STUDIES ON THE NATURE OF MALATHION RESISTANCE IN A POPULATION OF ANOPHELES STEPHENSI FROM SOUTHERN IRAN

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Abstract. Malathion resistance in a population of Anopheles stephensi originating from Bandar Abbas, southern Iran was found to be intermediate in its expression. Repeated backcrossing accompanied by selection with a dosage of malathion known to kill most susceptibles yielded results not entirely consistent with the resistance being dependent on a single gene mechanism, though the use of synergists indicated the involvement of carboxylesterase only.

Introduction

Manouchehri et al. (1975) reported on the laboratory selection of malathion resistance in Anopheles stephensi Liston from 2 localities in Iran from progeny of the survivors of the exposure of adults to 3.2% malathion for one hour. The actual existence of malathion resistant individuals of this species in the field in Bandar Abbas, Iran, was reported by Manouchehri et al. (1976) and subsequent increasing trends in this resistance by Manouchehri et al. (1976b).

This paper reports on a laboratory study of the nature of this malathion resistance in a population of An. stephensi originating from Bandar Abbas, southern Iran.

Materials and Methods

The strains of Anopheles stephensi used were:

ST/15—a standard strain derived from a population originating from Delhi, India in about 1947 and presumed to be susceptible to all insecticides.

SM55 and E316—sub-colonies derived from a population ST/ROK originating from Roknabad, Minab, Bandar Abbas, southern Iran and supplied by Dr. A. V. Manouchehri of the Department of Envi-
environmental Health, School of Public Health, University of Tehran, Iran. 1976.
Malathion impregnated papers (5% in olive oil/1% solution) used for testing
the adults were supplied by the World Health Organization. The synergists
triphenyl phosphate (TP) and piperonyl butoxide (PB) were provided by Dr. F. J.
Oppenwoth of the Laboratory for Research on Insecticides, Wageningen,
Holland. S,S,S-tribusyl phosphorothioate (DEF) was supplied by Dr. R. M.
Sawicki of Rothamstead Experimental Station, Harpenden, England. The impreg-
nated papers of these were prepared locally at the maximum non-toxic dosage
of the appropriate synergist.
Mosquito rearing, maintenance and the
experimental procedure were carried out
under controlled conditions of tempera-
ture (25–28°C) and RH (70–80%). Standard rearing procedures adopted for
anopheles species were followed. Test-
ing procedures involved the standard
WHO adult susceptibility test using less
than 1 day old adult males and females.
In the ST/ROK population the propor-
tion of malathion resistant individuals
was low at the time of receipt from the
field. There was 99% mortality after 1
hour's exposure to 5% malathion among
a sample of 246. Selection was therefore
continued in the laboratory, but on a very
gradual basis with a view to maintaining
the genetic variability of the population,
knowing it to have been derived in the
first place from a very few eggs. The pro-
gen from the eggs from the field were
maintained over a number of generations
until a desired population size was
reached. In each of the following 5 gen-
erations, a sample of the population to be
selected was exposed to 5% malathion for
15 min and survivors were returned for
mating with the unexposed stock popula-
tion. In each of the last 5 generations the
entire population was treated with the
same dosage of malathion. The process of
initially exposing only a proportion, fol-
lowed by exposure of the entire popula-
tion, was repeated gradually increasing
the exposure time. Exposures of 30 min,
1 hr and 2 hr to 5% concentration were
involved with a minimum of 5 genera-
tions between each extension of exposure
time. Initially when selecting, particularly
at 1 and 2 hr exposures, the survivors
were small in number. This necessitated
generations of unselected maintenance
to the densities were re-established to
enable continued selection. It was ex-
pected that such a selection procedure
would avoid any rapid elimination of
background genetic material which might
eventually contribute to the stabilization
of resistance in the population. In addi-
tion, such partial selection may simulate
to some extent the type of selection that
might occur under field conditions. The
resulting population was maintained un-
selected for 3 more generations and
identified SM35. It showed 11% mortality
after 1 hr exposure to 5% malathion
among a sample of 616.
The E136 sub-colony was derived from
SM35 by single family selection. The pro-
cess of sib-mating (brother-sister mating)
accompanied by susceptibility testing was
followed with a large number of families
until one was encountered where all the
adults emerging from an egg batch of a
single female gave no mortality after one
hour's exposure to 5% malathion.
Further sib-matings from this family were
followed through 3 successive genera-
tions before 8 families showing no mor-
ality after exposure to 5% malathion for
1 hr were pooled to give strain E136
which was then continually pressurized
with malathion to ensure full homozygosity.
To study the mode of inheritance of
the resistance, the ST/15 and E136 strains
and the progeny of the cross between
them were exposed to 5% malathion for 1
hour. The hybrid offspring were also
backcrossed to ST/15, the progeny being
exposed to the same dosage of malathion.
The survivors were again backcrossed to
ST/15 and their offspring again exposed
to 5% malathion for 1 hour. This proce-
dure was repeated for 3 successive
backcrosses in an attempt to distinguish monofactorial from polyfactorial inheritance.

To gain some indication of the detoxication mechanisms involved in resistance, a sample of the SM35 population was initially exposed to an appropriate synergist and subsequently to malathion. At the same time, a comparable sample of the same population was tested with malathion by itself.

RESULTS AND DISCUSSION

A sample of 1,054 adults of the ST/15 population exposed to 5% malathion for 1 hr produced a 98% mortality. The exposure of 80 adults of the E136 strain to the same dosage produced no mortality at all. The F1 progeny of the cross between them on the other hand showed 28% (among a sample of 111) and 31% mortality (among a sample of 129) from the reciprocal matings. On this basis malathion resistance in the E136 population can be considered to be intermediate dominant in its expression and thus differs from the almost completely dominant nature of malathion resistance reported for a population of An. culicifacies Giles from India (Herath and Davidson 1981a).

Five percent malathion for 1 hr discriminates susceptibility from homozygous resistance but not from the heterozygous state. Taking into consideration the average mortality of 29.5% of the heterozygotes and the 2% survival of the susceptibles at this dosage, the mortalities in the backcross progenies would be expected to remain at about 64% in consecutive backcrosses with selection if inheritance is dependent on a single genetic factor.

Tables 1 and 2 analyze the data from 7 families of the first backcross and 8 of the second. In the first backcross (Table 1) all 7 families show a significant departure from the expected mortality of 64%, 6 of them showing less than this figure. The overall mortality was in fact only 42%. In the second backcross (Table 2) 3 out of 8 families produced the results expected of a single gene hypothesis but 5 of them did not. Half the families showed less than the expected mortalities though the overall figure was the expected one of 64%. A third backcross was made but the offspring were reared together instead of in single families. Here of 858 adults tested 382 died, a mortality of 44%, again a significant departure from the expected ($\chi^2 = 101.60; P = <0.01$).

<table>
<thead>
<tr>
<th>Family number</th>
<th>Number tested</th>
<th>Number dying</th>
<th>$\chi^2$ (1:1 expectation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>123</td>
<td>47</td>
<td>25.92*</td>
</tr>
<tr>
<td>2</td>
<td>63</td>
<td>29</td>
<td>5.06*</td>
</tr>
<tr>
<td>3</td>
<td>49</td>
<td>40</td>
<td>3.22*</td>
</tr>
<tr>
<td>4</td>
<td>73</td>
<td>27</td>
<td>17.02*</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>21</td>
<td>11.20*</td>
</tr>
<tr>
<td>6</td>
<td>107</td>
<td>37</td>
<td>28.29*</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>14</td>
<td>11.08*</td>
</tr>
<tr>
<td>Total</td>
<td>510</td>
<td>215</td>
<td>75.58*</td>
</tr>
</tbody>
</table>

+ P = < 0.05.

Table 2. Single family results of exposure to the discriminating dosage of malathion of the offspring of the first backcross of hybrid (resistant Iranian x susceptible Indian) to the susceptible Indian population of Anopheles stephensi.

<table>
<thead>
<tr>
<th>Family number</th>
<th>Number tested</th>
<th>Number dying</th>
<th>$\chi^2$ (1:1 expectation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>320</td>
<td>204</td>
<td>0.01</td>
</tr>
<tr>
<td>2</td>
<td>336</td>
<td>205</td>
<td>0.96</td>
</tr>
<tr>
<td>3</td>
<td>186</td>
<td>133</td>
<td>3.30</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>36</td>
<td>16.68*</td>
</tr>
<tr>
<td>5</td>
<td>110</td>
<td>56</td>
<td>5.60*</td>
</tr>
<tr>
<td>6</td>
<td>105</td>
<td>86</td>
<td>10.78*</td>
</tr>
<tr>
<td>7</td>
<td>187</td>
<td>165</td>
<td>33.74*</td>
</tr>
<tr>
<td>8</td>
<td>256</td>
<td>134</td>
<td>15.24*</td>
</tr>
<tr>
<td>Total</td>
<td>1600</td>
<td>1019</td>
<td>0.048</td>
</tr>
</tbody>
</table>

* P = < 0.05.
These results are not consistent with the single gene hypothesis, nor do they strongly indicate polyfactorial inheritance, as there is no consistent rise in mortality over the 3 successive backcrosses. However, results from the synergist work favor a single gene interpretation (see Table 3). If a single gene is involved in this resistance the departures from the expected values may have been caused by a loss of susceptibles during the rearing procedure. However, individual family yields of pupae from eggs were not recorded (as they were in the case of similar studies with An. culicifacies (Herath and Davidson 1981a)).

Pre-treatment of the SM55 population

<table>
<thead>
<tr>
<th>Insecticides &amp; synergest</th>
<th>Exposure time in minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Malathion 5%</td>
<td>0(20)</td>
</tr>
<tr>
<td>TPP + α-malathion</td>
<td>17(111)</td>
</tr>
<tr>
<td>DEF + malathion</td>
<td>—</td>
</tr>
<tr>
<td>PB + malathion</td>
<td>4(96)</td>
</tr>
<tr>
<td>Control + malathion</td>
<td>2(50)</td>
</tr>
</tbody>
</table>

(Figures in parentheses denote the number of mosquitoes tested.)

with TPP produced strong synergism with malathion suggesting carboxylesterase (CE) involvement in malathion resistance (see Table 3). DEF produced no synergism. With PB there was continuous antagonism at all the dosages tested. This could be attributed to the inhibition of mixed function oxidases (mfo's) involved in the oxidative conversion of the P=S bond to P=O during the activation of malathion to the toxic malaoxon. There was no evidence to suggest any mfo involvement in malathion detoxication such as was found in multiple resistant populations of An. culicifacies (Herath and Davidson 1981a) and An. albimanus Wiedemann (Herath and Davidson 1981b).

ACKNOWLEDGMENTS

The work described was done while one of us (F.R.J.H.) was studying for a Ph.D. degree and while financially supported by the WHO and UNDP. Research on mosquito resistance and other genetic aspects in the London School of Hygiene and Tropical Medicine is partly supported by funds from the World Health Organization and from the British Medical Research Council. We are grateful to Miss J. Hemingway for her constructive comments.

References Cited


