Plasmodium Gallinaceum: Development in Aedes aegypti Maintained on Various Carbohydrate Diets 1

A. Burns Weathersby and Raymond Noblet 2

Department of Entomology, University of Georgia, Athens, Georgia 30602

(Submitted for publication, 8 February 1973)

Weathersby, A. B. and Noblet, R. 1973. Plasmodium gallinaceum: Development in Aedes aegypti Maintained on Various Carbohydrate Diets. Experimental Parasitology 34, 426-431 (1973). Fructose, galactose, glucose, maltose, melibiose, and trehalose were evaluated for their nutritional and survival values for Aedes aegypti. The development of the exogenous stages of Plasmodium gallinaceum was evaluated by counting and averaging the number of oocysts developing on the midguts of Aedes aegypti.

The nutritional and survival value for A. aegypti was greatest on glucose, sucrose, and fructose and lowest on galactose and melibiose. Mosquitoes maintained on fructose produced the greatest numbers of oocysts. Only two other sugars, galactose and melibiose produced more oocysts than their respective controls. Glucose and maltose, both of which had high nutritive and survival value for A. aegypti were less efficient than the control (sucrose) for the development of P. gallinaceum.

INDEX DESCRIPTORS: Aedes aegypti; Susceptibility; Immunity, Invertebrate; Plasmodium gallinaceum; Carbohydrates; Malaria; Nutrition; Chickens.

The mosquito infected with plasmodia is particularly well adapted for studies of vector–parasite relationships including the immune responses of the vector. This model also lends itself to the study of specific biochemical, physiological, and nutritional factors in the overall host-parasite relationship. Oocyst counts from the stomach wall provide an exact determination of the rate of infection in individual mosquitoes or populations of mosquitoes infected with plasmodia.

The intrinsic factors influencing the ability of a mosquito to transmit malarial organisms presumably are of primary importance. The extrinsic factors are such that many more species could serve as vectors if they were physiologically able to support the parasite. Intrinsic immunity of mosquitoes to the etiologic agents of malaria is little understood and the factors controlling this immunity have not been defined. Results thus far indicate resistant or refractory species possess substances antagonistic to a given species of malarial agent. Early experiments (Weathersby 1952, 1954, 1960) showed the factors determining susceptibility were systemic in nature rather than being localized in any particular tissue. Later results (Weathersby 1963, 1965, 1967; Weathersby and McCall 1968; Weathersby et al. 1971) indicated that these determinants of immunity were antiblastic in nature.

A variety of genetic, anatomic, and physiological factors as well as cellular and humoral
responses seem to be involved in invertebrate immunity (Tripp 1969). It is also clear that dietary components of invertebrate hosts are important in determining susceptibility to parasites, although the basis for this is not understood (Tripp 1969). The purpose of this investigation was to determine the survival and nutritive value of seven widely used carbohydrates for susceptible Aedes aegypti and to evaluate the development of the exogenous stages of Plasmodium gallinaceum in Aedes aegypti maintained on these carbohydrates.

MATERIALS AND METHODS

Laboratory strains of Aedes aegypti (L.) and the 8A strain of Plasmodium gallinaceum were used throughout the study. The mosquitoes were maintained in a climate room at 27°C and 70% relative humidity employing standard mosquito rearing techniques. The parasite was maintained by alternate passage from Aedes aegypti to White Leghorn cockerels.

The first part of the study involved feeding the mosquitoes various carbohydrates and determining their longevity and survival on these materials. The female pupae were placed into crystallizing dishes in groups of 100 and allowed to emerge into small, cylindrical Lucite chambers. Soon after emergence the mosquitoes were presented 5% solutions of one of the following carbohydrates: galactose, glucose, fructose, maltose, melibiose, and trehalose. Mosquitoes fed sucrose served as controls. The solutions were changed daily and each morning the dead mosquitoes were removed from the cages and counted to determine percent mortality.

The second phase of the study was to evaluate the development of Plasmodium gallinaceum in Aedes aegypti fed the different carbohydrates. The mosquitoes were infected at 7–9 days of age by allowing them to feed on chicks with at least 20% parasitemia and gametocyte counts of at least 10 per 10 oil immersion fields. All mosquitoes were fed on the same chick but based on the results of Ward (1963) they were not fed simultaneously on the same chick.

The mosquitoes were dissected in Hank’s balanced salt solution 5–7 days after exposure and stained with mercuric chromo to facilitate oocyst counting (Eyles 1950). Relatively long-lasting temporary slides were prepared by sealing the cover slips with clear fingernail polish or paraffin and Vaseline. Oocyst counts were the means used to evaluate the relative success of parasite development.

RESULTS

The ages at which 50 and 100% mortality were reached are shown in Table I. The mortality rates for Aedes aegypti were very similar for the first 9 days in the groups receiving glucose, maltose, fructose, trehalose, and sucrose but were considerably higher than those fed galactose and melibiose. Survival was greatest on glucose, sucrose, and fructose, respectively, and was poorest on galactose followed by trehalose and melibiose. Survival was best on maltose for the first 15 days and then fell below the levels for glucose, fructose, and sucrose.

Overall the maximum survival periods for Aedes aegypti (Table I) were in the groups fed glucose (84 days), sucrose (72 days), and fructose (63 days). The shortest-lived were those fed melibiose and galactose which reached 100% mortality at 51 days.

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. used</th>
<th>Days to mortality of 50%</th>
<th>Days to mortality of 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>300</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Galactose</td>
<td>811</td>
<td>11</td>
<td>51</td>
</tr>
<tr>
<td>Glucose</td>
<td>813</td>
<td>24</td>
<td>84</td>
</tr>
<tr>
<td>Fructose</td>
<td>819</td>
<td>22</td>
<td>63</td>
</tr>
<tr>
<td>Maltose</td>
<td>851</td>
<td>21</td>
<td>58</td>
</tr>
<tr>
<td>Melibiose</td>
<td>750</td>
<td>15</td>
<td>51</td>
</tr>
<tr>
<td>Sucrose</td>
<td>980</td>
<td>25</td>
<td>70</td>
</tr>
<tr>
<td>Trehalose</td>
<td>805</td>
<td>16</td>
<td>56</td>
</tr>
</tbody>
</table>
The nutritional value (Table II) was calculated according to the following formula using the 50% mortality values (Galun and Fraenkel 1957).

\[
\text{N.V.} = \frac{\text{Survival on Test Sugar} - \text{Survival on Water}}{\text{Survival on Sucrose} - \text{Survival on Water}}
\]

This equation gives a comparison to the sucrose control which is given a value of unity. Glucose, fructose, and maltose, respectively, had the greatest nutritional value for *A. aegypti*. Again galactose and melibiose gave poorest results.

Due to the wide range in numbers of oocysts produced by different mosquitoes, relatively large samples were used and the experiment was replicated five times. The sample mean was the primary statistic used to evaluate the results. Each experimental group was compared to its control for significance. The data were analyzed by the *t* test for significance between means. The *t* values obtained indicated that oocyst production in *A. aegypti* fed these sugars was not significantly different from their controls. However, pronounced differences did occur as shown in Fig. 1.

Mosquitoes fed fructose produced more oocysts per mosquito than did mosquitoes fed on any other carbohydrate used including sucrose. The mean number of oocysts was 156 for the fructose-fed mosquitoes and 104 for the controls. The average percent infection for all mosquitoes maintained on fructose was 93 with a range of 75-100% in the various replicates as compared to 98% for the controls which ranged from 94 to 100%. The number of oocysts ranged from 1 to 715 in the experimentals and from 1 to 706 in the controls.

Galactose gave surprising results in regard to oocyst production. The experimental mosquitoes produced a mean of 120 oocysts as compared to 110 for the controls. The overall percent infection was 88 in the mosquitoes receiving galactose and 94 in the controls. Oocyst ranges of individual mosquitoes fed galactose and fructose were quite wide (1-715) and similar to those in the controls (1-706).

![Fig. 1. Mean number of oocysts of *Plasmodium gallinaceum* produced per mosquito in *Aedes aegypti* fed 5% solutions of various carbohydrate diets.](image-url)
In general, fewer oocysts were produced in mosquitoes fed glucose. The mean in this experiment was 66 oocysts per mosquito and 89 in the controls. The average percent infection was 91 in the experimentals and 98 in the controls. The oocyst range is again wide (1–593) in keeping with the infection patterns previously described.

Maltose was least efficient of the sugars used relative to oocyst production. The average number of oocysts produced was 64 with a range of 1–518 in contrast to 110 in the controls which had a range of 3–514. Overall infection was 98% for the controls and 64% for the experimentals. This compound had a nutritional value of 0.71 and survival was good in those mosquitoes maintained on it, resulting in a low infection comparable to that in mosquitoes fed glucose.

Mosquitoes fed melibiose exhibited infections paralleling those maintained on galactose. There was a considerable difference in the mean number of oocysts produced, 103 compared to 75, whereas the percent infection was similar (99 in the experimentals and 97 for the controls). The range in the number of oocysts produced was slightly lower than in the previous groups.

Trehalose also yielded unexpected results. Mosquitoes kept on trehalose produced fewer oocysts than the controls (Fig. 1). These mosquitoes were only 77% infected as compared to 95% for their controls. Trehalose-fed mosquitoes produced a mean of 104 oocysts per mosquito and their controls produced 115. Trehalose, unlike melibiose, did not appear to enhance parasite development.

**DISCUSSION**

To date there is little information concerning the factors controlling either susceptibility or the physiological relationships between vectors and parasites. Intrinsic immunity of mosquitoes to parasites such as *Plasmodium* is very difficult to study due to the technical problems encountered. Our results previously cited clearly indicate that the refractory *C. pipiens pipiens* possesses a factor(s) antagonistic to *P. gallinaceum*.

Although our early experiments and results indicate there are factors antagonistic or antiblastic to exogenous malaria stages in resistant mosquitoes, there are also indications that susceptibility can be enhanced by addition of other agents. Nutritional factors should affect the developing parasites directly.

Naturally occurring humoral immune factors have been reported from insects (Briggs 1964; Stephens 1964) but these substances were not chemically characterized. Weiser (1969) states there is no direct evidence of antibody-like substances limiting protozoan development in insects. On the other hand, there is no direct evidence such substances do not exist. The above information is in agreement with the systemic antiblastic response of *C. pipiens pipiens* to *P. gallinaceum* discovered by Weathersby (1952) and further substantiated in later studies (1954, 1960a, 1960b, 1965; Weathersby and McCall 1968).

Terzian (1950, 1955) and Terzian and co-workers (1949, 1952, 1953, 1960) reported that acids, bases, salts, antibiotics, hormones, vitamins, and other factors affect the relationship between *A. aegypti* and *P. gallinaceum*. This indicated more of a nutritional or athreptic nature of susceptibility than the previously discussed studies. Terzian's work indicates that this is a complex relationship with many factors involved. It seemed logical to assume that these factors which could be manipulated and shown to influence susceptibility may be naturally occurring factors determining immunity in such systems.

The carbohydrates used in these studies were chosen because they had varying degrees of nutritional value and would therefore yield information concerning the ability of *A. aegypti* to utilize these compounds and their relative effectiveness for supporting *P. gallinaceum* under these conditions. Re-
results showed that the relationship between \textit{A. aegypti} and \textit{P. gallinaceum} is affected by the diet of the mosquito. A diet of glucose or maltose which supported excellent survival of the mosquito was less efficient than the sucrose control diet for the development of \textit{P. gallinaceum}. In contrast, melibiose and galactose resulted in poor survival of the mosquito but parasite development was greater than in their controls. These results suggest that parasite development is enhanced by suboptimal nutrition of the mosquito. An interesting parallel can be drawn here with the work of Brooke (1945) who reported that birds on a poor diet suffered more severe infections of malarial plasmodia than those on a normal diet. According to Von Brand (1952) the experimental evidence of the effects of deficient diets on the course of malaria even in the vertebrate host is contradictory, but that as a whole, if the animal is starved, so is the parasite, under natural or laboratory conditions.

Ages at which 50 and 100\% mortality were reached differed somewhat from the results of Galun and Fraenkel (1957). This difference was probably attributable to our larger sample sizes and to the strain of mosquito used in the experiments.

All these findings indicate nutrition which is only one of a large number of interrelationships between \textit{A. aegypti} and \textit{P. gallinaceum}, is very important in parasite development although it is probably not a major determinant of mosquito immunity. Investigations now in progress indicate that metabolites and other dietary components can greatly influence mosquito susceptibility to \textit{P. gallinaceum} in both susceptible and refractory species of mosquitoes.

References


WEATHERSBY AND NOBLET


