnishing assistance to the State Department of Public Health, and also has arranged with the WPA to allow five million dollars for mosquito control work, of which California will probably receive about six hundred thousand dollars.

For many years past, Congress has appropriated annually sums of money to prevent the spread of diseases such as typhus, yellow fever and smallpox, and the federal government has practically taken charge of control measures for yellow fever in the south. Beginning before the war, Congress has made special appropriations for public health services out of Social Security Funds. County health units have been established or strengthened in many parts of the country, and considerable funds used for the training of personnel as health officers, sanitarians and technicians.

I presume the Bureau of Sanitary Engineering, and you who are present at this meeting are thinking more of the mosquito problem in California, although as Professor Freeborn has said, we don't know what is going to develop in this field.

This project as contemplated for California is to be administered under the direction of the State Department of Public Health. One of the first things being done is to obtain all data as to the amount of ditching involved, the amount of materials necessary, and the number of men required to do the job. One project is already under way in San Diego County, and in Los Angeles and Orange Counties projects are now being worked up. Other mosquito control projects are contemplated around Fresno, Merced, Sacramento and Marysville.

"MOSQUITO BREEDING AND CONTROL
IN THE VICINITY OF MILITARY ZONES

by

R. F. Peters,
Mosquito Control Officer
State Department of Public Health
Bureau of Sanitary Engineering.

My presence here as newly appointed State Mosquito Control Officer affords an opportunity to acknowledge the many

enjoyable associations which I have had with most of you through dealings with the districts represented in the California Mosquito Control Association and through contacts with the various local Health Departments of California.

As a recruit in the ranks of the men who make war upon mosquitoes, I am most happy to be able to issue the following communique - "Despite heavy mosquito attacks on some quarters in California during 1941, the onset of winter appears to allow for sufficient time to muster a strong defense and a greater offense against the Culicid dive-bomber and pursuit formations".

From out of the sky come more airplanes, bombs and mosquitoes, for during the Autumn of this year there descended upon the State Department of Public Health, with no more humming or buzzing than the ticking of a teletype machine, word from Washington that the State Department had been named as "maternal" sponsor of a State-wide National Defense W.P.A. Mosquito Control Project. Upon due deliberation, Dr. Brown, Director, and Mr. Gillespie, Chief of the Bureau of Sanitary Engineering, drew up adoption papers and made arrangements for the rearing out and application of the control measures provided, as a part of the activities of the Bureau of Sanitary Engineering. In this regard, I guess I was named "God-father".

We were not taken wholly by surprise upon the presentation of this project, for the National Defense Areas of California had been the object of much of our survey work since more than a year ago. In general we had a fairly good idea of the control picture, but the two factors of constantly increasing military establishments in California and an unusually "unusual year" of about twice the average rainfall, did somewhat cloud the approach to control. In addition, the promised early assignment of U. S. Public Health Service engineers to survey and supervise project-units was somewhat slow in materializing. However, today three engineers are stationed in various sections of the State, lining up control programs, and at least two more are on their way here to augment the work. The program provides for supervisors and for W.P.A. projects to control breeding for a distance of 5 miles from military reservations.

In connection with the weather, Southern California probably experienced the most pronounced springtime pest infestation in many years. Collections of rain water which stood unusually long, allowed for mass breeding and a great pest by such little regarded mosquitoes as Culex tarsalis and Theobaldia inornata. Citizens of Long Beach and San Diego were kept busy slapping and spraying for several

months, until control measures and the effects of evaporation could catch up with the breeding. The City of Riverside, upon being swarmed over by Culex quinquefasciatus and Culex stigmatosoma in the Spring and Summer, declared an "all out" against these pests, and by a varied control program overcame the infestation. San Bernardino citizens received a rather continuous barrage of mosquito bites during the year, but they just gritted their teeth and bore them.

In Northern California, what early threatened to be a record crop of mosquitoes became greatly retarded, reaching a peak late in the Autumn and never amounting to general expectations. Until Autumn, very few Anopheline mosquitoes were to be found. In such locations as Marysville and Lerdo, where Anophelines have been general, little breeding was evident, and malaria was practically nil. One interesting development of this year has been the finding of Aedes lateralis, a common pest of the Northwest states, in several locations of the mid-Sacramento - San Joaquin valleys. Noticeable throughout California has been a large amount of breeding of pest mosquitoes in domestic places. The excess rainfall appears to have been most responsible for this.

One especially important reason for reduced mosquito breeding in Northern California this year seems to be the extensive occurrence of natural enemies of mosquitoes. May-fly larvae, water beetles and water bugs have seldom existed in such numbers. Gambusia affinis have enjoyed broad dispersal and they have been able to exert a pronounced control. Several exceptions to the effectiveness of Gambusia have been noticed on my trips, in which connection Gambusia and mosquito larvae have been found to exist in friendly association with each other. However, in one case, (which Mr. Robinson knows well), the natural condition of the pool was altered slightly by the presence of numerous mulberries and rat-tail maggots. In the other case which I have in mind at a location in San Diego, the Gambusia were so small that the larvae of Culex stigmatosoma seemed to be wriggling out in the open as if to dare the mosquito fish to take a bite. Elsewhere, however, nothing but extremely fine results are being obtained through control with Gambusia.

Returning to the subject of mosquito breeding in National Defense Areas, we are faced with no small undertaking. When once we think we have taken account of all the military areas in the State, we receive word that another is added. Perhaps now with the war, they will continue to increase in number. In all, at present there are 31 National Defense centers in California. Of these, 21 have a mosquito control need in greater or lesser amount. In the remote sites, the problems confronting us are either agricultural mosquito breeding, or breeding characteristic of

a wilderness. Although we are acquainted with the type of control required in the agricultural areas, the significance of pest or disease in a wilderness requires much further study. The Navy has been headquartered at important California harbors for some time and with these areas we are all quite well acquainted. However, a recently established Mosquito Boat base at Morro Bay presents quite a salt marsh breeding problem.

One of the most important breeding places in the vicinity of reservations, and the cause of breeding in more than one case in California already, is that created from the disposal of sewage. With a little attention to what happens to the effluent, this can easily be averted.

Some examples of National Defense centers in California which are particularly important, follow:

At Marysville the Army proposes to locate a large reservation which would lie in an area long known for malaria and where pest mosquitoes have been accepted as a matter of course. There, a network of drains, sloughs and agricultural ponds indicate the need for a sizeable program.

Around McClellan and Mather Fields in Sacramento, some work will have to be done in the dredger ponds, in creeks, sewage disposal drainways and in miscellaneous domestic locations. Little mosquito bother was had this year until the late Autumn, when considerable numbers of Anophelines descended to make life miserable for a couple of weeks.

Mare Island being partially salt marsh itself and being situated along the shore of San Pablo Bay, received considerable infestation of Aedes dorsalis this year. Tidal action adjacent to the Island and dredge water disposal on the island proved responsible for the breeding. Early plans to improve the drainage in this area have been discarded in favor of an emergency larvicidal project which is being worked up at present.

The Merced Airfield is located right outside the corner of the newly enlarged Merced Mosquito Abatement District. There, Harold Lilley will be faced with extending his efficient Gambusia control to protect Uncle Sam's fliers.

Fresno Bomber Base has been the pioneer military reservation to carry on control, mainly through the determined efforts of its Surgeon, Major Heine, who declared war on mosquitoes back in June of this year, and by the application of a well-organized program of brushing, clearing and oiling, greatly reduced the usually numerous mosquitoes in

the Clovis area. At present in the metropolitan area of Fresno, a Mosquito Abatement District is being formed, and efforts are now being made by the Bureau of Sanitary Engineering to set up a W.P.A. project unit for the protection of the Bomber Base, especially as a supplement to any work to be done by the District.

Territory in the vicinity of Lerdo Airfield has been troublesome from the standpoint of both malaria and equine encephalomyelitis, and preparations are being made to control this area. Although breeding is not widespread, when it occurs the effects are very pronounced.

Mosquito breeding in the Monterey - Fort Ord - Camp Roberts - San Luis Obispo areas is mostly a spring-time pest. Project-units in these areas are being drawn up to handle just such situations. In these locations it is believed that the greatest amount of long-term good result will be derived from the extensive planting of Gambusia affinis. One of the mosquitoes to watch in this general area will be Anopheles pseudopunctipennis, which is to be found almost everywhere. Present evidence does not incriminate this mosquito as a pest or as a carrier of malaria, but with men from malarial states headquartered in these reservations, this mosquito will bear watching.

Practically all of the Los Angeles area is eligible for W.P.A. mosquito control on the basis of the National Defense industries there. Problems range from the small domestic fish pond through oil-well sumps, drains and ditches, to salt marsh breeding. A comprehensive project-unit is being lined up for this area.

San Diego represents a bizarre picture of mosquito breeding, in which creek beds, river bottoms, sewer overflows, salt marshes and various miscellaneous domestic places are implicated. This has been the first project-unit to get under way.

One of the underlying reasons for conducting a W.P.A. mosquito control project in National Defense areas of California has been the occurrence of encephalitis in several of the locations selected for military reservations. Dr. Creel and Dr. Harrison, recognizing this potential danger to the military forces consider the occurrence of this disease in any sizeable amount to be sufficient basis for establishing mosquito control. As has been pointed out by the papers of this morning, the implication of pest mosquitoes in the encephalitis picture practically in itself is sufficient to suggest the need for mosquito control wherever pest mosquitoes occur in great numbers.

In applying control in the various military areas, we have to consider the following as fundamental to the inception of a project-unit:

1. The need of control - based upon the history, the occurrence of complaint.
2. The availability of W.P.A. labor, present and future.
3. The local receptiveness and desire for participation as a co-sponsor - this to be obtained in such a manner as not to work a hardship upon the co-sponsor.

Control performed under this project represents a change of view from that of any W.P.A. mosquito control program undertaken heretofore. Formerly mosquito control projects received no particular preference or priority, and any work performed had to show a definite permanent control in which no maintenance would be required. This present Statewide project receives a high National Defense priority and emphasizes the application of larvicides with a minimum of preparatory work. In other words, the emphasis is upon emergency suppression of acute sources of breeding. In order to ease the local financial burden of this work, a high man-month allowance of \$16.79 has been provided for non-labor items. This is about sufficient to purchase oil and rent transportation and spray equipment. What appears to be considerable of a problem is the W.P.A. requirement of freedom from claims of damage for work undertaken. In this regard it is necessary to obtain an easement or permit on every parcel of property to be worked on. Where work is done inside Abatement Districts or in counties where Health Departments having enabling ordinances which assume this obligation, the task is simplified. However, in the great majority of places, permits must be obtained.

In evaluating such a control program, a number of factors must be taken into account. In setting down unit costs for items of control such as tule grubbing, clearing and brushing, ditching, spraying oil and fish planting, it has been difficult to establish an approximate basis. The only useful references in California have been the C.W.A. project of 1934 and estimates of the cost of such work done in the mosquito abatement districts. Because of a different type of control being emphasized in the Southern United States, work accomplished there has been of little help in furnishing these estimates. Because of industry taking the best of W.P.A. workers, we have not been too certain of the amount of

work to expect of those remaining. This point seems important - that control of mosquitoes under the program contemplated is subject to a variable degree of efficiency as far as results are concerned. The curve resulting from a plotting of cost against time might arrive at a point which did not accomplish complete elimination of mosquitoes, but yet would accomplish effective control. From this reasoning we have been able to compromise on man-months in certain instances in order to get projects started. This is important because of the great shortage of W.P.A. laborers.

As yet, it has not been determined what effect the war and priorities will have upon these projects. It is expected that they will continue to be provided for in some degree and that they will continue to rank high as National Defense priority projects. Surely the matter of mosquito control could not be ignored and thus allow the possibility of an adverse health condition at home to affect our war efforts. There are a number of matters pertaining to techniques of control and policy which have yet to be ironed out, but the major ground work has been laid and the pattern of procedure has been established. This program represents something new to California in the field of mosquito control, and with the assignment of qualified personnel to supervise the project-units, such as has been the case thus far, there is no reason to believe other than that the military and National Defense areas shall experience freedom from the annoyance or the disease possibilities from mosquitoes.

I believe the Division of Entomology, especially Professor Herms, Lt. Aitken (now with Uncle Sam's army) and Bill Reeves, deserve much thanks for the highly specialized service they have been rendering through the identification of mosquitoes in this work. In closing, I should like to introduce to you the men assigned from the Public Health Service who will be responsible for keeping the National Defense centers mosquito free - -

1. L. E. Thompson
2. R. A. Berg
3. E. E. Ulrich

Herms: I want to call your attention to the fact that Dr. Alfred C. Reed of the University of California Medical School is to be the speaker at our banquet tonight. He will speak to us along the very lines we have been discussing today. His subject is to be "Problems Involved in the Control of Mosquito-borne Diseases under Tropical Conditions". I have known Dr. Reed for many years. He has had a very wide experience in the tropics.

I had some experience with Anopheles maculipennis freeborni.

The presence of Aedes maculipennis freeborni does not depend upon rainfall. It is a seepage water breeder. We find them principally where agricultural irrigation is not accompanied by adequate drainage. You may have very little rainfall but plenty of freeborni due to irrigation.

Gambusia in the eastern part of the United States are not altogether effective because Anopheles quadrimaculatus larvae are rather inactive. Gambusia will go after them when they move, but otherwise not. But Aedes maculipennis freeborni is much more active than Aedes quadrimaculatus, is therefore more effectively attacked by these fish, and so the planting of Gambusia freely is a helpful control measure under our condition.

In the control of Culex tarsalis and Culex pipiens, frequent inspections and detailed knowledge of conditions in an area are necessary and important. The general, large-scale operations we use against salt-marsh mosquitoes are not effective against these species. With them we must always be handling details. In particular, the control of Culex pipiens means tremendously detailed work. They may be abundant in a house and yet you will have to hunt for days before finding their breeding place in some concealed, even underground, place.

Freeborn: In regard to this problem of Culex tarsalis and Culex stigmatosoma, I have seen tarsalis and stigmatosoma come out of the same egg raft. I believe that you will have to consider stigmatosoma with all the interest that you have for tarsalis as a disease transmitter, and consider them as one species. There is apparently one thing that works to distinguish one from the other. I have bred stigmatosoma out of permanent, natural waters, such as ponds or marshes the water with tule fringes where the water stands the year round, whereas tarsalis comes out of contaminated places where we expect to find pipiens. When tarsalis breeds in the warm water of natural ponds where tules are, it will come out with the characteristics of stigmatosoma. It seems that

ecological differences in breeding places can and do produce minor anatomical differences in a mosquito species.

To distinguish Culex tarsalis from stigmatasoma, take a good look at the under side of the abdomen. Round, dark spots, somewhat indistinct, indicate stigmatasoma, whereas V-shaped markings indicate tarsalis; tarsalis will also have a longitudinal white stripe or series of spots on the outer side of the legs, but stigmatasoma will not show such stripes or spots.

I believe that a similar situation exists with Aedes dorsalis on the Pacific Coast. Our salt marsh Aedes dorsalis is identical with Aedes caspius, but our fresh water or inland Aedes dorsalis should be considered either a distinct species or a variety which breeds only in fresh water of a temporary type such as the water in rice fields, which may be rather alkaline, or in flood run-off from melting snow fields, as in the bottom lands along our rivers debouching from the Sierra Nevada foothills. It is apparently rather adaptable in its breeding habits.

Culex pipiens, which I mentioned a few minutes ago, is a domestic species. It is usually, though not exclusively, found in cities and towns. This is the mosquito you are apt to find in catch basins, elevator shafts and in the gutters. After Oroville put in its sewer system, they reduced very greatly the numbers of pipiens they had.

Reeves: Since my return from Yakima, I have been interested in doing some work with Culex Tarsalis. Mr. Gray has had his men looking for larvae but those I have received so far have been C. stigmatasoma. I haven't had sufficient experience in breeding C. tarsalis in the laboratory to want to say much. In Yakima typical C. tarsalis were found in all stages of breeding. This C. tarsalis mosquito is perhaps the most widely distributed of any species in this state. There are a lot of them in the Sierras. The larvae are found in either fresh or foul water. In some places they have been found breeding in water definitely alkaline in character.

Freeborn: Yes, they are widely distributed. Collections have been made at 7600 feet in Alpine County, and they are abundant in the central valley as well as in the Coachella and Imperial Valleys. Even high tides do not seem to deter them.

Herms: Culex tarsalis is dark grey or brown with broad white bands on the tarsi, and the proboscis is white-ringed. You can see the bands on the abdominal segments readily. This is the only mosquito you may find it difficult to differentiate from stigmatasoma, which does not have the white-

lined legs of tarsalis. I might also call attention to the "v" shaped ventral abdominal markings of the tarsalis as contrasted with the dark round or oval abdominal spots of stigmatasoma. These markings are somewhat variable, however, which makes it hard to distinguish between the two types. Culex stigmatasoma is seldom or never present in large numbers.

Reeves: While Culex stigmatasoma seems to prefer fresh water, it has been found in sewage water.

Gray: Yes, they have been found breeding in open sewage treatment ponds, and in foul water such as the wastes from the Holly Sugar Company's plant at Alvarado. Bill has been trying to get hold of tarsalis larvae and we have been trying to help him. We have gone to areas and locations where we have previously found Culex tarsalis for Tommy Aitken, but all the larvae we have obtained so far have turned out to be stigmatasoma.

This argument about tarsalis and stigmatasoma has not been settled yet, and apparently we can't settle it today. This discussion started out on one thing, and went off onto something else. We were discussing the matter of Federal assistance for mosquito control work.

Peters: I would like to hear what you think about the amount of preliminary work which needs to be done to set up such projects.

Herns: These are times of emergency. We do not have time to line ditches, or do many types of permanent construction under the circumstances, and it would seem therefore that the preliminary planning need not be minutely detailed.

Hayes: I think we have handled as much W.P.A. work as anybody here. I want to just back Mr. Gray and urge that we all give our friend Peters all the help we can if he is going to have supervision of these projects.

I want to say something about Major Heine as being able to go out and somehow get the money and get the work done at the Bomber Base at Fresno. I take pleasure in introducing Captain Johnson of the Air Base.

Johnson: I think we all have a high opinion of Major Heine. He certainly is a go-getter. We have apparently plenty of money for labor and supplies but we have no money for equipment. The present plans are to do as much preliminary work as possible before next summer. I am digging ditches in swamp land, clearing out sloughs, etc., but, if we don't get at least one piece of high pressure spraying

equipment, I don't think we will be able to make the control work completely successful, though conditions will be much better. I don't know the mosquito situation in the west and have to rely on others who do know. I think that there is a big improvement coming due to our efforts, and also in forming a mosquito abatement district at Fresno, which looks as though it would go through.

Bendel: Does the military personnel do the ditching?

Johnson: We now hire civilians. Until about a month ago we had soldier personnel working on it.

Campbell: Are there any questions with regard to Federal Aid money?

Gray: Is this to be a matter of application by the various districts and counties, or is the Public Health Service assigning so much money to particular districts?

Peters: It will be necessary to obtain someone as a sponsor, so that practically you will have to make an application for a project. The WPA will supply the labor and a certain amount for materials; the sponsor would contribute the supervision.

Robinson: Modesto, in Stanislaus County, is an intermediate stop-over point for a good many troops in transit. At present they have a temporary camp outside our district. Could they come under this arrangement?

Peters: Very probably.

Gray: We tried all this before and our experience in sponsoring such federal aid work was that it cost us more just to sponsor it than it would have cost us if we had done the work with our own men. It seems to me that a small amount of money placed in the hands of the superintendents who are getting good results and could get better with more money at their disposal would do the most good. In this way we could get results that would definitely change the situation for the better, with the least expenditure, the greatest speed and the most certainty, and we would avoid a lot of red tape and unnecessary report making.

Campbell: We have annexed a lot of new territory to our district, in the Walnut Creek area, but we will not get any tax money from it until after July, 1942. But the people in the annexed area will expect a considerable amount of control work to be done there this summer. Can we get any of this money to carry over next year in the annexed area?

Peters: Probably not, unless there is a military establishment in the annexed area.

Stewart: I wouldn't want to emphasize anything being said this afternoon, or change the subject, or appear to take exception to anything, but I think it might be well to give you my opinion of what has happened. Of course I speak without authority and everything I say is but my own personal analysis of the situation.

The emphasis in this afternoon's discussion has been placed in part at least upon the present situation of national defense and our part in it. It has been shown by each speaker that we are going to be faced with many problems--some old and some new. I think it is quite possible that those of you who have been engaged successfully in mosquito control may ultimately find themselves in other insect control work also. We talk about a lot of money from the W.P.A. and other sources to help out in this work. A lot of money has been released, but remember that it is also true that we are facing a period of strict economy. We are going to have to draw a sharp line of demarcation between luxury and necessity. Very soon this sharp line will be presented to us in this matter of abatement work. I believe it is quite possible we may be instructed, in view of the enormous national expenditures we are facing, that we must expend our available money for control of those mosquitoes directly concerned or at least very strongly suspected of being concerned with the transmission of disease. I can visualize the possibility of having to let up on the control of some species, in order to devote our energy and money toward those forms which may detract from military efficiency and defense industries. That is the whole keynote of preparedness, using the term in a broad sense. We must be preparing ourselves for the situation which has arisen and which no one of us completely understands.

This morning we mentioned our need for mental elasticity. I think here again in the matter of financing our work we must adopt the same attitude. It seems probable that this and similar groups will be charged with a great deal of responsibility in determining how we shall direct our efforts, and toward what specific ends. One of my greatest fears has been that there would be a possible or probable lack of coordination in this work. Perhaps the most important function we can perform immediately is to do everything we can to coordinate our viewpoints and efforts, so that when we do get more direct orders we can proceed with the minimum of lost time and with the maximum of efficiency. I am reminded in particular of one warning

that has been given today. Mr. Gray spoke about the danger of rigidity in our thinking and in our methods of work, and Professor Herms emphasized species sanitation. As an illustration of what is happening, at lunch today it was reported that in one locality there was an intense outbreak of Culex pipiens in a defense industrial plant, which seriously interfered with production. The source of breeding was found to be water in tire casings in an adjacent tire re-capping plant. A change in inspectors was probably the reason that this breeding was allowed to occur. We must make every possible effort to protect our defense workers from anything that will lessen their efficiency or interfere with maximum output. Never before has it been so important that there be no waste effort. Our energies and funds must be placed where they are most needed and where they will be of the greatest benefit.

Campbell: I would like to pause a moment to welcome Dr. Barry, the State Health Officer of Idaho. Have you a word for us, Dr. Barry?

Dr. Barry: I have nothing of particular interest to report at this time. We do have some problems of mosquito control and we are trying to meet them wisely and intelligently. We came down here for the purpose of trying to find out what we could do further or do better. I wish to introduce Mr. Luke and also Mr. Ward of our State Health Department.

Peters: I would like to urge that you all come to the morning session tomorrow. This matter of species sanitation is tremendously important and I believe you will find the material on exhibit in the laboratory both interesting and helpful.

Gray: Tomorrow morning Mr. Bendel will bring up the power spray rig that we are using. It will be parked as close to this building as he can get it so you will have a chance to get a good look at it. He will be there to explain about it and answer any questions you may want to ask him regarding his experience with it.

Campbell: May we have the report of the Nominating Committee?

Emerick: The Committee desires to nominate Mr. Nicholson of the Pine Grove District as our new incoming President and Mr. Peters as Secretary-Treasurer.

Campbell: How about a Vice President?

Emerick: We did not nominate one. If you like, we will meet again and do so, reporting to the Conference later.

Campbell: Yes; I think you should do that.

Thereupon it was duly moved, seconded and carried by unanimous vote that Mr. M. D. Nicholson be elected President, and Mr. Richard Peters Secretary, of the California Mosquito Control Association for the year 1942. The session then adjourned, to meet at 7:00 p.m. at the Durant Hotel, Berkeley, for the Annual Banquet.

After the banquet, the members of the Association were addressed by Dr. Alfred C. Reed, Professor of Tropical Medicine in the Medical School of the University of California, who spoke informally on the subject of "Problems Involved in the Control of Mosquito Borne Diseases under Tropical Conditions". The talk dealt principally with the problems of malaria, yellow fever and filariasis in the tropics, and in particular with the problems they present in relation to military operations in the South Pacific and eastern Asia combat areas. The talk was high-lighted by Dr. Reed's delightful humor at appropriate points.

SECOND DAY - TUESDAY DECEMBER 16, 1941

The Conference reconvened at 9:15 a.m. in Agriculture Hall, University of California, with President Campbell presiding.

Campbell: We will now have the pleasure of having Professor Herms give his annual review of the literature on mosquitoes and mosquito abatement.

Herms: On account of the amount of work I have had to carry recently, I have been unable to prepare this review, and have turned it over to Bill Reeves instead.

"REVIEW OF SELECTED LITERATURE
PERTAINING TO MOSQUITOES FOR 1940-1941"

by

William C. Reeves,
Division of Entomology and Parasitology,
University of California

Because of the absence of a formal review of the literature during our 1940 meeting it seems advisable this year to include at least the more important works for 1940 together with those of 1941. In this survey approximately 400 papers were encountered. Obviously it is not feasible to include the entire number so only those papers which appear to be of primary interest have been selected.

As usual the "Insect Pest Survey Bulletin" of the United States Bureau of Entomology and Plant Quarantine gives us an idea of the general importance of mosquitoes during the year, although the bulletin is only indicative of general trends. For the years 1940 and 1941 only 11 states submitted reports. The 1940 reports are of little importance to us at this time. No important records from the Pacific Coast were included so we shall pass on to the 1941 Bulletins. Mosquitoes from Oregon receive the first mention, Knipling reported an unusual February and March with some mosquito breeding. Adult Theobaldia were active on March 2. Aedes increpitus were found on March 11, 24 days earlier than previous records from the Portland area.

On April 20 Aedes dorsalis were reported as active in Utah. During May from Utah came reports that Aedes dorsalis and Aedes campestris were annoying to man and to recently shorn sheep in Sanpete County. In Missouri, pest mosquitoes had been annoying since the middle of May, through central Missouri, in areas close to favorable breeding grounds. In Oregon, Knipling reported the first larvae of Aedes lateralis and Aedes vexans were found at Portland on May 9. The Columbia river had the lowest peak of flood on record, resulting in a very low population of flood water mosquitoes.

In June many complaints were received in Central Missouri about mosquitoes. In Utah, Aedes dorsalis was very annoying to man and livestock in various parts of the state. In South Dakota, mosquitoes were reported much worse than usual throughout the state, being exceedingly troublesome in the northern third.

During July in Arizona Anopheles pseudopunctipennis was common at Benson, biting people out-of-doors early in the morning. This is an unusual record as in California this mosquito rarely bites humans. During this month the Pacific Coast again received notice when Mansonia perturbans was reported from Yakima, Washington. This was a new state record. At the same time, Knipling reported large populations of Mansonia perturbans from Scappoose, Oregon, once again a new state record. This species was by far the most abundant of the six species collected at the time.

In North Dakota mosquitoes reached their high peak of incidence in late June and remained abundant for several weeks. With heavy rainfall in early August, mosquitoes became abundant again, Aedes vexans and Aedes dorsalis predominating. In a stockyard seepage pond near Fargo, larvae were found to be 3000 per square foot of water surface. From Utah in August came the report that Aedes dorsalis and Aedes nigromaculis were abundant, attacking man, cattle, and horses in various parts of the state.

August 6 west of Logan and Benson these two species were particularly abundant. In this area horses had been dying of equine encephalomyelitis and it was believed that the deaths may have been caused by mosquito abundance. From Illinois came a report of severe outbreaks of mosquitoes associated with salt water sloughs near oil wells.

During September, Knipling from Oregon reported 254 Anopheles in one night's catch in a light trap at Portland. Mansonia perturbans was taken in a light trap on Lotus Island (Portland) during August. In Colorado, breeding was more active than usual at Denver, Fort Collins, Greeley, and Boulder. Reports of mosquitoes received from Denver and stockmen in southeast Wild County reported the infestation had continued longer than usual. Preliminary surveys showed that Culex tarsalis, Theobaldia inornata, Aedes dorsalis, A. nigromaculis, and A. vexans seemed to be the chief species. One hundred and sixty four cases of encephalitis in man and 39 deaths had been reported for the state up to early September. In Kansas, Culex apicalis were more abundant than at the same time the previous year in Manhattan. Missouri mosquitoes were extremely annoying throughout the state. In Florida Aedes taeniorhynchus was unusually abundant during the summer but decreased during September. Aedes sollicitans was present in the vicinity of Panama City and was found breeding in large numbers in a smallpond about 50 miles from salt water.

It is of considerable interest that during the summer of 1941 these reports indicated an unusual abundance of mosquitoes in the middle west at the same time as a sizeable epidemic of virus encephalitis.

A publication of very great interest because of the information it contains on mosquito control work is the Proceedings of the New Jersey Mosquito Extermination Association for 1941. This includes 26 papers, all of which apply directly to mosquito control work. The first paper, by T. J. Headlee, is on "New Jersey Mosquito Problems" in which he takes up mosquitoes and national defense, emphasizing the importance of mosquito abatement workers maintaining their high standards and extending their work for the protection of military organizations in their vicinity. Next he reviews the research problems which face the mosquito control worker, including studies of methods of mosquito control, repellents, and developments of trapping devices. Considerable attention was given to the recent addition of dry ice as an attractant, increasing the catches by five fold with maximum catches for one night in a single trap of 18,000 mosquitoes in New Jersey and 250,000 mosquitoes in Delaware. He also refers to larvicide developments, and attacks on the eggs.

In the second paper, by C. Filsinger, on the "Distribution of Aedes vexans eggs", he describes his technique of sod sampling, method of egg recovery, and results, showing that most eggs were present in sods offering good cover of grass and weeds.

H. A. Manzelli of Rutgers University presents a very interesting paper on "Studies of the Effect of Reduction of Surface Tension on mosquito pupae". This includes a study of the pupal transformation to the adult stage, the mechanism of respiration during the pupal stage, and a demonstration of the dependence of the pupae upon a high surface tension to maintain their equilibrium and normal respiration. If the surface tension is reduced by a wetting agent, the pupae can not rest at the surface in an upright position and therefore die. Reduction of the surface tension is also effective against egg rafts, and possibly the larvae. These findings might be applied in the development of insecticides to be used in places where additional dilutions will not occur.

F. C. Bishopp and J. L. Webb present "A Review of Contributions to Knowledge of Mosquitoes During 1940", including general developments in the country, techniques, taxonomy, encephalomyelitis, yellow fever, Anopheles and malaria, habits, biology and physiology, ecology, and control.

H. H. Stage and W. W. Yates report on "Comparative Resistance of Several Species of Mosquitoes to Larvicides" (work done in the Oregon area). They give the comparative toxicity of larvicides to Aedes, Culex, Theobaldia, and Anopheles larvae, the comparative resistance of Aedes and Culex larvae to larvicides, the comparative resistance of instars to larvicides, and the comparative resistance of Aedes, Culex and Theobaldia pupae to larvicides. Aedes vexans larvae were found to be slightly more resistant to oil, pyrethrum oil emulsion, and Paris green than those of Aedes lateralis, Culex pipiens and Culex tarsalis. Larvae showed about the same degree of resistance to oil, pyrethrum-oil emulsion, and phenothiazine, but larvae of Culex pipiens were more resistant to Paris green. Larval instars of Aedes, Culex and Theobaldia appeared to become progressively more resistant to larvicides after each molt. Aedes and Culex pupae were more resistant to pyrethrum-oil emulsion than to Diesel oil. Culex pupae were somewhat more resistant to Diesel oil and far more resistant to pyrethrum-oil emulsion and phenothiazine than Aedes and Theobaldia pupae.

The next paper, by Yates and Stage - "Factors that May Affect the Toxicity of pyrethrum-oil Emulsions as Mosquito Larvicides" - goes still more thoroughly into the

study of inconsistent results. They studied emulsifiers, the addition of organic activators such as sesame oil and amyl acetate, and found that additions of 5% increase the toxicity but probably are not economical. It was found that the temperature of storage and application were very important as toxicity is reduced if applied below 60°F. and accelerated at 60° to 68° F. The effect of organic debris, if heavy, is to necessitate retreatment.

J. M. Ginsburg reviews "The Present Status of Knowledge on Mosquito Oils and Larvicides". The characters which he gives for an efficient mosquito oil are high toxicity to larvae and pupae, quick, uniform spreading on all types of water surfaces, rapid penetration into the tracheal system of larvae and pupae, and stable, long lasting film on the surface. He recommends the New Jersey mosquito larvicide if petroleum oils are objectionable, and emphasizes the importance of considering the variants of temperature and surface tension of waters in use of oils.

Along with these papers are 14 reports on problems of various districts, the history and development of certain areas, economy in plant investment with eyes to the future of paying for mosquito control, and national defense considerations.

A number of other papers have appeared during the past two years dealing with the developments in larvicides. E. H. Hinman of T.V.A. read a paper on "The Place of Larvicides in Mosquito Control" before the 15th annual meeting of the Florida Anti-Mosquito Association, at Jacksonville, Florida, 1941. This appeared in the Georgia Malaria Bulletin, Vol. 4, No. 2. In this paper, he gives the specification for petroleum oils as taken from Ginsburg, discusses the use of pyrethrum, Paris green, borax, calcium arsenite, and copper sulphate, the importance of larvicides in a malaria control program, the importance of municipal control of pest mosquitoes, the use of larvicides in the control of salt marsh mosquitoes, the control of flood-water mosquitoes, and woodland species. He considers larvicides as temporary and a secondary control method. If they must be used, the problem is the selection of the best suited material. In summary he states: "In any well integrated mosquito abatement program the use of larvicides will find a place as an auxiliary or emergency measure. Since the final objective should be permanent elimination of the problem area, reliance upon their use should be continually decreasing. The selection of material and method of application can only be made upon detailed study of the immediate problem. In most situations, economic considerations will determine the extent and duration of this secondary measure of mosquito control".

The paper by A. G. Richards on "Differentiation Between Toxic and Suffocating Effects of Petroleum Oils on Larvae of the House mosquito (*Culex pipiens*)" in the *Trans. Ento. Soc.*, Vol. 67, No. 1087, presents a critique of experimental work, a summary and discussion covering the literature on the suffocation of mosquito larvae, and of cutaneous respiration in insects with particular reference to such respiration in water and in oil. It also includes brief notes concerning histopathological and similar studies with insects.

In the *Jour. of Econ. Ento.*, Vol. 34 for April 1941, Mentzer, Daigh, and Connell report on a subject of considerable interest to us, "Agents for increasing the toxicity of pyrethrum to mosquito larvae and pupae". They experimented with Yarmor Pine Oil, D.H.S. activator, and Thannite. By adding 5% D.H.S. activator, it was possible to use half as much pyrethrum as in standard "New Jersey Mosquito Larvicide" with no loss of toxicity. This decreased the cost and made the most satisfactory spray tested.

In the February, 1941 issue of the *Jour. of Econ. Ento.*, J. S. Kennedy discusses the "Lethal Concentration and Mode of Action of Copper Sulphate used as a mosquito larvicide". In experimenting with young larvae of *Anopheles maculipennis* var. *atroparvus* he showed that fatal concentrations of anhydrous copper sulphate are one part in 50,000 to 100,000 of an otherwise favorable artificial medium at 20°C. The main effect is a direct poisoning of the larvae, but in greater dilution the destruction of the food may play a greater part. The addition of much less copper sulphate is required to kill larvae in natural waters in which copper carbonate is precipitated probably because the larvae take in more copper when it is present in this solid form.

In the *Bull. of Ento. Res.*, Vol. 31 for 1941, a basic paper by G. I. Watson on "A Physiological Study of Mosquito Larvae which were treated with anti-malarial oils" is of interest. In this work he elucidates the reasons why mosquito larvae die when in contact with oils. He shows that if oil gains admittance to only one trachea the larvae may moult and survive; however, if it is in both trachea it interferes with moulting and the larvae die. The microorganisms on which the larvae feed are also killed by oiling. One of the fundamental functions of some larvicides is that the oil/water interfacial tension may be so small in an oil that spreads very widely on water that the tracheal flaps are not pulled open by it.

Several works on the use of Paris green have appeared. LeVan, in the Amer. Jour. Pub. Health, Vol. 30 for 1940 reports on "Measures Instituted for the Control of Aedes aegypti". They used Gambusia holbrooki in covered cisterns. In heavy infestations kerosene was applied every ten days in addition. For flower containers in cemeteries a pellet of a wet mixture of one part Paris green and four parts plaster of Paris was placed in each vase. Houses infested with adults were treated with pyrethrum spray.

Henderson and Howard in the Amer. Jour. of Trop. Med., Vol. 20, 1940 gives a progress report on "A Comparative Evaluation of Paris Green and Pyrethrum Emulsion as Anopheline Larvicides in Georgia". Their work shows that pyrethrum-oil emulsion is a competent Anopheline larvicide, the efficiency of which varies directly with its rate of application. That under the conditions of observation, the material cost of pyrethrum larvicide is from 10-15 times that of the amount of Paris green-line mixture required to produce equivalent mortality. Non-material costs would increase the economic differential in favor of the Paris green. An additional point of importance is that pyrethrum kills or injures invertebrate predators; Paris green does not.

Further developments have taken place in the work of "Disinsectization of Aircraft" as reported by C. L. Williams in the Public Health Reports Vol. 55 for 1940. This article explains the development of a special atomizing hand sprayer for application of pyrethrum sprays in airplanes. It is run on compressed air pressure.

During 1941 three papers on the value of mosquito-proofing for malaria control appear. Hewitt and Kotcher in the Public Health Reports, Vol. 56, report on "Observations on Household Anophelism in a Selected Group of Mosquito-Proofed and Non-Mosquito-Proofed Homes". They found that in general fewer mosquitoes were found in mosquito-proofed homes than in houses not so protected, although strangely enough mosquito-proofing did not seem to prevent mosquitoes from leaving houses.

In the Amer. Jour. of Hyg., Vol. 34, No. 2, R. B. Watson and Helen C. Maher gave "An Evaluation of Mosquito-Proofing for Malaria Control Based on One Year's Observations", their conclusions being that there are indications it is of considerable value. C. C. Kiker and H. E. Breedlove reported in the Amer. Jour. of Hyg., Vol. 34, No. 2, on "Mosquito-Proofing for Malaria Control from the Standpoint of Construction Costs". They found that by contracting for screening and mosquito-proofing the average cost was \$33.00 per house on 350 houses undertaken.

The effect of mosquito control measures on wildlife is a matter of constant interest to this group. Hinman et al; in Science, Vol. 94, for July 11, 1941, give the report of the T.V.A. technical committee on "Additional Cooperative Studies of the Relation Between Mosquito Control and Wildlife Conservation". Detailed study of the various species of plants and their importance in the production of Anopheles quadrimaculatus showed that with the possible exception of water-shield (Brasenia schreberi), which may inhibit larval production, factors other than individual species of vegetation are of greatest importance in determining the extent of Anopheline production in a given area. Structure and growth characteristics of the plants, the way they interact with the combined external factors such as floatage, water-level, surface, etc., are more significant. Experimental studies are described on the control of vegetation objectionable to malaria control and wildlife interests. The application of sodium arsenite from airplanes showed some promise. Utilization of an underwater weed cutter for certain plants gave good results. It was also shown that predation on Anopheles by Gambusia was sufficient to reduce materially the production of adults, although other control measures were necessary to supplement this.

Stearns, MacCreary, and Daigh in Bull. 225 of the University of Delaware Agric. Exp. Sta. for 1940 report on the "Effect of Ditching for Mosquito Control on the Muskrat Population of a Delaware Tidewater Marsh". In this very interesting bulletin they give the importance of the muskrat industry in Delaware, the effect of ditching on mosquito breeding, on the height of the water table, on the character of vegetative cover, and on the distribution of muskrat populations, their conclusion being that effective ditching of a productive muskrat marsh for mosquito control is definitely injurious.

A subject of continued interest to us is that of the value of intermittent irrigation in rice culture. R. B. Hill and F. J. C. Cambournac in the Amer. Jour. Trop. Med., Vol. 21 for 1941, report on "Intermittent Irrigation in Rice Cultivation and Its Effect on Yield, Water Consumption, and Anopheles Production". They found that intermittent irrigation with no water every 16-17 days, requires special preparation of the fields and of the irrigation and drainage systems,---and needs close supervision, but once the fields are set up for it the economic conditions for labor stimulate interest on the part of the growers, and the practice is spreading slowly. It is concluded that it is the only feasible method so far evolved for the control of Anopheles breeding in the rice fields of Portugal.

E. L. Bishop in Science, Vol. 92 for 1940, reports on "Cooperative Investigations of the Relation Between Mosquito Control and Wildlife Conservation". In the T.V.A. project, they studied the effect of Paris green and oils on vegetation and fish, and showed the accumulation of arsenicals in fish had not rendered them unfit for human consumption.

Most of the people at this convention have probably seen the two articles by Harold F. Gray on "Naturalistic Methods In Mosquito Abatement" in the Weekly Bulletin of the California State Department of Health for January 1941, in which he classified the methods into chemical, physical, and biological categories, with a summary of successful applications in various areas of the world under varying conditions.

W. A. Hoffman in Science, Vol. 94, 1941 reports beneficial results in the reduction of irritation of insect bites in "The Effect of Chloroform on Some Insect Bites", including mosquitoes.

In the Jour. of Econ. Ent., Vol. 33 for June 1940, P. Granett in "Studies of Mosquito Repellents" gives the test procedure and method of evaluating test data, and the relative performance of certain chemicals and commercially available mixtures as mosquito repellents. This type of information is becoming important for the protection of army troops in the field.

During the years 1940 and 1941 the addition to our knowledge of mosquitoes of the Pacific Coast area has been considerable. As previously noted, Knipling and Gjullin recorded Mansonia perturbans from the Pacific northwest. This mosquito is also present in California as was reported by William C. Reeves, "The Mosquito Genus Mansonia Blanchard in California", Vol. 17 of the Pan Pacific Ent. for January, 1941. It is recorded from Bakersfield, Holt, Galt and Landers. These collections were made in 1917 and 1919 and the specimens were with material which heretofore had not been determined. It has since been collected in Fresno, a record not included in the paper.

Another valuable contribution to our knowledge of California mosquitoes is that by Thomas H. G. Aitken "Notes on Aedes nigromaculis, (Ludlow), Aedes increpitus Dyar, and Culex territans Walker in California", in the Proc. Ent. Soc. Wash. Vol. 42, No. 7, 1940. In this paper 12 records are given for Aedes nigromaculis showing its distribution in nine counties of the state. It would appear that this mosquito is becoming common in the central valleys of California where it may be very pestiferous; in addition, it is an efficient vector of equine encephalomyelitis

in the laboratory. Aedes increpitus is reported as breeding in salt marshes in Los Angeles County, which is the first record of this type of breeding in California. In the Oregon salt marshes breeding by this species is fairly common. Three records of collections of Culex territans are given which are the only records in California subsequent to Dyar's unique capture in 1922.

Another paper by Aitken is "The Genus Psorophora in California" in the Revista de Entomologia Vol. 11, December 1940. This is the first detailed report of this genus in California. Psorophora confinnis is reported from the Coachella and Imperial Valleys of southeastern California and from southern Arizona. The larvae are found in irrigation overflow pools (as early as May). The adults appear during the months of June to September, and may be of considerable annoyance to local residents. Of systematic importance is the conclusion of the author that Psorophora columbiae, Psorophora tolteca, and Psorophora jamaicensis are only synonyms of this species.

A third genus is first reported for California by Reeves, in "The Genus Orthopodomyia Theobald in California", in the April, 1941 issue of the Pan-Pacific Entomologist. The species Orthopodomyia signifera was collected at Riverside, Riverside County, and Redlands, San Bernardino County. This species breeds only in tree holes. The author points out similarities between the California specimens and the eastern species Orthopodomyia alba, although there is no doubt the California specimens are Orthopodomyia signifera.

Aitken reports on "A New American Subgenus and Species of Aedes" in the April, 1941 issue of the Pan-Pacific Entomologist. This new subgenus and species Aedes (Kompiia) purpureipes Aitken was collected in lower California and has also been taken in southern Arizona.

A significant piece of work from the Pacific Coast and one which has already been discussed in the virus symposium appeared in Science for October 3, 1941, "Isolation of the Viruses of Western Equine and St. Louis encephalitis from Culex tarsalis Mosquitoes" by Hammon, Reeves, Brookman, Izumi, and Gjullin.

The final contributions to our knowledge of western mosquitoes are two works by Aitken. "The Anopheles Complex in California" from the Proceedings of the 6th Pacific Science Congress, Vol. 4, 1941 and his Ph.D. thesis on "A Clarification of the Anopheline Complex of Western America". In these works he recognizes Anopheles maculipennis occidentalis, Anopheles maculipennis freeborni, Anopheles pseudopunctipennis franciscanus, Anopheles pseudopunctipennis boydi, and Anopheles punctipennis, all from western North

America. In disease transmission, Anopheles maculipennis freeborni is the most important if not the only vector of malaria in the west. He considers Anopheles maculipennis aztecus as a Mexican species. Anopheles punctipennis is a stable species and a possible malaria vector, although of little importance. Anopheles pseudopunctipennis he considers as an unstable group, found in North and South America. This species is an efficient malaria vector in areas other than the United States. He believes that Anopheles punctipennis franciscanus is a northern race, the common form in California, and in addition Anopheles pseudopunctipennis proper also extends into California and probably interbreeds. This work has been a very valuable contribution to our knowledge of this important group, and has been favorably received by other workers in this field throughout the United States.

Now that the matter of races, varieties, and subspecies of Anopheles maculipennis has been precipitated, a paper of M. Bates in the *Ann. Ent. Soc. Amer.*, Vol. 33, No. 2 for 1940 on "The Nomenclature and Taxonomic Status of the Mosquitoes of the Anopheles maculipennis Complex" is of interest. He treats the subject from a worldwide viewpoint, although regretably the California complex is not included. In this work he proposes to raise the subspecies of this complex to species standing.

H. S. Hurlbut in the *Amer. Jour. of Hyg.*, Vol. 54, No. 1 for 1941, "First Instar Characters for Distinguishing the Common Inland Species of Anophelines of Eastern United States", makes possible a separation in the early stages without awaiting the complete larval development. This is a type of work of considerable value to field collecting.

W. K. Lawlor in *Public Health Reports*, Vol. 55, No. 9, presented "Notes on a variation in the eggs of Anopheles punctipennis Say". He considers the variation to be a possible seasonal variation.

Several interesting articles appear in the *Jour. of Econ. Ent.* for 1940 by Fisk and LeVan on mosquito collections by New Jersey light traps in different areas of southern United States. These are "Mosquito Collections at Charleston, South Carolina, Using the New Jersey Light Trap", and "Mosquito Collections at Brownsville, Texas". In these collections many new records were obtained for the areas investigated, adding to our knowledge of mosquito distribution.

The paper describing the work of Gjullin, Hegarty and Ballen at Portland, Oregon on "The Necessity of a Low Oxygen Concentration for the Hatching of Aedes Mosquito Eggs" appeared in the Jour. of Cellular and Comparative Physiology, Vol. 17, No. 2 for 1941. As most of the members of this conference will remember, Mr. Gjullin reported on this work at the meeting here last year. This paper is basic and of great importance.

Another work along similar lines appeared in the Jour. of Econ. Ent. in April, 1941 by W. A. Connell, "Hatching Response of Aedes sollicitans Eggs Under Selected and Controlled Environmental Conditions". Eggs of Aedes sollicitans were tested with infusions prepared from various plants, with water from various creeks, marshes, and with distilled water. Records were kept on which of these resulted in hatching of the eggs.

Another article in the April, 1941 issue of the Jour. of Econ. Ent. was by T. J. Headlee on "Further Studies of the Relative Effects on Insect Metabolism of Temperatures Derived from Constant and Variable Sources". This work was carried out on Aedes aegypti. It confirmed the general conclusions set forth in their preceding paper that the relative effect on insect metabolism of temperatures drawn from variable and from constant sources is dependent upon where in the gamut of the insects' normal temperature reaction these constant and variable temperatures lie. Also this work indicates that the underlying and governing factor of such differences as exist in the variable and constant temperatures is the accumulation of the required amount of heat regardless of constant and variable sources.

Some very good papers on the laboratory rearing of mosquitoes, and on mosquito larval nutrition, are of basic importance to mosquito work. In the Jour. of Econ. Ent. for April 1941, A. R. Buddington writes on "The Nutrition of Mosquito Larvae". He shows that autoclaved and Berkefeld-W filtered pond water supports the growth of the larvae of Aedes aegypti and Culex pipiens only as far as the 4th instar. Escherichia coli fails to support the growth of larvae to maturity, but Bacillus subtilis and Saccharomyces cerevisiae support the growth of larvae to maturity. Mosquito larvae develop under sterile conditions to maturity on alcohol-sterilized yeast, but cannot develop beyond the 4th instar on autoclaved yeast. The heat-stable factor of yeast is also present in autoclaved liver concentrate. Nicotinic acid and ascorbic acid do not replace the heat-stable factor in yeast. Mosquito larvae fail to develop beyond the 4th instar on a solute diet containing amino acids, vitamins, glucose and Osborne-Mendel salts. Mosquito larvae require for development to maturity under aseptic conditions at least 3 factors;

thiamin hydrochloride (B₁) riboflavin (B₂), and a heat stable factor in yeast and liver¹ extract.

M. Bates in the *Amer. Jour. Trop. Med.*, Vol. 21, No. 1 for 1941, "Studies in the Technique of Raising Anophele Larvae", attempts to find a combination of mineral salts and food that would serve as a satisfactory basis for rearing larvae under standard conditions. The results were not entirely satisfactory, but were a definite advance in technique.

R. L. Crowell in the *Amer. Jour. of Hygiene*, Vol. 32, No. 1 for 1940, "Insectary Rearing of Anopheles quadrimaculatus", gives a description of the air-conditioned insectary at Wilson Dam, Alabama, in which a self-perpetuating colony of Anopheles quadrimaculatus has been maintained since November, 1937. Pulverized dog biscuit is used as larval food. The results of the management technique and management of the colony of adult mosquitoes are described.

Still another article in the April, 1941 issue of the *Jour. of Econ. Ent.* is by D. MacCreary on "Comparative Density of Mosquitoes at Ground Level and at an Elevation of Approximately 100 feet". In this interesting study, New Jersey traps were operated in pairs one 4-5 feet above the ground and the other 80 feet high or higher at two locations in Delaware. One area was fresh water, the other salt marsh. In the fresh water area the lower trap took 2,556 females of 15 species in 54 nights. The upper trap took 87 females of 10 species during the same interval. On the salt marsh area the upper trap took 1,334 females of 11 species, and the lower trap 14,325 females of 15 species. On September 10th at Femoids (salt marsh) an unusually large flight took place and the upper trap caught 13,555 females and the lower trap the astounding figure of 250,000 females, which is really a catch.

Still another unusual report is that of A. D. Richards in *Ent. News* for October 1941, on "A Stenogamic Autogenous Strain of Culex pipiens in North America". This laboratory adapted strain of Culex pipiens is stenogamic, that is it mates readily in confined quarters, and it is autogenous, indicating that the adults do not require a blood meal to lay viable eggs. The adults will take a blood meal if it is available, but it is not necessary for their continued production of eggs.

In the *Lancet* Vol. 240 for 1941 a report of interest in National Defense appears by P. G. Shute on "A Species of Mosquito Infesting Deep Shelters in London".

Culex pipiens molestus has been found breeding in considerable numbers during the winter months in deep subway tubes where water has gathered. Breeding is continuous even when outdoor temperatures are freezing. During peace time this was not noticed; during war time when subway tubes were used as shelters it became a problem. Mr. Gray has been corresponding with Mr. Shute on this problem.

L. E. Rozeboom in the 1941 Proc. Okla. Acad. Sci. reports on "The Overwintering of Aedes aegypti in Stillwater, Oklahoma". The mosquito apparently successfully passes through the winter, and although an unusually severe winter might destroy all the eggs, the fact that it breeds in and around houses and barns probably would ensure its permanent existence. This fact is of interest to workers in California. Although this mosquito has apparently never established itself on this coast, if it did its elimination would probably be most difficult as in some areas favorable conditions for its continuous existence exist.

Vargas and Freire in 1940 report on a novel method of staining mosquitoes for studies of flight range, "New Methods of Measuring the Range of Anopheles". The addition of methylene blue to water in which mosquito larvae were submerged, of 0.5 or 0.25 parts per 1,000 for 18 to 48 hours, colored the thorax and abdomen of the adults emerging from it sufficiently that the blue color could be detected.

G. E. Smith, R. B. Watson, and R. L. Crowell in the Amer. Jour. of Hygiene, Vol. 34, No. 2, 1941, "Observations on the Flight Range of Anopheles quadrimaculatus Say", state that in their study of flight distance the greatest distance found was 2,700 feet from point of hibernation. This is a matter of possible importance in anti-malarial work.

To review the work on mosquito-borne disease for the past two years is an obvious impossibility in the limited time available. The entire time could be devoted to the work of the Rockefeller Foundation alone, or to a discussion of the Symposium on Human Malaria held at the national meetings of the American Association for the Advancement of Science in 1940 and published in book form. A few articles of particular interest have been selected, and these along with a brief review of the book "Human Malaria" will have to suffice.

A paper by W. A. Davis on "A Study of Birds and Mosquitoes as Hosts for the Virus of Eastern Equine Encephalomyelitis", Amer. Jour. of Hyg. Vol. 32, No. 2, was referred to at the meetings here last December, and this has been discussed at an earlier time this year in the symposium on encephalitis.

J. D. Fulton, J. E. Gruetter, R. O. Muether, E. B. Hauss, and G. O. Brown in the Proc. Soc. Exp. Biol., No. 1, "Observations Concerning Culex pipiens as a Possible Carrier of St. Louis Encephalitis", show that the virus does not survive for more than 10 days in the mosquito.

V. H. Cornell and W. A. Davis in the Proc. Soc. Exp. Biol., Vol. 42, No. 1, "Mosquito Transmission Experiment with Poliomyelitis Virus", were unsuccessful with Aedes aegypti and Culex pipiens, although they did not believe that the experiment was conclusive.

Some pertinent figures on "Losses from Malaria in Mississippi County, Arkansas" are presented by W. B. Grayson and R. E. Schirmer in the Georgia Malaria Bulletin, September, 1941. They showed a cost of \$54,054.90 for anti-malaria drugs and physicians' fees for treatment of malaria in the nine months period from January 1st to October 1st, and considered this figure as far from complete.

A matter of very great importance in the making of malaria surveys was brought out by H. S. Hurlbut and R. Hewitt, 1941 in "Sporozoites of Plasmodium lophurae, an avian malaria parasite, in Anopheles quadrimaculatus". Quoting them: "Considerable importance is sometimes attached to oocyst and sporozoite indices in Anopheles quadrimaculatus associated with endemic human malaria. It appears therefore that some method should be sought to distinguish between the exogenous stages of human and avian malaria in this species, since the findings reported here tend to invalidate the assumption that all oocysts and sporozoites which may be found are those of human malaria". This statement in itself is sufficient.

"A Brief Review of Needed Research in Malaria" in Public Health Reports, Vol. 55, No. 40 for 1940, contains the report of a committee of twenty outstanding workers in this field who met and outlined in a complete fashion the essential research in the field of malaria with the hope of discovering opportunities for coordinated effort in a program toward broader horizons of thought and research service. It showed the very apparent and compelling need for drawing together the natural and medical sciences in joint efforts to acquire basic knowledge upon which to develop more effective control. Those persons interested in malaria suppression may

be stimulated to an awareness of the disease and to constructive efforts directed to closure of these gaps, and those undertaking studies in malaria may give greater consideration to the completeness of particular programs of study rather than to diversity of studies within particular groups. Direction of concerted action along this line must surely lead to results of a gratifying nature.

Another approach towards drawing together and unifying the work on malaria is "Report on Terminology in Malaria", a Bulletin of the Health Organization of the League of Nations, Vol. 9, No. 2 for 1940. In this work terms are standardized in a glossary and explained. It is divided into three sections. The first deals with malaria parasites and infections to which they give rise. The second deals with malaria in the human community. The third deals with the insect vectors. It is stated that subspecies should be called varieties, also races should be called varieties.

A very timely paper by Dr. A. C. Reed, in California and Western Medicine, Vol. 53, No. 1 for 1940, is "Malaria: A Clinical Summary". He gives the history of malaria in California with the comment that in California "all types of malaria are still present, that the general distribution is approximately the same as a century ago, and that this endemic area lies chiefly in and contiguous with the central valleys of the San Joaquin and Sacramento Rivers." He goes on further to say "It is evident that the problem of malaria in California has been solved only in part, and that its eradication will require continuance of an intensive campaign. Decreased money and effort can only result in return to an incidence proportional to the ratio of the present population to that of a century ago". The article further points out that there is a definite increase of imported cases into California. The continuation of studies on immunity such as those of Taliaferro, Coggeshall and others offers today the most hopeful field for advance in the control of malaria. In spite of all the work on chemotherapy, prevention of mosquito bites is still of primary importance in prevention of malaria. This is facilitated by deterrent application, proper clothing, and proper use of bed nets.

M. F. Boyd published four papers in the Amer. Jour. of Trop. Med. during 1940. The first was on "Strains or Races of the Malaria Parasite". The second, "The influence of Sporozoite Dosage in Vivax Malaria", showed that the dosage of sporozoites exerts a significant effect on the subsequent infection; the heavier the dose the more severe the attack and the shorter the incubation period. These were followed by two basic papers on artificially induced

malaria.

In 1941, two further articles by this worker appeared in the same journal, the first on "Observations on the Blood Proteins During Malaria Infection", and the second "An Historical Sketch of the Prevalence of Malaria in North America".

J. Maier and L. T. Coggeshall in the Jour. of Inf. Diseases, Vol. 69 for 1941, on "Respiration of Malaria Plasmodia", continued their attack on the problem of malaria from the ground floor.

Coggeshall's report in the Proc. Indiana Acad. of Science, Vol. 50 for 1941, on "The Complement Fixation Reaction", explains a complement fixation test which has been devised as an aid to the diagnosis of chronic malarial infections. The antigen is prepared from red cells infected with P. knowlesi, a monkey malaria parasite which will fix complement in human malaria serum. The test is specific for malaria. In induced therapeutic malaria infections the test becomes positive about the second week of infection, and persists for approximately 5 months after parasites are no longer detectable in blood smears. The final evaluation will have to come from field studies in endemic areas, although the experimental studies suggest the test may have considerable merit.

In 1940 several papers appeared which were concerned with the possible winter carry-over of malaria infections in hibernating mosquitoes. Hinman and Hurlbut in the Amer. Jour. of Trop. Med., Vol. 20, No. 3, "A Study of Winter Activities and Hibernation of Anopheles quadrimaculatus in the Tennessee Valley", reported that they were working towards a study of the ability of malaria parasites to overwinter in hibernating females. Oocysts were discovered on the stomach of one specimen found in a cave on December 1st.

W. K. Stratman-Thomas in the same volume, No. 5, "The Influence of Temperature on Plasmodium vivax", stated that in certain localities the oocyst may survive the winter and complete its development in the following spring. He also reports that a temperature of 37.5C (99.5F) for 24 hours destroys all parasites in Anopheles quadrimaculatus.

Some papers on the development of chemotherapeutic agents for the treatment of malaria appeared in 1940 and 1941. Coggeshall, 1940, "The Selective Action of Sulfanilimide on the Parasites of Experimental Malaria in Monkeys in Vivo and in Vitro", in the Jour. of Exp. Med.,

Vol. 71, No. 1, showed that this drug was highly selective in its action against two species of plasmodia in the experimental animal. No therapeutic agent should be discarded on the basis of a negative result against one plasmodium, nor can such an agent be proclaimed as a universal remedy, regardless of its effectiveness against one type of parasite. Sulfanilimide apparently inhibits the respiration of P. knowlesi, a parasite with a high oxygen consumption, but not P. inui with low oxygen consumption.

Hegner, West, Ray and Dobles, 1941, "A New Drug Effective Against Bird Malaria", in the Amer. Jour. of Hyg., Vol. 33, No. 3, believed that since malaria parasites in birds have been found to react to drugs similarly to those in man it seems probable that hydroxyethylapocupreine dihydrochloride will be as effective against human malaria as it is against bird malaria. Its low toxicity is a distinct advantage, especially since it can be administered in larger doses. The effect of this drug on relapses, on sporozoites, and in the elimination of all parasites from the vertebrate host, remain to be determined. It is as effective as quinine hydrochloride and has a lower toxicity. It appeared to affect merozoites and gametocytes.

A report by W. N. Bispham on "Toxic Reactions Following the Use of Atabrine in Malaria" in the Amer. Jour. of Trop. Med., in 1941, stated "As the U. S. Army contemplated using this drug extensively, a study of reports of reactions was made and a detailed report covering all cases in which atabrine was administered was required". The reports were very favorable and the increasing use of the drug confirmed them. 49,681 cases were studied; 38 cases of severe toxic reaction were included. The relationship to blackwater fever is unknown. An increase in rapidity of removal of this drug should be of value in decreasing its toxicity.

In closing this discussion of malaria I would like to briefly review two symposia, one reported in 1940 and the other in 1941. The first was a series of papers presented before the National Malaria Committee meeting with the Southern Medical Association on November 21st to 24th in 1939 and reprinted in the Southern Med. Jour., Vol. 33, No. 8, for August 1940. Seven papers were published at this time. It will be possible only to list these papers and the authors, which will show the scope of the symposium: H. C. Clark, "Review of Recent Research on Drug Prophylaxis and Treatment of Malaria"; J. Andrews, E. C. Faust and R. B. Watson, "Recent Advances in the Epidemiology of Malaria"; S. F. Kitchen, C. G. Hugg, M. A. Roe, "A Review of Recent Experimental Studies in Malaria";

G. H. Bradley, R. E. Bellamy, and T. T. Bracken, "The Work of State Board of Health Entomologists in Malaria Control; Report of the Subcommittee on Entomology"; L. L. Williams, "Malaria Control Activities; Report of the Subcommittee on Malaria Prevention Activities 1939"; E. C. Faust and V. Parker, "Malaria Mortality in the Southern United States for the Year 1938"; and L. M. Clarkson, "Engineering Phases of Malaria Control".

The second symposium and by far the larger and more comprehensive is that previously mentioned "A Symposium on Human Malaria With Special Reference to North America and the Caribbean Region". This is the eighth symposium in a field of eight which have been held on public health and published by the American Association for the Advancement of Science. The symposium was organized by a joint committee from the Section of Medical Sciences of the Association, the American Society of Parasitologists, the American Society of Tropical Medicine, and the National Malaria Committee. The volume as it appears presents a systematic, comprehensive, authoritative and thoroughly documented discussion of the problems of human malaria in North America and the Caribbean region. This volume is especially timely because of the great importance of malaria to National Defense in the areas concerned, both the Army and Navy along with civilian persons being involved with the struggle against this disease. Forty two contributors presented forty four papers. A brief review of the scope of the different sections of the publication will be all there is time for.

M. F. Boyd opened the discussion with an "Historical Introduction to the Symposium of Malaria". This was followed by the second section, on "Parasitology", with six papers covering the distribution of malaria in North America, Mexico, Central America, and the West Indies; the taxonomy of the human malaria parasites with notes on the principal American strains; the morphology, life cycle, and physiology of Plasmodium malariae, Plasmodium vivax, and Plasmodium falciparum; and the detection and differential diagnosis of malarial parasites in the schizogonous and sporogonous cycles.

The third section was on "Anopheline Vectors of Malaria", contained seven papers covering the general morphology of Anopheles and the classification of the Nearctic species; distribution of the Nearctic Anopheles; bionomics and ecology of Nearctic Anopheles; the classification and identification of the Anopheles mosquitoes of Mexico, Central America, and the West Indies; distribution and ecology of the Anopheles mosquitoes of the Caribbean region; factors influencing infection of Anopheles with malarial

parasites; and the transmission of malaria by the Anopheles mosquitoes of North America.

The fourth section on "Epidemiology" is composed of four papers on cyclical variation in the incidence of malaria; topographical and related factors in the epidemiology of malaria in North America, Central America, and the West Indies; malaria and the community; and the role of Anophelines in the epidemiology of malaria.

The fifth section on "Symptomatology" contains five papers covering the infection in the intermediate host; symptomatology, general considerations; the infection in the intermediate host; the symptomatology caused by vivax, quartan, and falciparum malaria; and the infection in the intermediate host as caused by blackwater fever.

The sixth section is on "Pathology and Immunity" and is composed of five papers on some pathologic aspects of human malaria; the physiological pathology of malaria; immunity to human malaria, characteristics of immunity; the cellular basis for immunity in malaria; and humoral immunity in malaria.

The seventh section on "Treatment" contains four papers covering cinchona and its alkaloids in the treatment of malaria; antimalarials other than quinine; experimental chemotherapy in malaria; and a summary of ten years' observations on malaria in Panama with reference to control with quinine, atabrine, and plasmochin, without anti-mosquito measures.

The last and largest section of the book, Section eight, is devoted to "Control and Eradication". This includes twelve papers covering the planning of malaria control; methods and procedures of making a malaria survey; methods directed against adult mosquitoes in the control and eradication of malaria; housing with special reference to mosquito-proofing for malaria control; drainage and filling methods for mosquito control; the management of water for malaria control; petroleum products for mosquito control; Paris green and other stomach poisons as larvicides against mosquito larvae; naturalistic methods of malaria control; adaptability of control measures to the Holarctic fauna of Anopheles mosquitoes; the adaptability of control measures to the malaria vectors of the Caribbean region; and the anti-malaria program in North America.

A brief summary of the work in the various areas of the world leads to an appreciation of the scope of mosquito work in South America, Central America, India, China, Africa, or Russia. A detailed summary of each of the papers

from these various areas would necessitate a great increase in the length of this paper and is therefore impossible. From South and Central America we have received more contributions than any other area outside of North America.

An example of the interests in this area are the lists and keys of mosquitoes from various areas, Colombia, Mexico, Costa Rica, Venezuela, British Honduras, and areas of Cuba. Malaria surveys with recovery of Anopheles females of several species naturally infected with Plasmodia are reported from various regions. Anopheles bellator was found naturally infected in Trinidad. Anopheles albimanus, Anopheles vestitipennis and Anopheles darlingi in British Honduras, the very important Anopheles gambiae in Brazil, and still we have only mentioned a few of the more important. Descriptions of new species of mosquitoes come from many regions. Studies of distribution, and eggs of Anopheles mosquitoes are being carried out with consequent studies of races or subspecies, also studies of ecology, with special notes as to the possible importance in the control of malaria. Works are published clarifying the confused nomenclature and systematic positions of the various Anopheles thus establishing the true relationships of the various species. The development of specific anti-malarial legislation in South American countries is witnessed by the report of "The Venezuelan anti-malarial legislation and reclamation project". Detailed reports on the spread of Anopheles gambiae in Brazil and important developments in the fight against this worst of malaria vectors have been prepared. In mentioning the work to the south it is certainly necessary to include the reports of the Gorgas Memorial Laboratory in Panama and the various progress reports of the several workers from this laboratory. Anti-malarial and anti-yellow fever work in the Latin American countries at the present time is based on a scientific foundation, and the one agency responsible to a large degree for this is the Rockefeller Foundation. The Annual Report of the International Health Division of the Rockefeller Foundation, reviewing their work for the past year, is a wonderful source of information on the advances and type of work being carried on in this area.

From India come several journals devoted entirely or largely to the subject of malaria; The Journal of the Malaria Institute of India, The Indian Medical Gazette, Health Bulletins of the Indian Malaria Bureau, and Reports of the All-India Institute of Hygiene. During the past two years detailed accounts have appeared of studies on the important malaria vectors; control measures directed against the various species; ecological studies; studies of the rate of natural infection of the various species; and of special interest to this group would be the development

of control measures such as anti-larval flushing of rivers and streams, the establishment and rigid enforcement of half-mile oiling zones surrounding rubber estates, the use of native species of fish against larvae, the development and use of automatic siphons, the development and choice of mechanical sprayers for mosquitocide spraying directed against adults, the packing of fascines into drains and tributaries of ravines to prevent breeding of Anopheles, the use of water instead of dust for diluting Paris green in malaria control, using a portable spray gun to apply it, the natural control of Culicine mosquitoes by prawns in coal mines, and development of various other means of naturalistic control.

From Indo-China and China, the latter in particular, the amount of published material is great. A very pertinent paper on "The Choice of Emplacements of Camps and Their Unavailability for Malaria with Military Forces in the Field" by P. Menu and C. Taumanoff is based on studies in French Indo-China. Their study showed the importance in choosing camping places, of making topographical and entomological investigations first of all. This combined with medical prophylaxis preserved the sanitary state of troops in a highly endemic area. Great importance was laid on a knowledge of the particular types of water the malaria vectors breed in. Knowledge of this type is quite naturally of great interest to us at this time.

The great importance of the Burma Highway in China and the necessity to keep it open has resulted in detailed entomological studies of the area by workers of the U. S. Public Health Service, native workers, and Rockefeller men. Three reports have appeared; L. L. Williams in the Amer. Jour. of Trop. Med., Vol. 21, No. 1 for 1941; "Malaria on the China-Burma Highway"; R. C. Robertson in the Trans. Roy. Soc. Trop. Med. and Hyg., Vol. 34, No. 4, "A Malaria Survey on the China-Burma Highway"; and Robertson and T. L. Chang in the China Med. Jour., Vol. 58, No. 4, 1940, "Malaria Survey in Western Unnan Lungling Area and Lushih County". This work is being carried on still further by a group of 15 U.S.P.H.S. men now in the same area combating malaria as a railroad is built parallel to the highway.

Other works from China cover mosquitoes as carriers of Wuchereria bancrofti, the geographical distribution of mosquitoes in China, eggs of Anopheles, studies of ecology, and the importance of various species as vectors of malaria. Anopheles minimus appears to be the most important vector in the southern area.

From Russia come detailed works on Anopheles maculipennis and its importance as a vector of malaria, studies of the influence of temperature on the larvae, studies of road dusts and their desirable characters for carriers in Paris green dusting from aeroplanes, and attacks on adults in houses, along with considerable work on races, and the methods of controlling larvae.

From the African continent come reports of the difficulties encountered by the Italians in the colonization of Ethiopia. Great outbreaks of malaria accompanied the influx of soldiers and workmen, with many problems of control and prevention. Also part three of "Mosquitoes of the Ethiopian Region (Culicine adults and pupae)" by F. W. Edwards appeared in 1941.

From East Africa come detailed reports on malaria including review of history, the present position, cost of malaria, techniques used in making mosquito surveys, legal powers for eradicating mosquitoes, the use and results of many hundreds of tests on oils available in this area, and the use of native fish considered superior to Gambusia because of the fact that it is capable of resisting drought by depositing resistant eggs. These papers appeared in the East African Medical Journal, Vol. 17, No. 10 for 1941. Another paper in this volume was on "Yellow Fever and East Africa". During October, 1940 in southern Sudan over 15,000 cases occurred with more than 1,600 deaths for an area of 6,000 square miles. They are not certain that the epidemic depended entirely on Aedes aegypti as it was believed that jungle yellow fever may have been involved.

Few papers have come out of Europe during the past year, as would be expected. "Field Studies of the Anopheline Mosquitoes of Albania" by M. Bates appeared in the Proc. Ent. Soc. of Washington. R. C. Shannon and J. Hadjinicalao reported in the Jour. of Econ. Ent. on "Egg Production of Greek Anophelines in Nature".

The Annual Report of the Rockefeller Foundation for 1940 contains much information on most of these areas. This report is easily attainable and therefore will not be reviewed.

In finishing this review, there are a number of informative publications from various agencies of mosquito control which should be mentioned. These mostly include histories of the agency concerned, mosquito life history and habits, methods of mosquito abatement, and related information. Particularly attractive in the manner they are made up and presented are "Keeping Alameda County Free of

Mosquitoes" from the Alameda County Mosquito Abatement District, Oakland, California, and "An Informative Bulletin on Mosquito Abatement in Salt Lake City". The Dominion of Canada Department of Agriculture in Publ. 719, Circ. 172, "Mosquito Control in Canada" gives a four page account which is very general. Sinton and Shute in the Medical Memorandums of the Ministry of Health, No. 238, "Memorandum on Measures for the Control of Mosquito Nuisances in Great Britain" give a short review. A revised edition of "The Mosquitoes of Arkansas" by S. J. Carpenter appeared.

I regret that it is impossible to include all the papers which appeared during the two year period. The more important ones, as far as this particular group is concerned, have been included.

Stewart: Mr. Reeves calls attention to the finding of Mansonia perturbans in California. Mansonia perturbans is without doubt one of the most vicious biters in the eastern states. Their bites are rather venomous, and they are persistent, vicious biters. The control of this mosquito is a difficult problem, as they do not come to the water surface to breathe. The larvae insert their breathing tube into the hollow stems of certain aquatic plants and obtain their oxygen from these plants. Therefore oiling the water surface is ineffective in controlling Mansonia perturbans. You must resort either to biological control by extermination of the oxygen-bearing plants, which is practical under some conditions, or you must use toxic materials which will poison the water or sink to the bottom.

Herms: Is there anything particularly different in the breeding habits of Aedes dorsalis and Aedes nigromaculis?

Reeves: Aedes nigromaculis seems to be taking over where Aedes dorsalis was formerly prevalent. Aedes dorsalis appears early in the spring and Aedes nigromaculis come later.

Peters: On well flooded fields we have taken Aedes nigromaculis about the month of April or May when both Aedes dorsalis and Aedes nigromaculis were abundant. At the Fresno sewer farm probably Aedes nigromaculis is the most vicious biter. We found them there in droves, and they were terrible in Fresno. They are primarily a day-biting mosquito.

Herms: They bite principally early in the morning and toward evening.

Peters: Some of us were dressed in light clothes and some in dark and we found the light clothes attracted the Aedes

nigromaculis.

Reeves: That is generally true of any Aedes mosquito, Aedes increpitus, for instance.

Herms: Heretofore Aedes increpitus has been looked upon as an alpine or snow mosquito, but it is also found in ocean shore marshes.

Reeves: They are found in the Santa Cruz Mountains also, where it breeds in the stream beds. In the Pacific Northwest it breeds out in the pastures in overflow pools or irrigation ditches.

Herms: I am sorry we must curtail this discussion, but we have an exhibit set up in Room 204 for you to study. Bill Reeves got up at six this morning to set up this exhibit, and he has also done a very fine job in preparing the key for "The Identification of California Mosquitoes" which is now being distributed to you. Using this key, you can follow through the working exhibit and follow out a complete and systematic scheme of identification of our California mosquitoes.

In past years Tommy Aitken has done most of the work on these exhibits. We miss Tommy, but we are glad that he is now doing a job of his own for the U. S. Army, being at present stationed in Puerto Rico. But we have found a capable successor in Bill Reeves, and the present exhibit is one of the best we have ever had. We will now adjourn to Room 204 in this building.

Reeves: This Key I have intended to be merely an aid to a rapid field identification of our mosquitoes. It is not a systematic identification. It is impossible sometimes to make a complete identification out in the field, but it can be of great importance to be able to make a tentative diagnosis of the species. There are a few important identifying characteristics, and in the laboratory the exhibits of these characteristics are numbered the same as in the key. If you start with specimen No. 1 and follow through with the keys, you will have a chance to really study each specimen, and follow the scheme of identification

NOTE: "The Identification of California Mosquitoes" by William C. Reeves was given only to those persons attending the Conference, and is not reproduced in these Proceedings.

The group then spent the remainder of the morning in the laboratory working on identification characters of mosquitoes.

At 1:30 p.m., the Conference re-convened in Agriculture Hall.

Campbell: We will now have a paper on "Changes in Legislation Affecting Mosquito Control Operations" by Harold F. Gray.

Gray: Two years ago the Association organized a Legislative Committee, which at our meeting last year reported on desirable changes in the laws under which we operate. The committee members had bills introduced into the 1941 State Legislature, and by working cooperatively according to a general plan agreed to, we were able to get the several amendments passed without appreciable difficulty, and they were signed by the Governor and went into effect September 13, 1941. The original amendments were designed to enable districts to operate outside of their boundaries where necessary, to make the power to inspect specific instead of implied, to prevent interference with or damage to our work, and in the case of pest abatement districts, to provide an optional basis of assessment.

Without consultation with us the Marin District, which seems to prefer to play a lone hand in all matters relating to mosquito control, had introduced a separate amendment relating to the method of defraying expenses of Trustees, and the method of withdrawal of funds. As there was no objection, their bill was tacked onto our bill in the Senate.

I wish to thank all the members who worked on this legislation, primarily by contacting their own Assemblymen and Senators. While all pitched in and helped, Chester Robinson and Fred Hayes were particularly helpful.

CHANGES IN LEGISLATION AFFECTING
MOSQUITO CONTROL OPERATIONS

by

Harold F. Gray, Engineer,
Alameda County Mosquito Abatement District

The 1941 Legislature of California passed certain acts which amend various sections of the Health and Safety Code relating to mosquito abatement districts and to pest abatement districts. In addition, at least two general

statutes were passed which have relation to the work of such districts.

MOSQUITO ABATEMENT DISTRICTS

The act which amends the mosquito abatement district law is Chapter 314, Statutes of 1941. The original sections of the Health and Safety Code affected by this act are shown in the left hand column below, and opposite in the right hand column are these sections as now amended. Section numbers refer to Health and Safety Code section numbers. Section I of Chapter 314 amends Section 2248 of the Health and Safety Code as follows:

2248. The members of the district board shall serve without compensation; but the necessary expenses of each member for actual travelling in connection with meetings or business of the board shall be allowed and paid.

2248. The members of the district board shall serve without compensation; but the necessary expenses of each member for actual travelling in connection with meetings or business of the board shall be allowed and paid. In lieu of expenses, the district board may by resolution provide for the allowance and payment to each member of the board of a sum not exceeding five dollars (\$5.00) per month for expenses incurred in attending business meetings of the board.

This amendment provides an alternate method of compensating members of the Board of Trustees for travelling expenses in attending business meetings of the Board. Instead of filing an itemized statement of expenses, board members may receive in lieu of such actual incurred expenses a monthly allowance of not to exceed \$5.00; provided, that the Board of Trustees must first, by formal resolution, entered in the minutes, authorize this method of defraying expenses of members of the board for actual attendance at meetings.

Section 2 of Chapter 314 amends Section 2270 of the Health and Safety Code as follows:

2270. The district board may

(a) Take all necessary or proper steps for the extermination of mosquitoes, flies, or other insects in the district.

(b) Subject to the paramount control of the county or city in which they exist, abate as nuisances all stagnant pools of water and other breeding places for mosquitoes, flies, and other insects in the district.

(c) Purchase such supplies and materials and employ such labor as may be necessary or proper in furtherance of the objects of this chapter.

(d) If necessary or proper, in the furtherance of the objects of this chapter, build, construct, repair, and maintain, necessary levees, cuts, canals, or channels upon any land in the district, and acquire by purchase, condemnation, or by other lawful means, in the name of the district, any lands, rights of way, easements, property or material necessary for any of these purposes.

2270. The district board may:

(a) Take all necessary or proper steps for the extermination of mosquitoes, flies, or other insects either in the district or in territory not in the district but so situated with respect to the district that mosquitoes, flies, or other insects from such territory migrate into the district.

(b) Subject to the paramount control of the county or city in which they exist, abate as nuisances all stagnant pools of water and other breeding places for mosquitoes, flies or other insects either in the district or in territory not in the district but so situated with respect to the district that mosquitoes, flies and other insects from such territory migrate into the district.

(c) Purchase such supplies and materials, employ such personnel and contract for such services as may be necessary or proper in furtherance of the objects of this chapter.

(d) If necessary or proper, in furtherance of the objects of this chapter, build, construct, repair, and maintain, necessary dykes, levees, cuts, canals, or ditches upon any land, and acquire by purchase, condemnation, or by other lawful means, in the name of the district, any lands, rights of way, easements, property or material necessary for any of these purposes.

- (e) Make contracts to indemnify or compensate any owner of land or other property for any injury or damage necessarily caused by the use or taking of property for levees, cuts, canals or channels.
 - (f) Do any and all things necessary or incident to the powers granted by, and to carry out the objects specified in this chapter.
- (e) Make contracts to indemnify or compensate any owner of land or other property for any injury or damage necessarily caused by the use or taking of property for dykes, levees, cuts, canals or ditches.
 - (f) Enter upon without hindrance any lands, within or without the district, for the purpose of inspection to ascertain whether breeding places of mosquitoes, flies or other insects exist upon such lands; or to abate public nuisances in accordance with this article; or to ascertain if notices to abate the breeding of mosquitoes, flies or other insects upon such lands have been complied with; or to treat with oil or other larvicidal material any breeding places of mosquitoes, flies or other insects upon such lands.
 - (g) Sell or lease any land, rights of way, easements, property or material acquired by the district.

Every sale of real property pursuant to this subdivision shall be made to the highest bidder at public auction after five days notice given pursuant to Section 2204 of this code, and at such place within the district as the district board shall specify.
 - (h) Do any and all things necessary or incident to the powers granted by, and to carry out the objects specified in, this chapter.

These amendments provide, first, that the district may operate outside of its official boundaries if it is necessary to do so in order to adequately protect the inhabitants of the district from mosquito annoyance; second, confers the right to inspect in specific terms, instead of by inference as heretofore; third, provides for the sale of district property where desirable or no longer required. It should be noted that the power to inspect is limited to lands; it does not confer the power to inspect the interior of dwellings.

Section 3 of Chapter 314 amends Section 2274 of the Health and Safety Code as follows:

2274. Whenever a nuisance specified in this chapter exists upon any property in a district, the district board may in writing notify the record owner, or person in charge or in possession of the property, of the existence of the nuisance.

2274. Whenever a nuisance specified in this chapter exists upon any property either in the district or in territory not in the district but so situated with respect to the district that mosquitoes, flies or other insects from such territory migrate into the district, the district board may in writing notify the record owner, or person in charge or in possession of the property, of the existence of the nuisance.

This amendment makes it possible for districts to serve notices to abate mosquito breeding nuisances upon owners or occupants of property outside the district as well as within the district, and under the provisions of the related sections 2271-2273 and 2275-2289 of the Health and Safety Code proceed to abate the nuisance either under the method prescribed in said sections, or by any other method prescribed by law, and also to require that adequate steps be taken to prevent a recurrence of such breeding.

It is recommended that this section be used with especial care, and that definite proof be available that mosquitoes from breeding places outside of the district do actually migrate into the district in sufficient numbers to constitute a public nuisance, that is, a nuisance affecting a considerable number of people. It is probable that the possession of this power, if tactfully explained, may be sufficient in practically all cases to obtain abatement without actually proceeding to legal action.

Section 4 of Chapter 314 adds a new Section 2292 to the Health and Safety Code, as follows:

2292. Any person who obstructs, hinders or interferes with the entry upon any land mentioned in this article of any officer or employee of the district in the performance of his duty, and any person who obstructs, interferes with, molests, or damages any work performed by the district, is guilty of a misdemeanor.

The purpose of this new section is to provide a more direct method of handling the occasional cases where entry is refused, or where malicious damage to district work is done. It is recommended that this section be used with discretion, and where possible as a preventive of obstreperous interference, rather than as a punitive measure.

Section 5 of Chapter 314 amends Section 2312 of the Health and Safety Code to read as follows:

2312. The funds shall only be withdrawn from the county treasury depository upon the warrant of the district board signed by its president or acting president, and countersigned by its secretary.

2312. The funds shall only be withdrawn from the county treasury depository upon the warrant of the district board signed by its president or acting president, and countersigned by its secretary. However, if the county in which the district is situated has adopted a requisition system covering the withdrawal of funds for the purchase of services or supplies, the district board may, by resolution, adopt such system and make withdrawals in accordance therewith.

This amendment makes it possible for districts situated in counties which have a requisition system for withdrawal of funds from the treasury, to comply with the practice of that county. At the present time, only a few districts would be affected by this amendment.

PEST ABATEMENT DISTRICTS

The law relating to Pest Abatement Districts (Chapter 8, Division III, Health and Safety Code), has been amended by three separate acts of the 1941 Legislature, respectively Chapters 333, 334 and 361. The original sections of the Code are shown on the left, with the amended sections on the right, and are placed in the order of the code sections, irrespective of their order in the 1941 acts.

(Added new section)

2803. Any person who restrains, hinders or threatens any officer or employee of a district in the performance of his duties as such officer or employee is guilty of a misdemeanor.

This section is similar to the first part of the new section 2292 of the mosquito abatement district law.

(Added new section)

2822.5. The petition shall state the basis on which the property of the district shall be taxed for district purposes, which shall be either on the basis of area or on that basis of assessed valuation.

See remarks concerning Sections 287, 2871.5 and 2871.7, which also comment on this section.

2853. The district board may: 2853. The district board may:
(a) Purchase supplies and other personal property. (a) Unchanged.

(b) Employ necessary labor. (b) Unchanged.

(c) Acquire by purchase, condemnation or otherwise, in the name of the district, any lands, rights of way, easements, or other real property necessary for the district. (c) Unchanged.

(d) Make contracts to indemnify or compensate any owner of land or other property for any injury or damage caused by the exercise of the powers conferred by this (d) Unchanged.

chapter or of powers incident thereto.

(e) Sue and be sued.

(f) Do everything necessary to carry out the powers conferred by this chapter and carry out the objects of the formation of the district.

(e) Unchanged.

(f) Enter upon any property in the district for the purpose of inspection and control work and for the same purpose may enter upon property adjacent to the district which is a breeding place or is susceptible of being a breeding place from which infestation may spread into the district.

(g) Do everything necessary to carry out the purposes conferred by this chapter and carry out the objects of the formation of the district.

The added sub-section (f) in the amended law confers the power to inspect, within or without (adjacent to) the district.

2871. The board of supervisors shall levy, annually, a tax sufficient to raise the amount required for the purposes of the district. The rate shall be uniform and based on area of land, regardless of assessed valuation. The county assessor of each county shall prepare an assessment roll showing the names and addresses and the acreage owned by each person owning land within a district, which roll shall be the basis for the tax provided for herein. If the rate has been fixed by the organization petition, the rate fixed by the board shall not exceed that rate.

2871. The board of supervisors shall levy annually, a tax sufficient to raise the amount required for the purposes of the district. If the rate has been fixed by the organization petition, the rate fixed by the board shall not exceed that rate.

(Added new section)

2871.5. If the petition states that the property shall be taxed on the basis of area, the rate shall be uniform and based on area of land, regardless of assessed valuation. The county assessor of each county shall prepare an assessment roll showing the names and addresses and the acreage owned by each person owning land within a district, which roll shall be the basis for the tax provided for herein.

(Added new section)

2871.7. If the petition states that the property shall be taxed on the basis of assessed valuation, the board shall determine the rate of the tax by deducting 15 per cent from the total assessed value of the property in the district appearing upon the assessment roll and then dividing the amount required to be raised by the remainder of the assessed value.

In the original pest abatement district act, Section 2871 required the assessment base for taxes to be assessed valuation only. In 1939 this was changed (Chap. 449, Stats. 1939) to an assessment base of area only. The foregoing sections, together with Sec. 2822.5 previously noted, permit the basis of taxation to be either assessed valuation, or area, as the petitioners of the district desire, and the added new section 2875 following permits the citizens of the district to change the basis of taxation, by petition and hearing, at any time after the formation of the district.

(Added new section)

2875. Upon application of registered voters within district equal to the number required for a petition to initiate proceedings for the organization of the district, and after notice published as prescribed for notice of hearing on a petition for organization and a hearing on the

matter, the board may change the basis upon which the property of the district shall be taxed from one permitted basis to the other. Thereafter in ensuing tax periods the basis as changed shall be the basis of taxation for the district.

(Added new section)

2901. At any time after the incorporation of a district, upon application of such persons as could have initiated proceedings for the formation of a district composed of the land sought to be annexed, land contiguous to the district may be annexed by the board of supervisors upon like procedure, notice and hearing as provided for formation of a district.

This section provides an alternate method of annexation, supplementing the previous procedure (Sec. 2900) by petition of adjacent property owners only. It makes possible an annexation of territory over the objection of a minority property interest.

GENERAL LAWS RELATING TO DISTRICTS

Two new general laws of the 1941 Legislature are also of interest to us.

The first is Chapter 739, Statutes of 1941, which makes available a simple procedure for the dissolution of any district which has ceased to function, and is no longer required.

If such a district has no debts or bonds, and has not exercised its functions, then either the county assessor or county tax collector may make a written request to the Board of Supervisors to have such district dissolved. If the Board of Supervisors approve, then the District Attorney takes the dissolution procedure into the Superior Court.

It should be noted that this method is applicable only to those districts whose taxes are assessed and/or collected by county officials.

Probably the only mosquito abatement district which could be affected by this act at the present time is the Fair Oaks District in Sacramento County, which I understand has been inactive for many years.

The other general law is Chapter 465, Statutes of 1941, which provides that two or more districts, of any type, may jointly by contract exercise any common power or powers. Cities and counties have heretofore possessed and exercised this right.

This act would make it possible for two or more mosquito abatement districts to join together in their operations, so as to work more effectively. For example, it would be possible for the inadequately financed, small districts in Shasta County to join with the Redding District, and by pooling their funds, equipment and resources do a much more effective job of mosquito control than has been possible individually. That combination appears to be the most obvious and advisable, but others may be found to be desirable, for example, the Three-Cities, Pulgas and Mataro districts.

Campbell: Fred Hayes was programmed to give a paper this afternoon on the effect of priorities and national defense on mosquito control operations. However, he had to return to Bakersfield where he is to be installed tonight as Master of his Masonic Lodge, so I will ask Mr. Gray if he has any information on this matter.

Gray: I have a substitute paper to present, but in regard to priorities, all local governmental agencies are entitled to an "A-10" priorities rating on all materials for ordinary operations. On orders for such materials you should type or stamp the following:

Material for Maintenance, Repair or
Operating Supplies. Rating A-10
under Preference Rating Order P-100,
with the terms of which I am familiar.

Mosquito Abatement District

Superintendent

The above certificate must be signed, of course.

For capital equipment and certain specified materials, a separate request on Form PD-1 of the OPM must be prepared and filed. This procedure is rather complex

and I shall not attempt to describe it, as I don't understand its complexities. Instead, I will present Mr. Thomas F. Kelley, a graduate student in the Division of Entomology and Parasitology here at the University, who has recently completed a report on a survey of mosquito breeding in certain cemeteries in Alameda County. This report was prepared for the Alameda County Mosquito Abatement District by Mr. Kelley, with the assistance of Mr. Pedro Galinda, also a graduate student, under the direction of Professor Herms and myself.

MOSQUITO BREEDING IN CERTAIN CEMETERIES
IN ALAMEDA COUNTY, CALIFORNIA

by

Thomas F. Kelley,
Division of Entomology and Parasitology,
College of Agriculture,
Berkeley

The purpose of this investigation was to ascertain the extent of mosquito breeding within cemeteries. For a number of years the Alameda County Mosquito Abatement District, for which District this study was conducted, had been aware that there was an appreciable amount of mosquito breeding in certain cemeteries in the District, but the resulting nuisance was relatively minor. Therefore, in view of the type of personal sentiment involved, and the possibility of arousing violent resentment and prejudice, the District forebore taking any drastic steps to abate mosquito breeding in the cemeteries, though it did receive helpful cooperation from at least one cemetery.

Recently conditions have changed appreciably, and a reconsideration of past policy has been necessary. Some of the species of mosquitoes breeding in cemeteries are probable vectors of encephalitis, and with the establishment of large military posts and war industries in the District, it was decided that the situation must be appraised as a possible danger to the nation's war effort. The first step decided on was a survey of the species of mosquitoes breeding in cemeteries, their relative numbers, and the types of breeding places. This part of the work, here reported, was performed by the speaker with the assistance of Mr. Pedro Galinda of this Division.

Three typical cemeteries were selected for study, in this paper being designated as A, B and C. Cemeteries A and B are located in the heart of a large city (Oakland),

while Cemetery C is located in a rural area.

While the problem in all three cemeteries was basically the same, the surrounding conditions were sufficiently different to require reporting separately. Basically in all three, mosquito breeding in flower containers on graves is the principal problem.

CEMETERY A

This cemetery is by far the largest of the three, and required the longest time for examination. Essentially it can be subdivided into two sections, since it consists of newer and older parts, which produce two different problems.

While the cemetery as a whole is extremely well watered the year round, thus providing a good medium for mosquito breeding should suitable containers exist, yet it is apparent that the graves in the newer plots are more frequently watered and hence afford more opportunity for breeding than do the graves in the old plots. Furthermore graves in the newer plots are placed closer together, lack upright head stones, and occupy less space per grave, thus also increasing the potential number of breeding containers in a given plot.

Due to the need for a good source of "cheap" water for use in the maintenance of the grounds, the stream flowing through the cemetery has been dammed to form a chain of three reservoirs. Here the impounded water might offer a source of mosquito breeding, but it appears to be kept somewhat in check by Gambusia which are present in the water. However, along the edges the grass in certain places is too dense for the Gambusia to penetrate, and in such locations occasional Dixids were found breeding. This is of some importance in that at the near-by rest house two adult specimens of Anopheles maculipennis occidentalis were found. These specimens were in perfect shape and gave every indication of having emerged somewhere in the vicinity. In view of the finding of Dixids and Chironomids breeding in the dense grass it would seem likely that Anopheles maculipennis occidentalis was also breeding there in small numbers, though a careful examination along the shore line failed to disclose any Anopheline immature stages. A survey of these reservoirs early in the season might reveal the presence of Anopheline larvae.

It is also of interest to note the collection of a single specimen of Anopheles pseudopunctipennis franciscanus in the same rest house where Anopheles maculipennis occidentalis was taken. There are several ponds in the cemetery containing

mats of green algae and very few Gambusia, which may very well become suitable breeding grounds for Anopheles pseudopunctipennis franciscanus during the summer.

CEMETERY B

This cemetery adjoins Cemetery A, hence one might expect to find similar conditions existing. In reality it is a different problem due chiefly to economic conditions governing the upkeep of the graves.

Cemetery B, lacking a reservoir from which to draw its water supply, coupled with the fact that many of its graves were independently owned or were old and abandoned, presented a considerably different problem than did either Cemetery A or Cemetery C. In approximately one-half of the cemetery a steady supply of water was lacking, and as a result most of the water in the containers in this section had evaporated during the dry season and afforded little opportunity for breeding. Only in the part of the cemetery subject to "perpetual care" did we find mosquitoes breeding in fair numbers. In the unwatered sections breeding could occur only during or shortly following the winter rains, but at that time might reach a high level due to the large number of exposed cans, bottles and other containers present on the graves. A tin can dump in the rear of the cemetery would also provide excellent breeding grounds during the rainy season. A removal of all unused cans in the old section would undoubtedly check this seasonal breeding to a large extent. In the new section, where apparently continual breeding occurs to some degree, the general recommendations intended for all the cemeteries, and found in the last part of this report, might be of value.

CEMETERY C

As may be determined from an examination of the data, breeding in the containers in this cemetery was, surprisingly enough, rather light despite the fact that the percentage of containers holding water was much higher in this than in either one of the other two cemeteries. An explanation of this discrepancy may be found in the fact that, on the average, more containers had flowers in Cemetery C than in either Cemetery A or B.

Here, as was the case at Cemetery A, the problem of an abundant source of "cheap" water has been solved by the damming of a small stream which runs along the southern boundaries of the cemetery property. This reservoir with its impounded water situated in a narrow canyon some quarter of a mile to the south east of the cemetery is apparently the source of considerable mosquito breeding. Although the actual number of immature stages collected along the edge of the reservoir

was small, probably due to the lateness of the season, numerous Anopheline pupal cases were found and examination of overhanging trees, wooden bridges and adjoining farm buildings housing domesticated animals and birds, disclosed a considerable number of adults. The following Anopheline species were collected both in the adult and immature stages: Anopheles maculipennis freeborni, Anopheles maculipennis occidentalis and Anopheles punctipennis. The finding of both freeborni and occidentalis in the same locality and breeding in the reservoir together is of fundamental importance and should be investigated much more thoroughly in the spring and early summer. Culex apicalis was also collected in the buildings and may well be breeding in the reservoir.

DISCUSSION OF THE SPECIES COLLECTED

The most common of the species collected was Culex pipiens, which represented about 65% of all the larvae and adult collections. It was found at the three cemeteries usually in those containers with rather clean water. Although we stayed around its breeding grounds until late in the evening on several occasions, we were never attacked by this mosquito.

Culex stigmatosoma

About 4% of the mosquitoes collected were of this species. It was common at Cemetery B but rather rare at the others.

Culex tarsalis

Only about 1% of both adults and larvae collected were of this species and it was not found in Cemetery B at all.

Culex apicalis

Only three adults of this species were collected. They were found resting inside a chicken coop located back of Cemetery C.

Theobaldia incidens

This species was also found at the three cemeteries and it represented about 20% of all our collections. This species was more commonly found in foul water than Culex pipiens, and on one occasion it was found breeding together with rat-tailed larvae (Eristalis tenax) in very foul and murky water. We observed this mosquito approaching to bite at dusk in fair numbers at both Cemetery A and B.

Theobaldia inornata

Two adult specimens of this species were collected in a rest house at Cemetery A.

Aedes varipalpus:

This species is represented in the collection by a single adult specimen taken on the wing at Cemetery C. Tree holes were very numerous in the many live oaks growing on the sides of the canyon above the stream which runs along the southern boundary of the cemetery, and in the spring months they should provide excellent breeding places for this mosquito.

Anopheline mosquitoes

Represented about 5% to 10% of all mosquitoes collected as adults.

Anopheles punctipennis

The most common of the Anophelines collected at Cemetery C. Adults of this species were taken from hollow trees, holes in the ground, chicken coops, stables, garages, etc. It is of interest to note that several second and third instar larvae of this species were collected in the reservoir, on the 26th of October.

Anopheles maculipennis occidentalis

This species was found in a rest house at Cemetery A, and as adult and pupa at Cemetery C.

Anopheles maculipennis freeborni

This species was only found at Cemetery C. Adults were secured from a stable, while two specimens were reared from pupae collected at the reservoir.

Anopheles pseudopunctipennis franciscanus

One adult of this species was collected in a rest house at Cemetery A on October 10th.

INFLUENCE OF THE GENERAL TOPOGRAPHY OF THE
PLOTS ON MOSQUITO BREEDING IN THE CEMETERIES

The general topography of each individual plot seems to play a great part in determining the potentialities of the plot as a source of mosquito breeding. Those plots located on the slopes of the hill at Cemetery A seemed to hold considerably less water than the plots that were completely on flat ground. A very striking example may be found by comparing plot 65, which is situated on a steep slope, and plot 58, which is on flat ground. The average number of containers full of water per grave in plot 65 was only 0.44, while in plot 58 it was 1.05. These last figures compare very favorably with those obtained for the entire Cemetery C (1.19 containers full of water per grave), which is located at the bottom of a series of hills on very flat ground. These figures seem to indicate that in those plots situated on a slope, surface runoff removes a considerable portion of the water sprinkled on them. Breeding in these plots, as we ascertained by a careful survey of plot 65, is then practically restricted to those containers located at the foot of the hill where most of the surface water accumulates.

On the other hand water sprinkled on those plots situated on very flat ground normally accumulates in the containers so that practically every available container has some water in it, thus greatly increasing the potentialities of the plot as a mosquito breeding place.

GENERAL RECOMMENDATIONS

Regarding general control measures as it concerns mosquito breeding in the containers in the three cemeteries, it would seem that to be effective they should be both simple and inexpensive, especially when considered over a period of time. Of the possible measures that might be taken, the most logical appears to be that of instituting a reversible flower container which is essentially one container within another, the whole unit being sunken into the ground; the inner one is used as the flower container, and when the flowers wither away and are discarded by the caretaker, he may then empty out the water and reverse the inner container. The bottom of this inner container will then serve as a top or cover and thus help to prevent the accumulation of water in the containers and the breeding of mosquitoes therein.

These containers while having a higher initial cost than the ordinary can containers commonly in use at the cemetery, are more durable, more convenient, and are

certainly less apt to serve as resting places of various slugs, snakes, frogs and "black widow" spiders such as were found by us in the course of the survey, and which might be extremely repulsive and shocking to certain people.

The recommended containers are sold by Cemetery A for \$0.75 each, while the can type more commonly in use retail at from \$0.25 up. The cemetery management appears quite willing to allow the neighboring florists to handle the sale of these reversible containers, and also appears willing to support any rulings requiring their use.

While more of the recommended containers are in use in Cemetery A than anywhere else, yet their use at the other cemeteries would be just as efficient in bringing about a marked reduction of mosquito breeding, and all the advantages in their use should be pointed out to the directors of these two cemeteries.

Oiling of the water in the containers does not appear to be feasible. The oil film would tend to be destroyed as a result of frequent sprinkling of water the year round, and in the rainy season the additional rain water would also have to be considered. As a result, frequent oilings would be necessary, and the cost of labor and materials would tend to mount excessively over a period of time, while still failing to provide a permanent solution to the problem. A clear picture of the situation may be had by considering that in the new section of Cemetery A alone there are actually some 7,600 containers full of water which would need frequent oiling.

SPECIFIC RECOMMENDATIONS

CEMETERY A

That some control measures should be undertaken here in the near future is evident if we consider that approximately 25,000 adults are emerging from the cemetery grounds every week. Since a considerable number of these mosquitoes are probably attracted to human habitations, they undoubtedly constitute a problem in those houses adjoining the cemetery.

In the old section of the cemetery, the problem is not as serious as in the newer plots since very few of the graves have flower containers. Even when present, containers in this section are not apt to harbor mosquito larvae since most of these plots are located on the slopes of the hills, so there is considerable surface runoff and very little water remains in the containers. Plot 43, which is one of the old plots, has 24 urns which are potentially very good breeding

places for the various species of mosquitoes present in the cemetery. A survey of these urns revealed the presence of Theobaldia sp. larvae (probably Theobaldia incidens) in eight of them. These urns should either be removed or oiled occasionally.

During periods of high water the Gambusia present in the reservoirs should be sufficient to hold breeding in check. However, during a period of low water which apparently occurs in the late summer and early fall, the Gambusia may not be able to penetrate the dense grass in the shallow water along the margin of the reservoir. At such times a supplementary application of oil to these areas might be effective.

A thorough investigation of the stream feeding the reservoirs and of the ponds with abundant green algae growth should be undertaken in early summer to determine whether Anopheles pseudopunctipennis franciscanus is breeding in such places.

CEMETERY B

That portion of the cemetery under perpetual care is fairly well kept up and the general recommendations would apply. The other and somewhat larger portion of the cemetery, lacking as it does perpetual care and a source of "cheap" water, is not kept up and has many potential breeding places in the form of old bottles and cans which should be removed and discarded before the rainy season.

In the rear of the cemetery is a tin can dump which might well serve as a breeding place during the rainy season and which should be cleaned up or oiled. One of the factors contributing to keep mosquito breeding in this cemetery at a low level is the close proximity of Cemetery A which, due to its abundant water supply and consequent high humidity, has a number of ideal resting places for adults* which probably attract a considerable number of gravid females, concentrating much of the oviposition around these resting places.

(* These resting places consist of a small rest house, two large spillways between the reservoirs, and numerous vaults scattered throughout the cemetery).

CEMETERY C

The main source of breeding, aside from the containers, appears to be the reservoir. Here three different Anophelines were found breeding together. No Gambusia fish were noted in the reservoir and their introduction might well prove the simplest method of controlling the breeding that occurs there. The general recommendations would apply to the cemetery proper.

Chart No. 1

Cemetery	Total Number of Graves	Average no. of containers per grave			Average no. of larvae per container (1)	Total number of larvae (2)
		Holding Water	Empty	Both water and larvae		
A	12,700 ⁽³⁾	0.60	0.41	0.24	25	76,200
B	? ⁽⁴⁾	0.33	0.28	0.04	27	? ⁽⁴⁾
C	6,000	1.19	0.27	0.15	17	15,300

(1) This average includes only those containers holding water

(2) This number represents an estimation based upon the sample

(3) This number refers only to the new section

(4) No records are kept of the number of graves in this cemetery

Chart No. 2

Topographical data	Cemetery and Plot Number	Average number of containers per grave			Average number of larvae per container (1)
		Holding water	Empty	Both water and larvae	
Plot located on very flat ground	A Plot 58	1.05	0.23	0.46	29
	C all plots	1.19	0.27	0.15	17
Plot located on steep slope	A Plot 65	0.44	0.40	0.07	33

(1) This average includes only those containers holding larvae

Species of Mosquitoes Collected	Cemetery in Which Species Was Collected	
	<u>As Larvae or Pupae</u>	<u>As Adults</u>
<i>Culex pipiens</i>	A, B, C	A, C
<i>Culex stigmatosoma</i>	A, B, C	A, C
<i>Culex tarsalis</i>	A, C	A, C
<i>Culex apicalis</i>		C
<i>Aedes varipalpus</i>		C
<i>Anopheles maculipennis</i> <i>freeborni</i>	C	C
<i>Anopheles maculipennis</i> <i>occidentalis</i>	C	A, C
<i>Anopheles punctipennis</i>	C	C
<i>Anopheles pseudopunc-</i> <i>tipennis francis-</i> <i>canus</i>		A
<i>Theobaldia incidens</i>	A, B, C	A, B, C
<i>Theobaldia inornata</i>		A

Bendel: If the container is reversed, just how are you going to get it out?

Kelley: The bottom is depressed, with a flat strap across it, below which you can insert a finger and easily lift it out.

Gray: I want to call your attention to one interesting thing. Mr. Kelley brought out the point that in one of the cemeteries they made a large collection of Culex pipiens, both larvae and adults, and then he went on to say that although he and Mr. Galindo were around this cemetery several times until fairly late in the evening they were never bitten by these mosquitoes. A year ago, on page 281 of Herms and Gray - "Mosquito Control", we called attention to the work of Jobling in England on Culex pipiens. He found two varieties of the species, one of which - Culex pipiens pipiens - does not bite humans, breeds in relatively clean water, and hibernates in winter: the other, designated as Culex pipiens molestus, attacks man, breeds by preference in foul water, and does not hibernate. So far as I know, no one has worked on this problem in the United States. On the fact cited by Mr. Kelley we strongly suspect that we have these two varieties here. Perhaps Professor Herms will have a student who wants to do some research work on this.

Herms: The Alameda County Mosquito Abatement District deserves a good deal of credit for sponsoring this survey. I don't believe there are many, if any, publications dealing with the subject. This report is quite revealing and shows that something ought to be done about this problem. The Alameda County Mosquito Abatement District received the whole-hearted cooperation of the cemetery authorities and of all concerned. You did a fine piece of work, Tommy.

Kelley: There was something in "Public Health Reports" about the breeding of Aedes aegypti in cemeteries. The subject was treated quite thoroughly. But that wasn't a general survey.

Gray: For a number of years we have known that we had mosquito breeding in these cemeteries, two of which are in the heart of Oakland. Residents along the edges of these cemeteries have been mildly annoyed by these mosquitoes at times. Mr. Miles has struggled with the problem and got some results, but the situation is still a long ways from what we would like to have it. But we have had so many other problems on our hands that we didn't want to tackle this situation, in which we could easily get crossed up with feelings and prejudices in regard to the disposal of the dead. People are very sensitive and touchy on that point and we could get ourselves into a difficult spot.

Recently, when all this new information became available that certain Culex and Aedes species were possible vectors of encephalitis we felt it was necessary to take action as a matter of self protection. We felt it was necessary to at least get the facts as a basis upon which to proceed. If we had the facts we would have a much stronger leverage to work with. I thought the cemetery authorities would be cooperative, but some owners of graves might be strongly opposed to any effective control measures. With the facts in hand we believe we can now put up a more convincing argument for better mosquito control in cemeteries.

Campbell: We are now to have a symposium led by Harold F. Gray in which a lot of very practical problems of interest to all of us are to be discussed.

Gray: Apparently what the Program Committee had in mind was a discussion of practical problems in connection with our regular work. Why they picked on me to lead on this I don't know. First of all, Roland Bendel of the Alameda County District has something to say about our new power spraying equipment which he is using.

Bendel: This summer the Alameda County Mosquito Abatement District put into service a power spray rig for oiling marsh and dairy areas, roadside ditches, etc.

A brief description of this rig is as follows: The whole assembly is mounted on a Ford Model "A" chassis, cut down and shortened to size. The pump assembly is a Bean No. 3 Orchard Sprayer, consisting of pump, tank and accessories mounted on a steel frame base. The pump is driven by a one half horse power Cushman "Husky" four cycle gas engine. The pump itself is of the two-cylinder plunger type, and can deliver oil at about 400 pounds pressure; but we usually operate at 150 pounds.

The pump takes suction from a fifty gallon tank, and discharges into 300 feet of 7/16 inch high pressure hose, which is mounted on a hose reel, behind the supply tank. If necessary, 300 feet of hose may be added, or 300 feet of 1/2 inch pipe can be laid out and oil pumped directly into the pipe, and hose attached to the far end. This latter method is used only where considerable extra "reach" is needed for marsh oiling. The discharge is about one half gallon per minute.

The cost of the entire equipment, spare hose, pipe and fittings was about \$580.00.

The machine is towed to the area to be sprayed. Three men usually comprise the crew. When more hose lines

are laid out, an extra man per every addition hundred feet is added.

When a long stretch of hose is to be laid out, we first lay out the entire length, then as the nozzle man works back and forth, the hose is reeled back on the machine to take up the slack.

The cost of this operation is about \$5.00 per acre. This may seem a bit high, but it must be borne in mind that the areas oiled in this manner are usually hard and difficult to reach by other methods, and hand oiling would run considerably higher. Also, we have not had this machine in service long enough to obtain conclusive cost figures.

The advantage of this rig is that work can be done more quickly and with less effort; also, the oil can be sprayed with more force, thus getting down through weeds to the water surface more effectively.

The locations where this can be used to the best advantage are as follows: Marsh areas, where the rig can be towed close enough to reach the area involved; dairy drain pools, which are sometimes one acre in area; roadsides, where the rig is towed along the road, and the operator, standing in the rear, can spray as we roll along.

We have the machine outside, and you will all have a chance to look it over, and I will do my best to answer the questions you may ask.

Robinson: How long does it take to lay out the hose and get into operation?

Bendel: It depends upon the length of hose and the kind of ground. Up to three hundred feet, where we pull off the automatic hose reel, it takes only long enough to walk out with the hose. Beyond three hundred feet it will take quite a little time.

Robinson: Using the pipe would be more trouble.

Bendel: We expect to use the pipe only when we have to run more than six hundred feet, which is the limit of our present high pressure oil-resistant hose, which we cannot get more of at the present time. We expect to use this pipe, much of which we already had on hand, as a feeder line close to the pump. We have a cut-off from the hose reel and can connect the pipe direct to the pump; at the far end of the pipe we have a double male brass fitting with pipe thread on one end and hose thread on the other, so that we can connect our

hose to the end of the pipe.

Gray: We expect to use the pipe only in situations where we must reach over six hundred feet.

Bendel: We use six hundred feet of hose and so far have had no difficulty.

Reeves: How long are the pipe lengths?

Bendel: About ten feet.

Robinson: Can't you get a quick-acting type of coupling?

Gray: There are several types of such couplings for low pressure work, but I don't know any hose quick coupling that will stand up to three hundred pounds pressure.

Mapes: On the Alvarado Gun Club property we used this equipment to spray kerosene and pyrethrum extract, and put on so much more than formerly that we killed the grass. The duck club operators were slightly miffed about it, but it came out alright in the end.

Emerick: We just use hand pressure pumps. We have lots of borrow pits to contend with along the highways and railroads. We figured that by the time we dragged the hose around all the poles we would be nothing ahead, but could use hand pressure pumps to better advantage. We use the Hudson barrel type with one strap which goes over the shoulder. With just one strap, when a man falls into the creek it falls off his back and he is free. This will happen occasionally but not often.

Robinson: We have a Dodge pick-up with a pressure tank. We use that by going down to the service station and borrowing air. We do our catch basins and gutters with this. It has a long pipe with a nozzle and we shoot as we go by.

Miles: We use motorcycle equipment in my division. This is at our warehouse in Oakland. I will be at the office at nine o'clock tomorrow morning and if any of you are interested in seeing this, if you will meet me at the office I will be glad to take you and show you this piece of equipment.

Gray: You really should see our new Oakland depot. We are quite proud of it.

Chester Robinson has been doing some experimental work on larvicides, and he may have some new information for us.

Robinson: Larvicides have been cussed and discussed. However, in the early work of a new district, I think it is very important to have, in addition to oil, a larvicide that will work. It need not be the most efficient larvicide, but we found in our first year that when we went to the farmers they immediately said they didn't want us to use oil, and they would ask what we were using. If we had told them we were going to use Diesel oil they would have ordered us off the place, but when we told them we used a material something like fly spray mixed with water, they had no objection. Now that they have found we do not damage their crops, we can use oil where appropriate without any trouble. But the advantage of the larvicide has been that by its use we can get the farmers' consent and cooperation, instead of having an argument.

So far all types of larvicides are a long ways from perfect. We have been using a concentrated material put out by the Sherwin-Williams Company, which is quite effective where you have a power sprayer. At times we have used it under unusual circumstances. For example, a septic tank in Modesto missed our inspection and a neighbor across the street who was going to have a barbecue rang me up and said that the mosquitoes were terrible and she expected a big crowd at her barbecue. A great many of the people in Modesto have barbecue pits in their yards. We call them our out door living rooms. Well, we went out on the afternoon of the barbecue and mixed up the larvicide, using a little more pyrethrum than usual. We sprayed thoroughly around the shrubbery and the flowers. She had her party and there were no mosquitoes, so she was well pleased. We find that a little public relations work of this kind once in a while is quite valuable.

This year we used about 10% of the amount of larvicide used previously. A good many of the places that were bad previously we had pumped and drained, and so required no spraying, but our educational work with the farmers, and the good relations established by the use of pyrethrum larvicide at the start of the work, has enabled us to use Diesel oil now, especially at the end of checks where the crop is under water practically continuously and there is nothing of economic value. The farmers have now become acquainted with us and our work and realize that we are out to help.

The California Spray Chemical Company about two months ago left me some samples. We have tried out some three hundred samples this year gotten out by different companies. These were different combinations of materials they felt were of good larvicidal character. Some

proved to be good and some did not. The California Spray Chemical Company has developed an oil-soluble emulsifying agent which is far superior to the agent used in the Sherwin-Williams product. From the standpoint of mix and spread, I think this is going to be superior to anything we have had. We have only used it experimentally so far. We made some laboratory tests which were satisfactory and we made a few sprayings in the field and they too were apparently satisfactory. Since that time, we have received from the east a five gallon sample of a new product developed back there. So far we have made no experiments with this.

Gray: Who else has used a pyrethrum larvicide?

Emerick: We found we didn't get good results with it.

Bendel: My experience has been that one time it kills and the next it don't.

Robinson: Yes; we found that too. We had to send several drums back. The stuff got as thick as syrup. If we mixed it and used it right away we got good results, but later it was not much good. For example, two years ago we had about half a tank left in our power sprayer one night. The next day we used it to spray on the golf course. About three hours after we sprayed, the larvae were all just as happy as ever. Then we mixed up a fresh batch and sprayed again, and that did the job. They were all killed.

I think the answer is that if your larvicide is made up with any water you will have a deterioration of the pyrethrum, but if it is an oil suspension with an oil-soluble emulsifier it will keep until diluted with water for spraying. Then it must be used at once.

Gray: There is no necessity for us to buy these emulsified products. Those of us who have power sprayers can buy the concentrated pyrethrum extract, and add our own oil and soap or emulsifier, and by running the pump and jetting the material back into the tank, you can make up your spray emulsion on the ground as you need it. I believe some of the districts in New Jersey and Florida are doing this. The material cost will be less, but the power and labor cost more.

Robinson: The new larvicide we are trying is not pyrethrum. Pyrethrum has gone up in price quite a bit recently.

Gray: I see that Mr. Oliver is now in the room, and if there is no objection I will ask Mr. Oliver at this point to answer questions about his colored photographs of mosquitoes, concerning which a question was raised yesterday morning.

(Mr. Oliver then stated what he was prepared to furnish at various prices, and after discussion the matter was referred to Professor Herms and Mr. Reeves for technical approval be-

fore further action would be taken.)

Gray: Mr. Emerick is our fish expert, and I think perhaps he has some interesting experiences along this line about which he can tell us. How about it?

Emerick: I don't know that I have anything new to say. It has been some time since I started raising mosquito fish and planting them here and there where needed. I have been putting quite a few out. As to anything new other than the sewage disposal plant at Calistoga, my work has been planting them in lily ponds and streams of water to protect people from the mosquitoes which would otherwise breed there. I have endeavored to plant them in sewage disposal ponds. I remember in 1935 when we went to Marysville I made the suggestion of planting them in the Yuba City ponds across the river. One of the men said it couldn't be done on account of the lack of oxygen, but in 1933 I had started at the Calistoga Sewage Farm. This is a series of large shallow ponds, the sewage running from one pond to another until it largely evaporates or is absorbed by the soil, the effluent from the final pond, which is clear and stable, running into the Napa River. It covers about five acres. I first started the fish in the lower pond, and then as they got used to it, they were moved from pond to pond every six months until finally they were moved to the sixth pond where the sewage enters from the septic tank. The fish went into this pond and even into the tanks themselves. They are still doing a wonderful lot of work. We keep the grass cleared away from the edge of the ponds by pasturing a small flock of sheep along the banks.

There was some talk yesterday about Gambusia not affecting Anopheles. The only place where I have found that to be the case was where there was considerable algae and where there is a certain water lily with a cup that is large enough for them. You will find a number of larvae inside the cup where the fish cannot get at them. The cup is large enough for the larvae but too small for the fish. We also have breeding in the algae along the edge where it becomes too dense. Wherever the larvae are in the open water I don't think you will have any trouble. We have planted these fish in barrels of water left standing for fire purposes and they have done very well. I have several watering troughs I began putting fish in thirteen years ago. There are still plenty of fish in these troughs. This is especially true back in the foothills. After the heavier oiling each spring I make a tour of all the farms and see that fish are planted in the troughs and springs so far as we can get to them on foot, on horse back or by machine. If you want a horse, the farmers will always furnish one for this work. Down in the lower part of our district we have cooperation which includes the use of a tractor when

we need it. This sort of thing makes our work a pleasure. By the cooperation of the farmers we have been able to cut our budget more than half.

Stewart: Possibly there is some misunderstanding of what Professor Freeborn said yesterday about Gambusia not killing off Anopheles. He was referring to Anopheles quadrimaculatus in the south and in the east.

Emerick: What has been said about the difficulty of using fish where there is a lot of algae is true, but you can control the algae by the use of blue stone. We can use blue stone to kill off the algae, but you may have to replace the fish afterwards. Where practicable, remove the fish first. In some places there is a water hyacinth which clogs the streams and causes a great problem in the south.

Herms: Mr. Emerick has done one of the finest bits of pure scientific work ever done anywhere in the adaptation of these fish to places which we have considered unfavorable. What he has done is a real scientific achievement. I think we are all deeply indebted to him for being a real practical scientist.

Gray: I took some students down to our breeding ponds on the Starr Estate in the southern part of Alameda County one day. Unfortunately for me I had Tommy Aitken with me. What did Tommy do but proceed to dip out several Anopheles maculipennis occidentalis larvae out of the breeding ponds. The Gambusia were all over the pond, but here were Anopheles larvae in with them. Was my face red! In the early spring the fish are still somewhat lethargic and that was the answer, but I had just been telling the students how marvelous these fish were when Tommy found the larvae.

Bendel: I think you have all seen pictures where a cat will be making a playmate out of a mouse. This is the same sort of thing that happens once in a while with the mosquito fish. We have had horse troughs alive with Gambusia and alive with larvae in one or two instances. We had this happen in Warm Springs. In one place there was a very large trough. It was in what is known as Morrison Canyon. Culex tarsalis breeds there. We have also gotten them in the salt water where the water is gradually changed. We have one slough where the water in spring is fresh and later gets salty. Gambusia seem to survive the change.

Robinson: We have found that the Gambusia we get out of different places vary in their ability to control larvae. Some are much more active than others. We have a trough about twelve feet across and fourteen feet long that someone

donated to us saying that we could have it for our mosquito fish. That made it real handy for us. We planted a thousand fish and replenished the supply from time to time. One day Mr. Adams, on whose property the pond was located, said that he wished we would come and look at the pond. We found it contained not only fish but mosquito larvae and also rat-tailed maggots. There was a mulberry tree over the pond. Mr. Peters said the leaves falling into the pond reduced the oxygen content, which had stupefied the fish.

Bendel: That is true where the pond is under a tree. You will find plenty of larvae, and it may be hard to keep fish alive in such a pond. I remember one pond where the water was practically black from decayed leaves from the tree overhead.

Gray: The leaves also may have put a different food supply in the water that the fish took instead. If the Gambusia had been feeding on this they may have been so full they wouldn't pay any attention to the mosquito larvae. It would be like a cat fed on liver--the cat won't bother about mice.

Miles: What would Professor Herms contribute to that?

Herms: You can utilize fish in the final tanks of sewage treatment plants, as Mr. Emerick has shown. Mosquito control measures should be instituted at every sewage treatment plant as they are apt to breed mosquitoes in unused tanks or channels. The use of Gambusia is one very good way. Tanks that are actually in service are not apt to show any breeding but, if they are out of service for a time they should be watched.

Emerick: You will have failure in lily ponds under black walnut trees; also under gum trees. They create a poison that will kill off the fish. Trees surrounding a pond may make quite a difference. Watch your trees as well as your ponds. I have found Aedes varipalpus breeding in any type of container set under a tree, as well in tree holes and in stumps where the timber had been cut off and grown up again into several branches.

Peters: Merced County perhaps has more Gambusia in it than any other county in the state. Perhaps Mr. Lilley can tell us about how he uses fish.

Gray: I was just about to ask him about his annexation campaign for his District. Perhaps he can answer both questions.

Lilley: We have the problem of large quantities of irrigation water. If it weren't for the fish we couldn't take care of it. Around Merced there are close to two thousand acres of irrigated pasture land. We use the mosquito fish freely and get good results. Our principal difficulty is that when people move sometimes the garden pond is neglected and the fish die. If we don't happen to notice it, we may get a crop of mosquitoes which is a local annoyance.

With regard to our annexation campaign, we have long realized that our District was too small to properly protect our people. There were large breeding areas under irrigation outside our boundaries from which mosquitoes came into our District. Last year we thought conditions were ripe for annexation. First we contacted our Chamber of Commerce, and by the use of facts, fairy tales and imagination sold them on the idea. The Secretary asked that a survey be made, which was made by Sid Dommies and presented to the Chamber of Commerce on November 7th. We thought we were already to shoot with the annexation petition, and the District Attorney told us to go ahead.

We had a big meeting scheduled, but the District Attorney failed to show up, so it had to be put over to the next afternoon. After that, from November 7th to January 7th, 1941, nothing was done, so we turned to the Junior Chamber of Commerce to help us circulate the annexation petitions. They didn't want to begin the job that Sunday, and the next two Sundays it rained hard. Then there followed the firemen's annual banquet, which incapacitated some of the boys. But we finally started out, but although they were shown the boundaries, some of the signatures were outside. They fooled around and only got a name here and there.

Finally we got the Chamber of Commerce to hire a man on the first of March, and he got 150 signatures. With these the hearing was held by the Board of Trustees, and as the only person to appear was the man who circulated the petition the District Board voted the area to have been annexed. But with all the delays we could not get it done before the dead line of the first of February, and so we can levy no taxes on the annexed area until next year. Then we will be able to step right on it.

Gray: I want you to notice that Mr. Lilley said they had to hire someone to go out and get signatures. I think you will find that is the usual case.

Robinson: We have started annexation procedures in our District. We have printed petitions to annex everything east of the San Joaquin River in Stanislaus County. We had everything signed up except for the signatures in Turlock where

the Chamber of Commerce had promised to back the situation. But they were as slow as in Merced. Then the Japs came along, and that put a bomb under us. At Turlock everybody was on a defense committee or something else. Now I don't know how we are coming out unless we can get the Chamber of Commerce to hire a couple of people to get five or six hundred signatures. The annexed area is about seven hundred square miles with about sixty million dollars valuation.

Gray: Are there any other districts that have had an idea of enlarging?

Emerick: I would suggest a little moral support for Sonoma County. There is still considerable work to be done there.

Andrieux: There hasn't been any demand for enlarging our district so far. The Sonoma Board of Supervisors gives us some money for work outside our district, but last year it wasn't enough to do much with. The opposition to annexation comes from the owners of the big ranches on marsh land, who don't want to pay the tax.

Gray: What has been done in Sonoma County has helped Alameda and Marin and San Francisco a lot in recent years. The only mosquitoes we get from Sonoma County now are just a few stragglers. This situation is an illustration of the fact that a fairly good job done sometimes makes progress to a first-class job very difficult.

Our next subject is also interesting and perhaps we'd better get on to it. Mr. Pangborn of Solano County is to talk to us about their experiences with re-flooding.

Pangborn: Since Mr. Rush has been ill I haven't felt like taking on any new projects, as I have been pretty busy trying to take care of both ends of our County. The properties with which we first experimented have been in good condition now for four or five years. Our district has something like a hundred thousand acres of marsh. We have the problem of flooding both for irrigation purposes and for the duck clubs. We have a property on the extreme west boundary of the marsh consisting of about 150 acres which we can now flood at any time and never get larvae of Aedes dorsalis. If we leave it alone for two or three weeks, we get a few Theobaldia and a few Culex tarsalis.

The idea of controlled reflooding is to flood these lands and then drain them quickly so as to clean out the eggs by successive hatchings until all eggs are hatched.

On a piece of property of about four hundred acres which we thought was cleaned of Aedes dorsalis, inspection showed there were lots of larvae. We knew the water level hadn't dropped, so we were sure the larvae were not Aedes dorsalis. We took samples, and Tommy Aitken identified them as Culex tarsalis. This was a few miles from Suisun Bay and the water was salty.

Wherever possible on our marshes we use reflooding and redraining, plus the Gambusia, and don't attempt to oil unless absolutely necessary.

Gray: Will you please be so good as to take a word of greeting to Fred Rush? We miss him at these conferences.

G. Mapes: Is it possible for Aedes dorsalis eggs to be completely submerged, and then after the water has drained off for them to re-hatch?

Pangborn: Yes. There is no question but what you can flood any area that has Aedes dorsalis eggs and some will hatch and some will not.

Freeborn: The distribution of Aedes dorsalis is practically world-wide. It is one of the two principal pest mosquitoes in this region. It hatches in the late spring and continues through the fall. They are a problem to all of us. They have them up in Washington and as far to the south as Monterey. Along the coast it breeds in salt water, but in the Central Valley it is a fresh water breeder. Sometimes it is found breeding in fish ponds in northern California.

Herms: As a salt marsh breeder you will usually find it in pools that are affected by the high tides. It comes later than Aedes squamiger but you may find it in the same pools. It prefers a higher salinity. It is a migrating mosquito.

Reeves: A number of times in the laboratory we have taken a known number of eggs, say a hundred Aedes eggs. Of course we do not get 100% hatch even by regulating the temperature of the water. By submerging we usually get only 40% or 50% to hatch. Oftentimes it is necessary to flood four or five times before the majority hatch. I thought that was shown very well by Rush's paper several years ago. One flooding will get a large number, but it is necessary to reflood three or four times. I remember he told of one case where they flooded seven times. It was on a large area used for grazing cattle. They had previously had a lot of trouble with this place. At the time of his paper (1938) he reported that this property had been flooded for the seventh time and inspection showed no larvae were to be found.

Emerick: This method will only work on the eggs already on the marsh. Where adjacent areas are not under control, your purpose is defeated, as the marsh can be re-seeded by mosquitoes from adjacent marshes.

Gray: Aedes dorsalis favors a higher alkalinity than most species. It has been found in alkaline hot springs.

Herms: Yes; I have seen them right on the edge of the water flowing out of such springs, and the water was very hot, too.

Reeves: It is readily possible for Aedes eggs to lie dormant for many months and then when they are wet they will hatch out. Contact with water at least once is necessary before they will hatch. They may require a number of wettings.

Emerick: On one of our reclaimed islands the levee broke, allowing the water to go over it for eight months. So far as we were concerned, from the mosquito abatement standpoint, we didn't care. There was no breeding because there was about five feet depth of water in and out every day. They finally rebuilt the levee, and put in a new gate. The balance of water that stayed on the land hatched a beautiful crop of mosquitoes. This island had been dry for seven years.

Gray: We know the eggs will last over ten years. Mr. Stover's experience proved that.

Our next topic for discussion is rice field control. Mr. Butler from the Durham District will start us out on that.

Butler: Our greatest problem in the Durham District is pest mosquitoes from the rice fields and sloughs. Lots of mosquitoes used to find their way in to us from the rice field nearby. We used fish and yet we had the larvae and the mosquitoes. We have three big ponds where we raise these fish which I use to stock the rice fields as soon as flooding begins.

Since the 1937 flood the rice field people have been levelling their fields more carefully and they have been getting far more rice per acre and a lot less mosquitoes. This year I know people are really able to sleep outside. They do get bitten once in a while but not at all as they used to. Now that we have the rice fields well levelled, with plenty of fish in them, we have no trouble at all. Another thing that has helped us is the use of a big drag-line by the irrigation people to clean out the sloughs and tules.

Our district was organized originally for malaria control. When the State Land Colony was proposed at Durham about 1917, the people were told they could not have the colony unless they organized a mosquito abatement district to control the malaria. From 1914 to 1920 we had more malaria than almost any other place in the state, an average of about 150 cases a year. Between 1921 and 1930 we cut this down 8 cases. In 1935 Butte County as a whole reported only 1 case. In 1938 it rose to 20 cases due to the influence of migratory labor from southern states, but since then malaria has dropped back to practically nothing in our area.

Mosquitoes and malaria can be a great detriment to any region. I remember that back in 1909 they held a big meeting in Oroville in our county to promote the growing of oranges and olives. One old fellow eighty five years old told that meeting that before they tried to get people interested in putting their money into orange and olive orchards they had better get rid of the malaria, or the scheme would be a failure. So they appointed a committee to investigate, got Professor Herms up there, and that was the start of mosquito control in our county.

Gray: Are there any topics which any of the members wish to bring up for discussion?

Framme: I believe the Alameda County District has a way of accumulating money in their budget from year to year. I get turned down every time I try it and I'd like to know how to go about it.

Gray: It's a form of skullduggery. When we first started out we realized we had to have cash money with which to operate from the first of July until taxes became delinquent in December. We went to the District Attorney and asked him if we couldn't establish a cash basis fund to carry us over this period. He said "It's not permitted in your law, therefore you can't do it". I had a different theory, that if the law didn't prohibit you from doing a thing, you ought to be able to do it. But our District Attorney held that if the law doesn't give specific authority for an act you are not permitted to do it. So I had to think up some way of accomplishing our purpose, feeling sure there must be some way by which it could be done.

This is the procedure which we have used successfully and without legal objection for the last ten years. When we set up our annual budget each May, we work out first what we call our "working budget" covering the items of expense which we anticipate will be required during the next fiscal year. These budget items are grouped under the main headings of (1) Salaries and Wages; (2) Maintenance and Operation;

(3) Capital Outlay. Say our working budget totals \$40,000, of which Salaries and Wages requires \$30,000, Maintenance and Operation \$9,000, and Capital Outlay \$1,000.

We also determine that we will need \$15,000 to carry us on a cash basis from July 1 to December. This \$15,000 we add to our working budget of \$40,000, giving us a gross budget of \$55,000. In adding the \$15,000, we split it up between the items in the budget, one-half (\$7500) being added to Salaries and Wages, and one-half being pro-rated among the subsidiary items of Maintenance and Operation. We add nothing to Capital Outlay, as we have to itemize each item in this account, and therefore we cannot successfully camouflage the addition.

This gross budget is what we present to the Board of Supervisors, stating (as in the illustration just given) that our District will require \$55,000 as shown in this budget, and the Board of Supervisors is asked to levy a tax sufficient to produce that amount of money (\$55,000), less whatever money is left in our fund after all bills for the current fiscal year have been paid. Suppose after our accounts have been audited in July we find we have \$16,000 cash left over. At the end of July we certify to the Board of Supervisors that we had \$16,000 left over as an unexpended balance (which remains in our fund for our uses only), and that the net amount required to be raised by taxes is \$39,000 (that is, \$55,000 minus \$16,000).

In other words, our procedure is something like the sale discount of the Hebrew merchant who put the discount onto the price first and then took it off,

I believe I have been occupying the rostrum long enough with this symposium, so I will now turn the meeting back to President Campbell.

Campbell: Are there any further items to be discussed?

Peters: I want to inquire about gasoline prices. I understand Mr. Hayes has worked out some special procedure by which he gets it for about six cents a gallon. His price on Diesel oil is also very low. It would be helpful if we could get data on the prices the different districts pay for some of our principal items of expense.

Gray: I understand Mr. Robinson is getting Diesel oil for 1/2 cent. less than we are. I wish I knew how he does it. We are about as close to the refineries as he is. If Mr. Peters will collect this information as to prices and quantities used in the different districts, it will help us all to get better prices.

Miles: I move that this conference send a Christmas greeting by wire to Tommy Aitken, wishing him luck. He is now in Puerto Rico and I am sure he will appreciate a word from us. I also suggest a letter be sent to Fred Rush, wishing a speedy recovery from his illness.

(There being no objection, it was so ordered).

Campbell: The Nominating Committee failed to appoint a Vice President and agreed to re-convene and nominate one.

Emerick: We would like to name Mr. Menefee as the Vice President. Upon being seconded, Mr. W. Percy Menefee, Superintendent of the Delta Mosquito Abatement District at Visalia was elected Vice President by unanimous vote.

Campbell: Shall we have a one or a two day conference next time?

Emerick: Personally I believe we get more out of a two day conference. In a one day conference there isn't time for any of the details it is worth while for us to discuss.

Campbell: If there is no objection, we will have a two day conference in December, 1942.

Mr. Hayes was to have given us some information on national defense matters. In his absence, has anyone anything to say on this matter?

Robinson: In this national emergency I think there are none of us not willing to do all we can to cooperate with the government and with each other. Some of us have pumping equipment and practically all of us have pick-up trucks. I phoned the Fire Department and offered our equipment for water or for chemicals. We are not using our large truck this winter, which is available for the service, hauling sand, etc. Our men that have phones and can be called, are available and the Farm Board has a key to our garage. The Fire Department will have the use of our equipment on call throughout the twenty four hours of the day. The idea struck me that it might be well if other districts who have these trucks do the same thing, offering their trucks for instance in carrying sand and helping with incendiary fires.

Campbell: I think a number of us already have done this. It is what we all should do.

Gray: Some of our equipment is quite useful in the case of incendiary bombs and also in demolition work that comes

afterwards. Our five gallon cylindrical pumps are useful too. You cannot throw water on the magnesium incendiary bombs, but a fine spray will accelerate their combustion and control the fire as well. These sprayers are also useful in combatting fires started by either thermite or petroleum type bombs. If you have any "Pyrene" or other carbon tetrachloride fire extinguishers, do not use them on high-temperature bombs, or you will generate phosgene, a very poisonous gas. For a similar reason "Foamite" should not be used, as the carbon dioxide will be broken down to poisonous carbon monoxide.

May I suggest that if we have any air raids, it will be necessary to keep track of all bomb craters and the basements of demolished buildings, for example. Water will accumulate in these places. I suggest that not only do you control mosquito breeding in these places, but that it will be interesting to keep them under observation to see what species of mosquitoes breed in them. I have had some interesting comments from England on mosquito breeding resulting from bombing raids.

Emerick: In my district we have signed up for twenty four hour emergency service as needed, and I know many of our districts are doing similar things and helping where they can in national defense.

Peters: Am I to proceed to collect the annual dues as was done this past year, making the same assessment?

Campbell: That is my understanding.

I now want to thank you for the splendid cooperation you have all given, and to thank you for the honor it has been to serve you as President of the California Mosquito Control Association this year.

I now take pleasure in turning the meeting over to our incoming President, Mr. Nicholson.

Nicholson: I don't know much about this but I'll do my best. May I ask what we are going to do about publishing the Proceedings?

Gray: As has been the custom, the Alameda County Mosquito Abatement District will transcribe and edit the Proceedings. This year we will not cut the stencils, but will expect the Association to pay for that work, as well as for the mimeographing, assembly, addressing and postage.

Heretofore we have sold extra copies of the Proceedings for only fifty cents. I suggest that they are worth more than that, and advise a price of \$1.50 per copy.

Peters: In talking it over, most of us have felt that \$1.50 was a lot of money. Wouldn't a charge of \$1.00 be more fitting? It seems to me it would be a good idea to print on the cover that the price is \$1.00.

Gray: All right, if that is the concensus of opinion, I move that a price of \$1.00 a copy for the Proceedings be established and that this price appear on the cover.

Miles: I will second that.

Bendel: I present a resolution that the University of California and especially the Division of Entomology and Parasitology of the College of Agriculture be thanked for their courtesy and cooperation. A copy should be sent to the President of the University.

Nicholson: There being no objection, it is so ordered, and the Secretary will send the letter of appreciation.

Robinson: I would like to include the fellows who set up the fine exhibit for us. The boys who helped set them up did a fine job.

Nicholson: If there is no objection, this also will be done by the Secretary.

Has Dr. Stewart anything to say in closing?

Stewart: I came to this conference with my mind a big question mark. I was uncertain about many things, and somewhat worried as you all are. But I was principally concerned as to the new demands which are going to be made upon us, and whether we are going to adjust ourselves quickly enough to handle the situations which we may face. I was afraid that some of us might not have sufficient mental elasticity to cope with changing conditions. But now I am tremendously relieved. I am leaving this meeting with absolute confidence that we are not only going to make the necessary adjustments, but that you already have done so. I think this has been a bang-up conference.

Nicholson: Professor Herms?

Herms: It has been a great pleasure to have you here as the guests of the University of California. There is a fine spirit of cooperation among you men, and the University and

my Division are always glad to help you in every way we can. Good luck to you all.

Nicholson: The Twelfth Annual Conference of the California Mosquito Control Association is now adjourned.

ROSTER OF ATTENDANCE

Alameda County Mosquito Abatement District, Oakland

Robert V. Dell, Trustee
Harold F. Gray, Engineer
Margaret A. Prefontaine, Clerk
Albert H. Miles, Foreman, Division No. 1
 John A. Duffey, Field Man
 George Fisher, Field Man
L. Percy Mapes, Foreman, Division No. 2
 Rex C. Lyndall, Field Man
 Ivan Best, Field Man
Roland Bendel, Foreman, Division No. 3
 Harley A. Dennis, Field Man
 M. J. Bernardo, Field Man

California State Department of Public Health

M. Dorothy Beck
Sidney F. Dommies, Jr.
Richard Peters

Contra Costa Mosquito Abatement District No. 1, Martinez

Ernest Campbell, Superintendent

Delta Mosquito Abatement District, Visalia

W. Percy Menefee, Superintendent

Dr. Morris Mosquito Abatement District, Bakersfield

Fred L. Hayes, Superintendent

Durham Mosquito Abatement District, Durham

F. M. Butler, Superintendent

East Side Pest Abatement District, Modesto

E. Chester Robinson, Superintendent
O. C. Adams
G. Kooy, Inspector
Floyd Smith, Inspector

Glenn County, Willows

P. V. Harrigan, Agricultural Commissioner

Idaho State Health Department, Boise

E. L. Berry, M.D., State Health Officer
H. Luke
Paul C. Ward

Los Angeles County Health Department

E. J. Bumiller, Chief Sanitary Inspector

Los Molinos Mosquito Abatement District, Los Molinos

Charles Pramme, President
Ivan V. Mattoon, Superintendent

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Yolo County Health Department, Woodland

S. M. Anderson

OBITUARY

Just as the stencil cutting for these Proceedings was being completed, we received word that Fred W. Rush, Superintendent of the Solano County Mosquito Abatement District died at his home in Suisun, California, on Tuesday, February 24, 1942, after a prolonged illness. He had been Superintendent since the organization of the District in 1930. He had not only done an excellent work in organizing and administering his District, but he was one of the first men to apply practically and successfully the principle of controlled reflooding as a method of eliminating hatchable Aedes eggs on salt marshes. He was personally likeable, most cooperative with his fellow workers, and esteemed by all. He was President of this Association in 1939.

THE IDENTIFICATION OF CALIFORNIA MOSQUITOES

By

~~William C. Reeves, Division of Entomology
and Parasitology, University of California~~

The purpose of the following list and keys of California mosquitoes is to serve as an aid to field identification. The keys are annotated with remarks concerning the breeding habits of the various species; as a knowledge of these habits can be of great aid in making a tentative field diagnosis. In taking a specimen through the larval keys it is often possible to reach a decision by using the type of larval habitat instead of morphological characters. Characters used in these keys, although often microscopic in size, are the most simple available. It must be understood, however, that a definite diagnosis to species is based only on thorough systematic study with the use of proper keys and microscopes, and not by a field key of this type.

Profuse illustrations of key characters serve as an aid to interpretation. There is no reason why the adults of the common species in California cannot be diagnosed in the field with the aid of nothing more than a 12 to 20 power hand lens. The keys in many parts follow those presented at the 1939 conference by Dr. S. B. Freeborn and Dr. T. H. G. Aitken. The recent addition of our California mosquitoes of several genera and species has made necessary the reworking of several of their keys; also, larval keys and numerous illustrations have been added.

The genus Uranotaenia has been omitted from these keys because of its rarity.

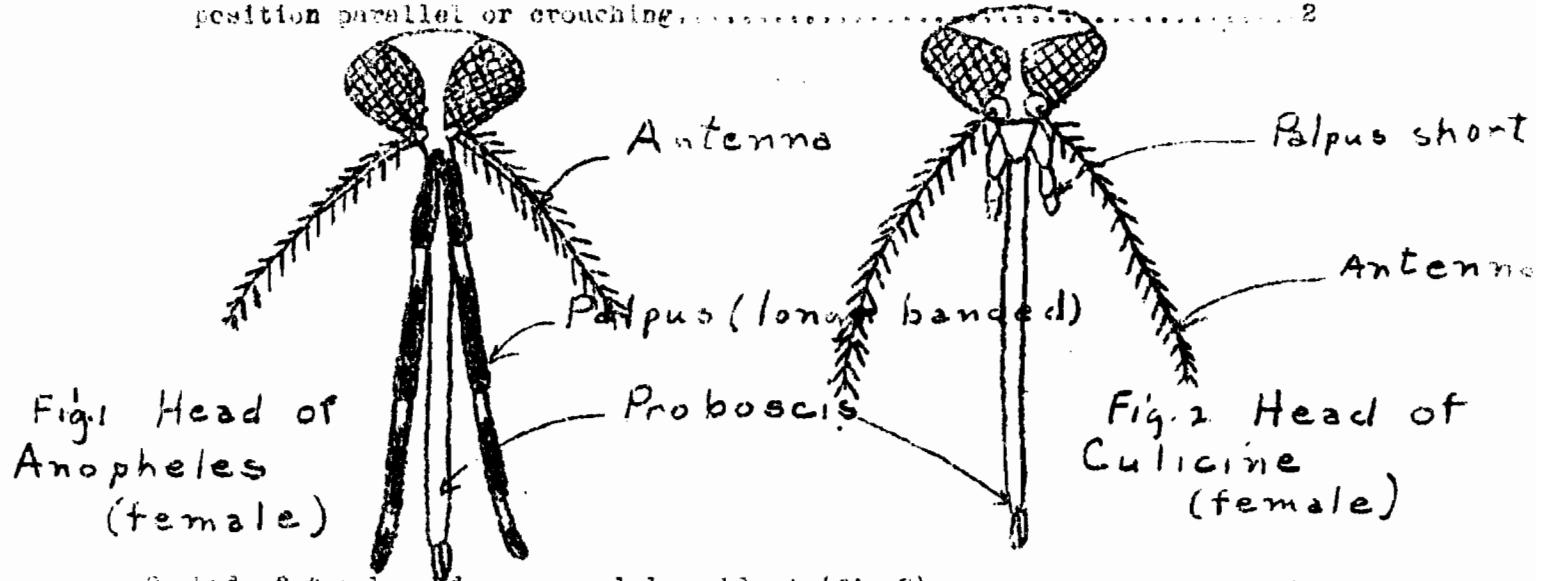
Adult and larval specimens have been arranged in the laboratory for study by use of this key. A number of qualified persons (marked by the wearing of white laboratory coats) will be there to answer your questions and assist. Do not hesitate to ask questions.

-
- 3. Anopheles trifasciatus (Say)
 - 4. Anopheles trifasciatus (Say)
 - 5. Anopheles trifasciatus (Say)
 - 6. Trinotus polytor Dyar
 - 7. Theobaldia macrakenae (Dyar & Knab)
 - 8. Theobaldia incidens (Thomson)
 - 9. Theobaldia inornata (Williston)
 - 10. Theobaldia impatiens (Walker)
 - 11. **Orthopodomyia signifera (Coquillett)
 - 12. **Mansonia perturbans (Walker)
 - 13. **Psorophora confians (Arribalzaga)
 - 14. **Aedes nigromaculis (Ludlow)
 - 15. Aedes taeniorhynchus (Liedermann)
 - 16. Aedes incroptus Dyar
 - 17. **Aedes flavescens (Lindley)
 - 18. Aedes fitchi palustris (Dyar)
 - 19. Aedes squamiger (Coquillett)
 - 20. Aedes dorsalis (Meigen)
 - 21. Aedes cataphylla Dyar
 - 22. Aedes communis tahoensis (Dyar)
 - 23. Aedes hexodontus Dyar
 - 24. Aedes impiger (Walker)
 - 25. **Aedes lateralis (Meigen)
 - 26. Aedes ventrovittis Dyar
 - 27. Aedes varipalpus (Coquillett)
 - 28. Aedes vexans (Meigen)
 - 29. Aedes cirereus hemiteles Dyar
 - 30. Culex apicalis Adams
 - 31. Culex stigmatosoma Dyar
 - 32. Culex tarsalis Coquillett
 - 33. Culex erythrothorax Dyar
 - 34. *Culex federalis Dyar
 - 35. Culex territans Walker
 - 36. Culex pipiens Linnaeus
 - 37. Culex quinquofasciatus Say
 - 38. Culex anips Dyar

- * Change of name since Proctor, 1926.
- ** Additional species since Proctor, 1926.

1. Length of palpi over surface of proboscis (fig.1); resting position of palpi Anopheles

Palpi of females less than half as long as proboscis (fig.2); resting position parallel or crouching..... 2



2. End of female abdomen rounded or blunt (fig.3)..... 4

End of female abdomen pointed (fig.4)..... 5

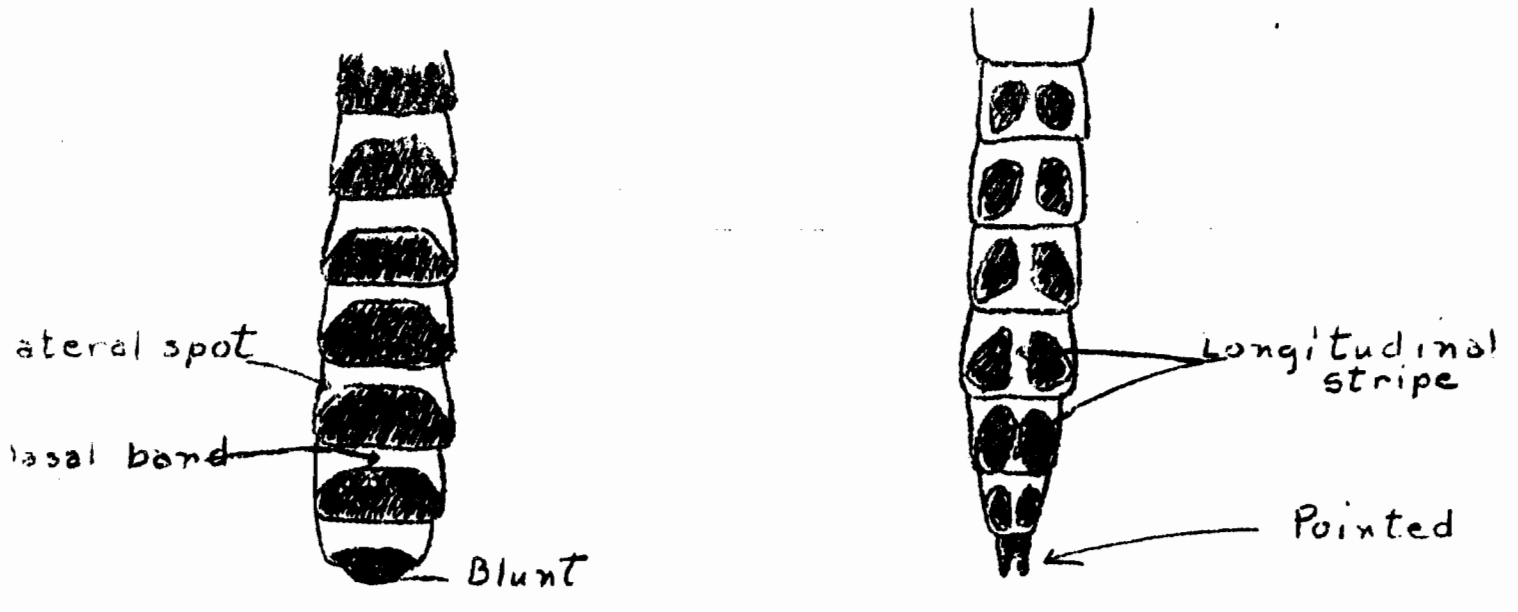


Fig. 3
Culex pipiens - abdomen

Fig. 4
Aedes dorsalis - abdomen

... of the ...
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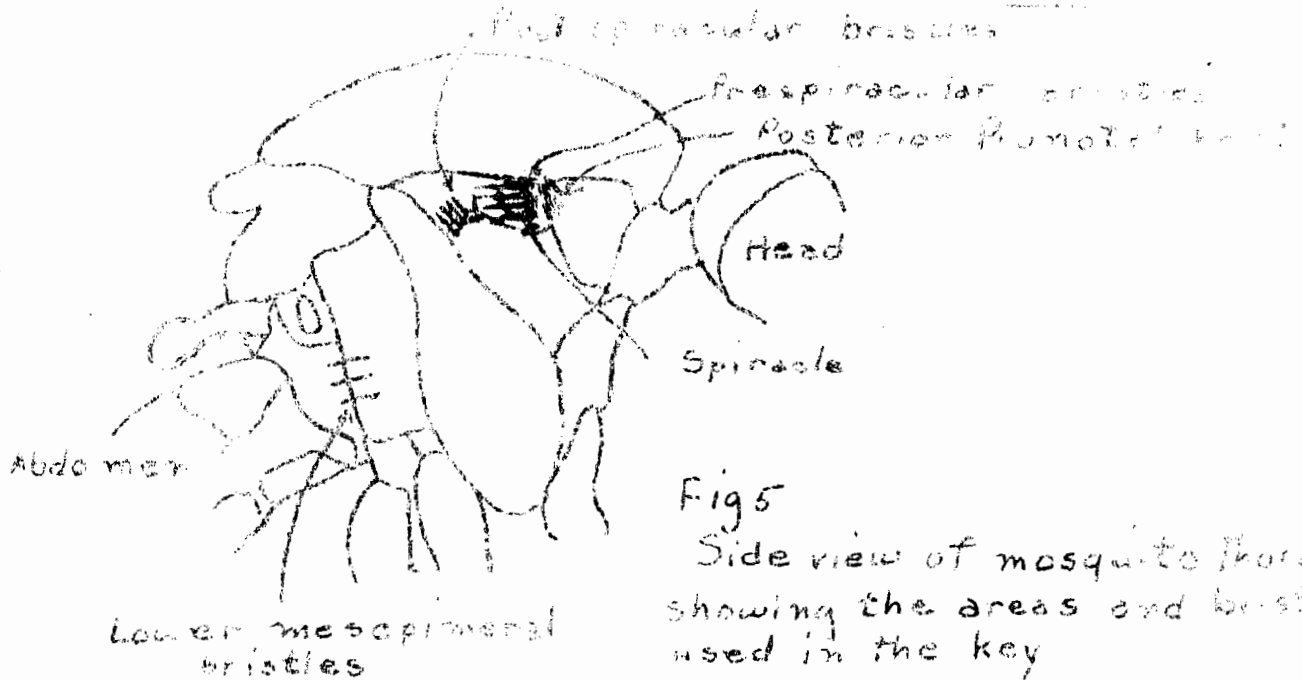


Fig 5
 Side view of mosquito thorax
 showing the areas and bristles
 used in the key

4. Prespiracular bristles present (Fig. 5); cross-veins nearly in line (Fig. 6); large spiracle (the one with spotted wings) ... Theobaldia
 Prespiracular bristles absent; cross-veins separated by length of posterior one (Fig. 7) 5

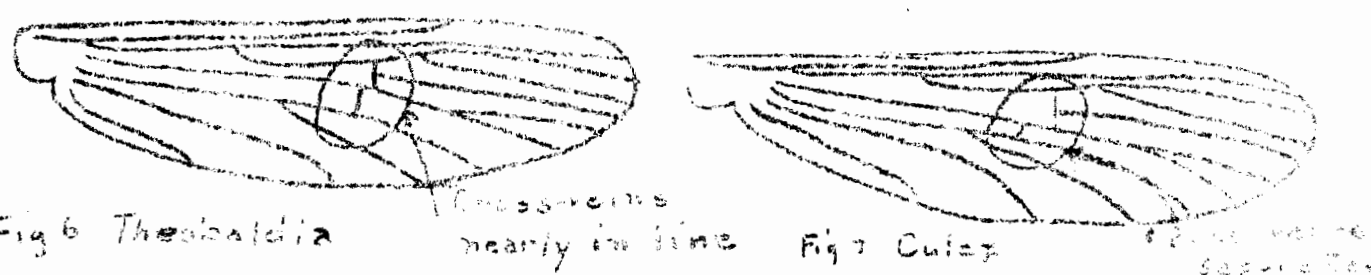


Fig 6 Theobaldia Cross-veins nearly in line Fig 7 Culex Cross-veins separated

5. Thorax dark brown with narrow longitudinal lines of silvery white scales; tree hole breeders Orthopneustie
 Thorax darker with narrow longitudinal lines of silvery white scales against a dark background; rarely breeding in tree holes
6. Wings with large anal cell, black light and dark (Fig. 8); 1st hind tarsal with a white ring at the outer end (Fig. 9); lower legs with bristles Leptotarsus
 Wings with small anal cell, black light and dark (Fig. 10); 1st hind tarsal with a white ring at the outer end (Fig. 11); lower legs with bristles

1. To have a siphon with a pecten.....

1. Insect with a siphon breathing tube (fig. 6) having lying parallel with the surface of the water.....

with elongate breathing tube (fig. 7) having bending at an angle with the water surface.....

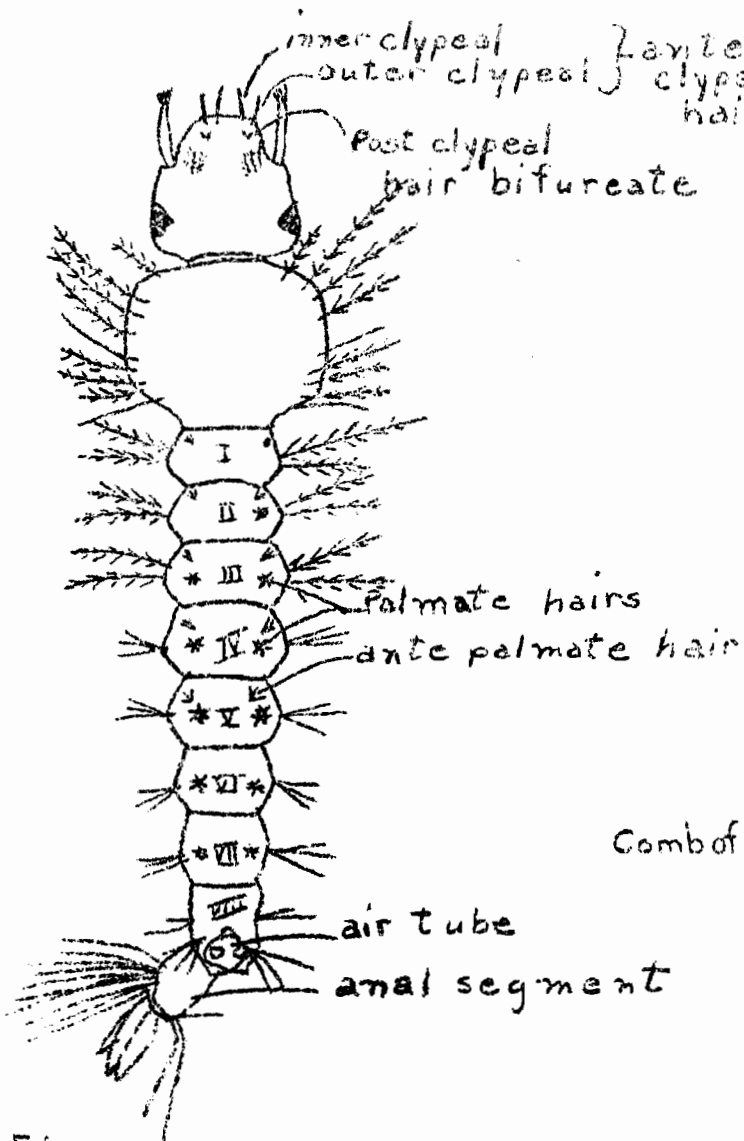


Fig. 6
Anopheline larva

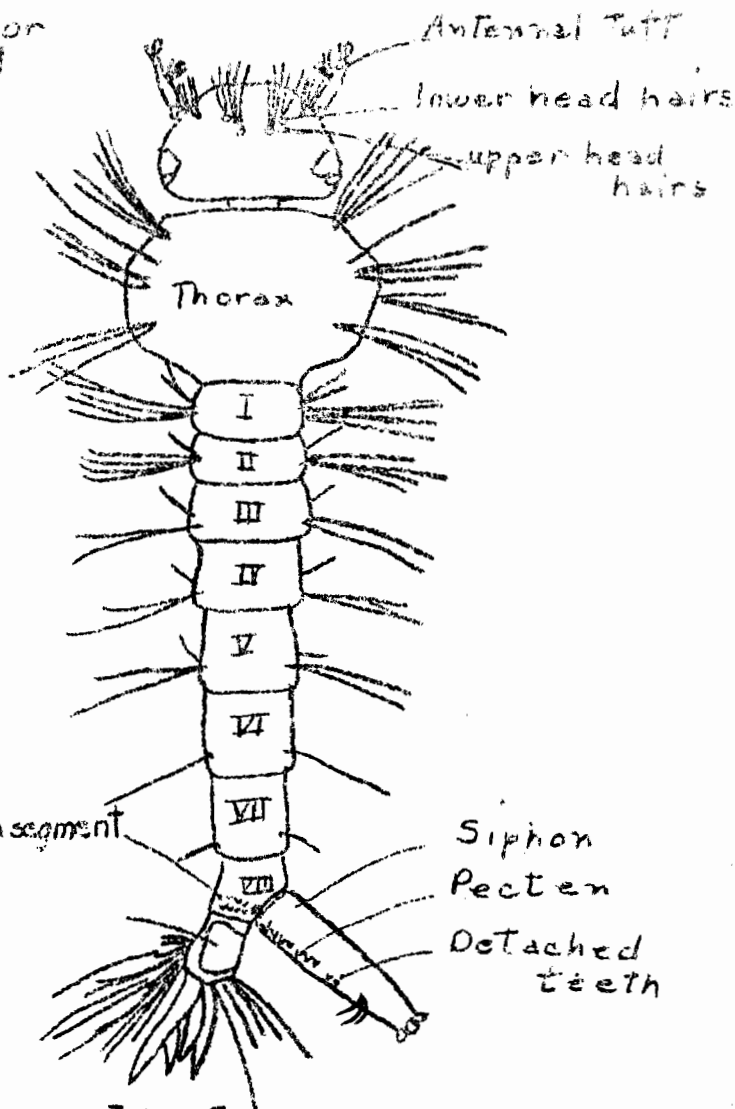


Fig. 7
Culicine larva

- 2. Air tube without pecten (figs. 8&9); breeding in unusual places such as tree holes, or with the siphon inserted in roots of aquatic plants....3
- Air tube with pecten (figs. 7&10).....4

3. Distal teeth of pecten replaced by long-toothed projections for piercing the walls of respiratory tubes (fig.8), which are not growth of the pecten itself.....Mansonia

Air-tube cylindrical not attached distally (fig.9); living in tree holes.....Orthopodomyia

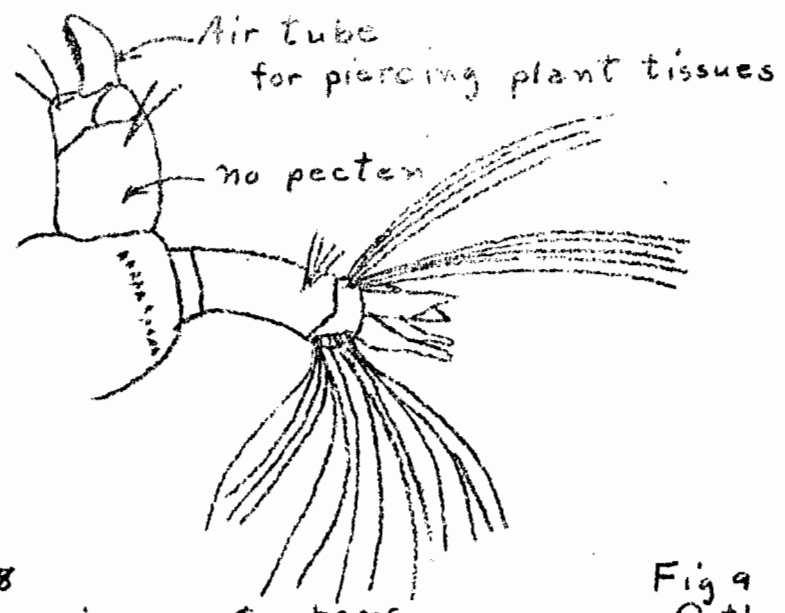


Fig 8
Mansonia perturbans

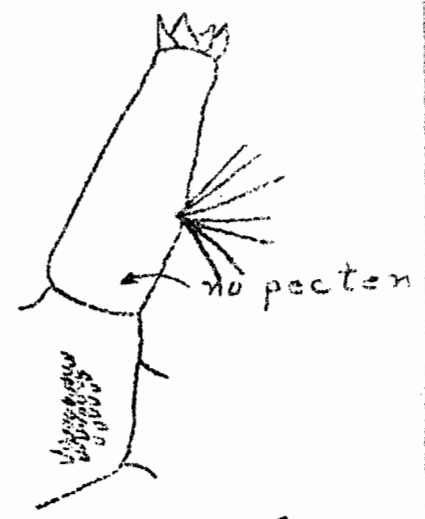


Fig 9
Orthopodomyia signifera

4. Distal teeth of pecten replaced by long hairs; single pecten hairtuft at the base of tube; robust larvae (fig.10).....Theobaldia

None of pecten teeth replaced by hairs; usually smaller larvae when mature.....5

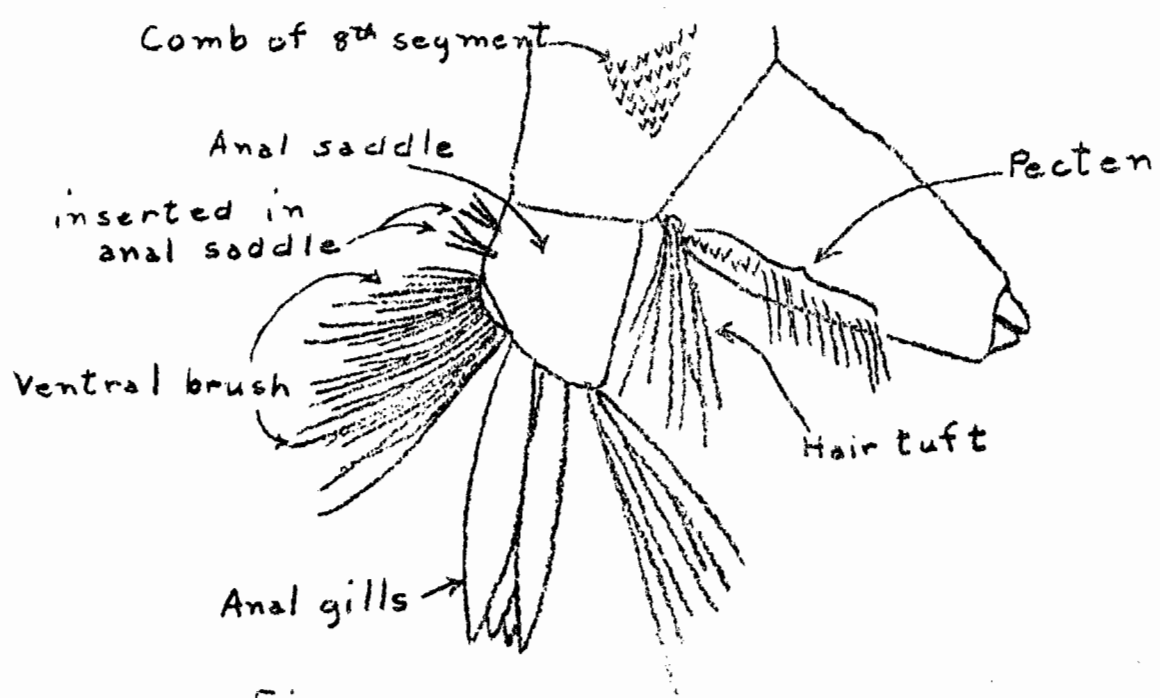


Fig 10
Theobaldia incidens

- 5. Breathing tube with a single hair tuft on each side (fig. 11) ... Culex
- Breathing tube with a single hair tuft on each side 6
- 6. Air tube inflated (fig. 14); comb of eighth segment consisting of a very few scales placed on a sclerotized plate; anal segment ringed by the dorsal plate, with hair tufts piercing the ring; (southeastern California only) Psorophora
- Air tube not inflated (fig. 15); comb of eighth segment consisting of many scales arising individually, without plate; anal segment not ringed by the dorsal plate, or, if ringed, the hair tufts posterior to the ring (widely distributed) Aedes

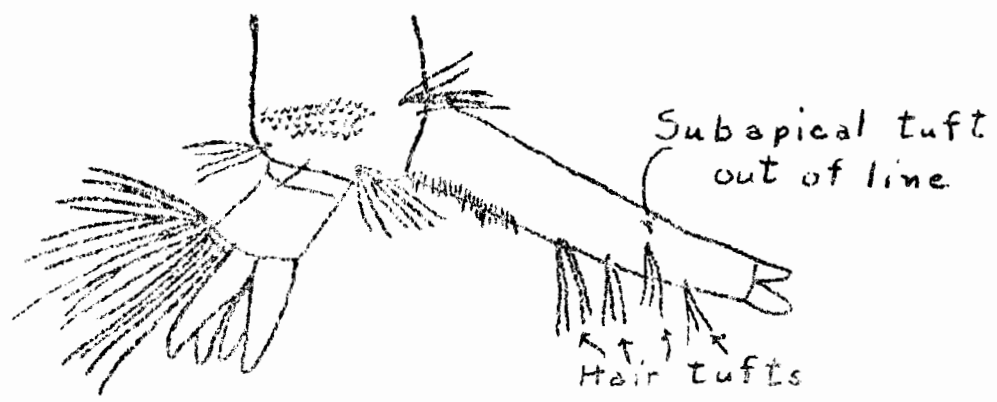


Fig 11
Culex pipiens

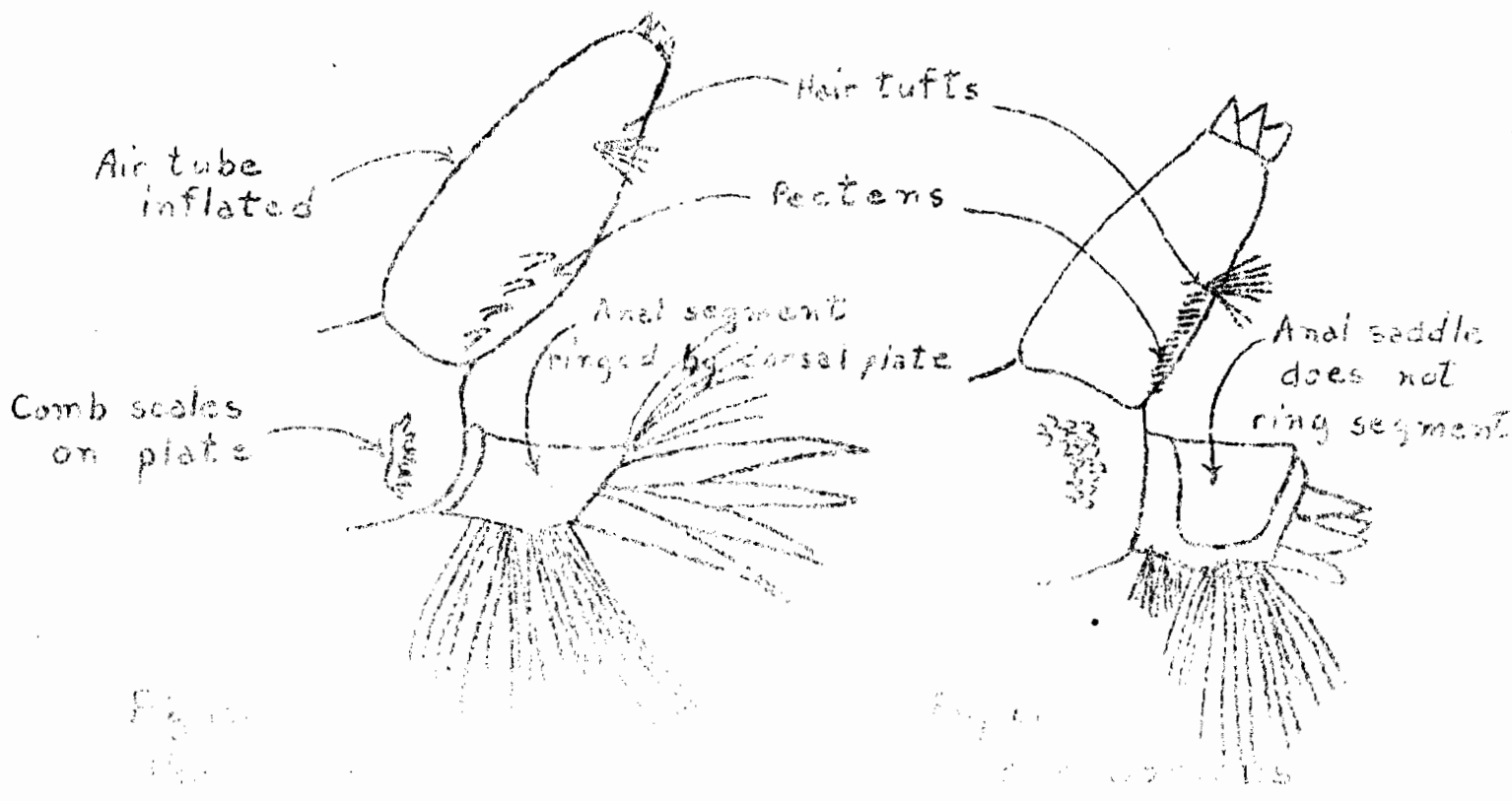


Fig 14

Fig 15

4. Wing scales unicolorous (southern coast and inland) maculipennis freeborni
 Wing scales bronzy or silvery in a spot at apex of wing (coastal from San Luis Obispo north) maculipennis occidentalis
5. Palpi with narrow white bands (fig. 10) .. pseudopunctipennis franciscanus
 Palpi unbanded punctipennis

Key to California Species of Anopheles Larvae

1. Anterior clypeal hairs (inner and outer) simple (fig. 6) (larvae usually in sunlit pools in algae along streams which are drying up)..... pseudopunctipennis franciscanus
 Anterior clypeal hairs (outer) branched 2
2. Antepalmar hair of abdominal segments IV and V usually two to three-branched (fig. 6); post-clypeal hair usually bifurcate at base (fig. 6) (larvae usually in clear shaded pools) punctipennis

Antepalmar hair of abdominal segments IV and V usually one-branched; post-clypeal hair multiple-branched (larvae in ponds and lagoons along coast) maculipennis occidentalis

Antepalmar hair of abdominal segments IV and V usually two-branched; post-clypeal hair normally two-branched near base or middle (larvae in fresh seepage water and roadside pools) maculipennis freeborni

Key to California Species of Theobaldia

1. Dark species with distinct black spots on wings; legs banded 2
 Relatively lighter species (brownish) without distinct wing spots; legs unbanded 3
2. Cross-veins of wings scaled; apex of wing pale bronze-colored; first four tarsal segments of fore- and midlegs ringed (relatively rare; dark woodland pools) macrorackenae
 Cross-veins unscaled (see other fig. 6); apex of wing dark; first two tarsal segments of fore- and midlegs ringed (very common; practically all water, except brackish and salt)..... incidens
3. Cross-veins scaled; wings without spots; basal abdominal bands, dull yellowish, broad, widen laterally to form lateral stripe (meadow pools; frequently found around farms) inornata
 Cross-veins unscaled; wings with indistinct spots at forks of veins, basal abdominal bands, pale whitish, narrow, not widened much laterally (woodland pools in mountains; rare) impatiens

Key to California Species of *Trichotria* Larvae

1. With the two anterior tufts of the ventral brush inserted in a cleft in the chitinization (fig. 14) (uncommon, dark woodland pools).....*macrackenae*

With these tufts puncturing the chitinization of the anal segment (fig. 10).....2

2. Both head tufts multiple, alike (rare; woodland pools in mountains).....*impatiens*

Lower head tuft with three long hairs, or if more, with two or three hairs stouter and longer, upper multiple.....3

3. Basal pecten teeth of air tube slender with one or two appressed denticles (fig. 15), (very common; all water, except brackish and salt).....*incidens*

Basal pecten teeth of air tube with three or four stout outstanding denticles (fig. 16), (many types of water meadow pools, brackish, and salt marsh).....*inornata*

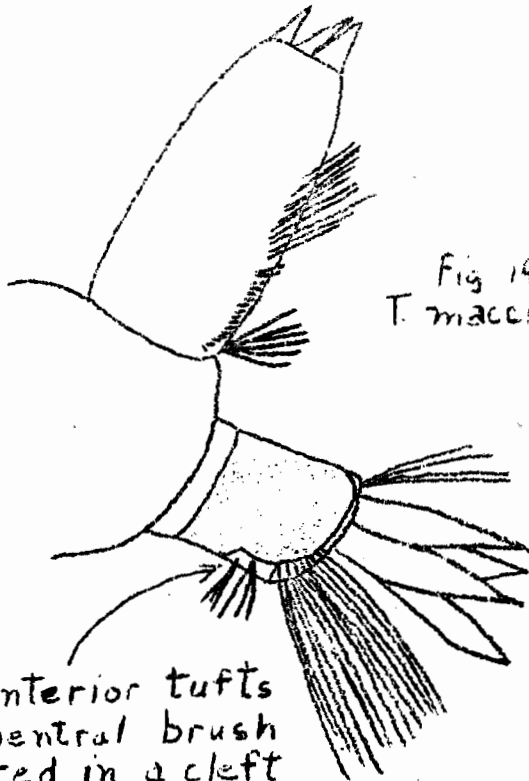


Fig 14
T. macrackenae

Two anterior tufts
of ventral brush
inserted in a cleft

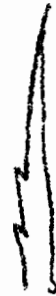


Fig 15
Pecten tooth of
T. incidens



Fig. 16
Pecten tooth of
T. inornata

Key to California Species of *Culex* Adults

1. Legs with pale bands (fig. 17).....2

Legs unbanded.....4

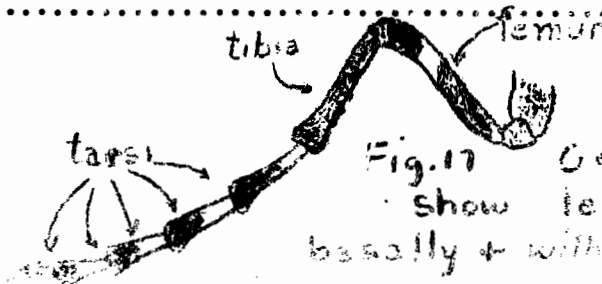
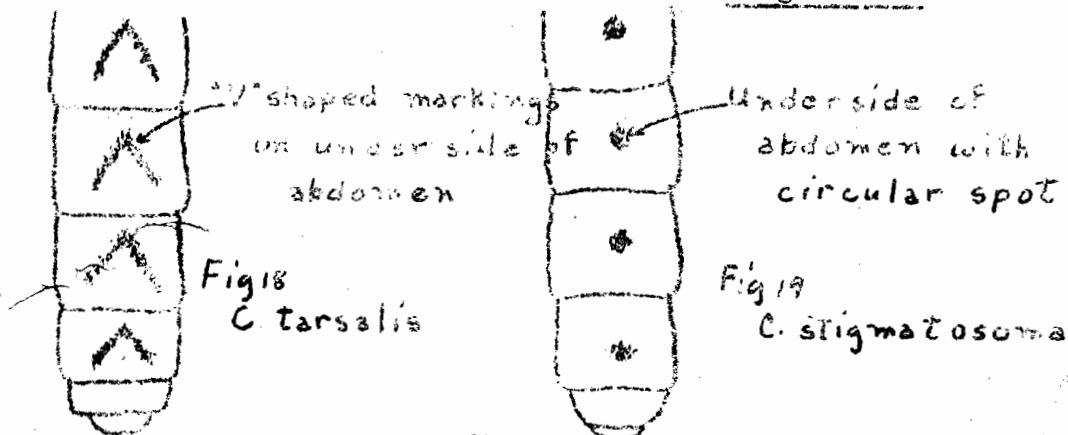


Fig. 17 General diagram to
show legs tarsi banded
basally + with banded femur

longitudinal white stripe on side of thorax on under side of legs
 "V"-shaped markings on under side of abdomen (fig. 18) (very common;
 variety of breeding places) tarsalis

legs without longitudinal stripe or spots; abdominal spots on under
 side of abdomen circular (fig. 19) (relatively common; predominantly
 fresh water pools) stigmatosoma

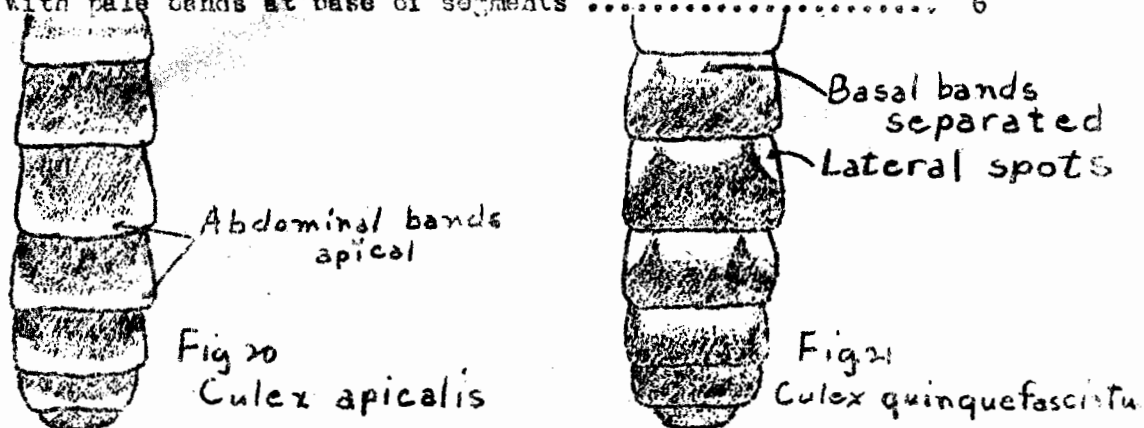


4. Abdomen without pale bands, only lateral spots (rare) ... anips, federalis

Abdomen with pale bands 5

5. Abdomen with pale bands at apices of segments (fig. 20) (relatively un-
 com., lucy; dark woodland pools and lake margins) apicalis

Abdomen with pale bands at base of segments 6



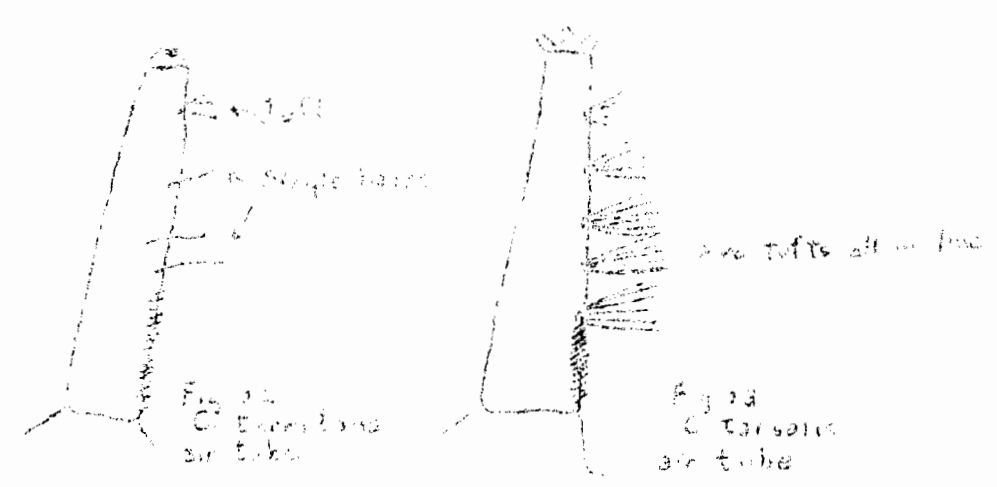
6. Thorax foxy-red; abdominal pale bands very narrow (relatively rare;
 shaded ponds and stream pools) erythrothorax

Thorax brown or greyish; abdominal bands broad 7

7. Basal abdominal pale bands connected with lateral spots (fig. 30) (common,
 particularly in towns; variety of breeding places) pipiens

Basal abdominal pale bands not connected with lateral spots (fig. 21),
 (quite common in south; variety of breeding places) quinquefasciatus

with an apical tuft of hairs. The subdorsal hairs of the third and fourth abdominal segments single or in threes.



- 3. Subapical tuft of air tube 7xl, dorsal out of line (fig. 22) 5
- Subapical tuft of air tube 7xl 5
- 4. Subdorsal hairs of third and fourth abdominal segments double (fig. 21) 5
- Subdorsal hairs of third and fourth abdominal segments single or in threes 5
- 4. Air tube 7xl, tapered erythrothorax
- Air tube 5xl, fusiiform (fig. 21) pinions
- 5. (3) Subdorsal hairs of third and fourth abdominal segments single quinquefasciatus
- Subdorsal hairs of third and fourth abdominal segments in threes stigmataeum
- 6. (2) Air tube 4xl, (fig. 23) subdorsal hairs of third and fourth abdominal segments in threes tarsalis
- Air tube 7xl, subdorsal hairs of third and fourth abdominal segments double sericalis

The larvae of E. anips and C. federalis are unknown.

2. Breeding in high mountain areas (altitude 10,000 ft.) (flood and irrigation water).....Aedes vexans
- Breeding in high mountain areas (altitude 10,000 ft.) (flood and irrigation water).....Aedes vexans, Aedes albopictus, Aedes canadensis, Aedes albopictus, Aedes albopictus
3. Tarsi with rings at both ends of segments.....4
- Tarsi with basal rings only (fig. 17).....5
4. Wing scales bi-colored predominantly gray; tarsal rings yellowish (dull-colored); abdomen frequently with longitudinal pale dorsal stripes (fig. 2) (salt marsh and flood irrigation water).....Aedes albopictus
- Wing scales dark (blackish); tarsal rings distinctly white; abdomen without longitudinal stripes; small nose pits (tracheoles).....Aedes albopictus
5. Wing scales broad and swollen (fig. 24), giving fuzzy, speckled or sooty appearance (salt marsh).....Aedes albopictus
- Wing scales normal (fig. 25).....6



Fig 24 Broad swollen wing scale

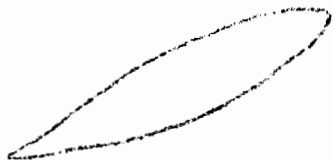


Fig 25 Normal wing scale

6. Tarsal rings very minute brownish and hard to see; basal abdominal pale bands indented in center (fig. 26) (flood and irrigation water).....Aedes vexans
- Tarsal rings normal; abdominal bands, if present, not indented.....7

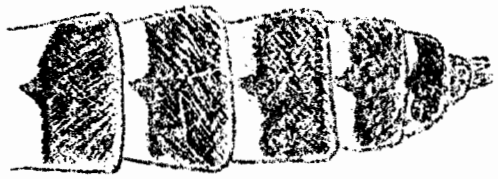


Fig 26 Abdomen of Aedes vexans (Showing indentations of basal bands)

- Abdominal bands absent; large dusky yellowish species (irrigation water northeastern counties) flavescens
- Basal abdominal pale bands present; usually smaller, darker species .. 8
8. Proboscis usually ringed; abdomen may have dorsal longitudinal stripe (fig. 4) 9
- Proboscis without pale ring; abdomen only with basal segmental bands (frequently snow-pool breeders) 10
9. Abdomen with dorsal longitudinal pale stripe, each segment appearing to have two black spots; proboscis not always ringed (irrigation water) .. nigromaculis
- Abdomen without longitudinal stripe, each segment with narrow basal band; proboscis ringed (southern salt marsh) taeniorhynchus
10. Wing scales chiefly black, few white ones on extreme anterior margin (meadow, river and lake pools, brackish water on coast) inorepitus
- Wing scales with many white ones (shallow grass pools of Sierras and northern counties) fitchi palustris

Key to California Species of Aedes Larvae

1. Pecten with detached teeth outwardly (fig. 7) 2
- Pecten without detached teeth outwardly (fig. 13) 7
2. Anal segment ringed by dorsal plate (fig. 12) (irrigation water) nigromaculis
- Anal segment not ringed by dorsal plate (fig. 13) 3
3. Pecten teeth present beyond hair tuft (snow mosquito) cataphylla
- Pecten teeth not present beyond hair tufts 4
4. Comb patch of 8th segment about 8-9 scales; air tube short and stout 2x1 (snow mosquito) ventrovittis
- Comb of about 10 plus scales; air tube more elongate (fig. 7) 5
5. Comb patch scales 3 rows deep (irrigation water northeastern counties) flavescens
- Comb patch scales 2 rows deep (fig. 7) 6
6. Air tube 3.5x1; lateral abdominal hairs single beyond second segment; lower head hairs multiple (snow mosquito) cinereus hemiteles
- Air tube 3x1; lateral abdominal hairs multiple; lower head hairs in 2's or 3's (fig. 7) (flood and irrigation water) vexans
7. (1) Anal segment ringed by dorsal plate (fig. 12) 8
- Anal segment not ringed by dorsal plate (fig. 13) 9
8. Air tube short, 1.5x1; upper and lower head hairs single, comb patch of 8th segment of about 18-20 scales (southern salt marsh).. taeniorhynchus