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AUTHORITY

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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland
The question, with respect to the physiological genetic peculiarities of animal forms, far removed from one another, is compelling the attention of the investigators more and more towards an attempt to solve it. For invertebrates, there are some questions which have still only been slightly investigated, e.g., the significance of hormones and vitamins for these organisms. There are a series of very contradictory statements, especially for the representatives of insects. Thus, it still remains undecided, up to the present, whether hormones are found in insects, and if so their nature, how they act and where they are formed. Likewise, the question is still disputed as to whether insects need vitamins.

In a review of investigations, J. Zabinsky (1928) has indicated that roaches require no supplementary factors in their food in order to maintain existence and that they develop normally for three months when their food is completely free from all vitamins.

Cuyens (1912), Laub and Northrop (1916), Northrop (1917) and Roar (1923) have shown, that Prophila melangaster only requires vitamin B. Richardson (1925) believed that the case was the case for nectar for insects. On the requirement of insects for lipid soluble vitamins, however, there is no consistent opinion. Sweetman and Palmer (1923) incline to the viewpoint, that these vitamins are not required by insects, while L. Mill and T. Borrett (1922) believe, on the basis of
their very interesting research, the necessity of vitamin E for the development of the female genital system of the Apis mellifica.

The contradictory statements on the importance of lipoid soluble vitamins for the organism of insects induced and instituted experiments to determine the part which vitamin E played in the vitality of the male germ cells of Periplaneta orientalis.

**Material and Method**

We used 234 adult males of *P. orientalis*, that is, 154 test and 80 control animals. They received synthetic food consisting of the following:

- Casein: 18%
- Starch: 54%
- Lard: 22%
- Cod liver oil: 2%
- Salt mixture: 4% (C. Collum (185))

In addition to the above, they received 5% brewers yeast. The control animals, in addition to the basic food, received 2% dry lettuce leaves, which are rich in vitamin E. Both the test and control animals were given water. The roaches took very well to the finely ground, synthetic food.

The food used for feeding the roaches had been previously tested with rats.

After the roaches had received the synthetic food for a certain period of time, they were dissected. The ejaculatory duct was, with the accessory gland, laid upon a slide and disintegrated in a physiological common salt solution. The movement and form of the spermatocoea, freed thereby, were examined microscopically.
We see from Tables I and II, that the vitality of the spermatozoa of roaches, which were fed with food not containing vitamin E, as well as that of the animals receiving vitamin E, remains without any noticeable disturbance of their movability or structure.

**TABLE I**
**Test, 154 animals**

<table>
<thead>
<tr>
<th>Period of test, In days.</th>
<th>Number of dissected animals</th>
<th>Condition of the spermatozoa</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-15</td>
<td>6</td>
<td>Normal</td>
</tr>
<tr>
<td>21-30</td>
<td>11</td>
<td>&quot;</td>
</tr>
<tr>
<td>32-45</td>
<td>5</td>
<td>&quot;</td>
</tr>
<tr>
<td>47-59</td>
<td>18</td>
<td>&quot;</td>
</tr>
<tr>
<td>61-91</td>
<td>11</td>
<td>&quot;</td>
</tr>
<tr>
<td>105-114</td>
<td>3</td>
<td>&quot;</td>
</tr>
<tr>
<td>121-126</td>
<td>17</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

**TABLE II**
**Control, 50 animals**

<table>
<thead>
<tr>
<th>Period of test, In days.</th>
<th>Number of dissected animals</th>
<th>Condition of the spermatozoa</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>14</td>
<td>Normal</td>
</tr>
<tr>
<td>20-30</td>
<td>9</td>
<td>&quot;</td>
</tr>
<tr>
<td>40-65</td>
<td>4</td>
<td>&quot;</td>
</tr>
<tr>
<td>81-113</td>
<td>12</td>
<td>&quot;</td>
</tr>
<tr>
<td>123-140</td>
<td>6</td>
<td>&quot;</td>
</tr>
<tr>
<td>140-146</td>
<td>6</td>
<td>&quot;</td>
</tr>
</tbody>
</table>
The period of the test, about 130 days, was greater than the time, after which the male rats, with the same feeding conditions, no longer brought the spermatozoa to maturity.

These results made it necessary to investigate the following questions:
(1) Is the maintenance of the vitality of the spermatozoa of roaches conditioned by the vitamin stored in the body, or (2) that the germ cells of Periplaneta orientalis require no addition at all of vitamin with their food?

In order to solve this problem, we carried out tests on 1500 animals which were divided into 3 groups: the first (700 animals), received synthetic food without vitamin E, the second (control, 400 roaches), received synthetic food with the addition of vitamin E, in the form of lettuce ground to a powder, the third (control, 400 roaches), received, for one month, ordinary food made up of fresh vegetables and bran. As in the first test, the vitality of the spermatozoa, in all three groups, was preserved independently of the food and period of testing (3 months).

Towards the end of the test, the roaches were subjected to ether. The animals of each group were separated. We now had the task of ascertaining the content of vitamin E in the organism of the fixed roaches, that is, both the vitamin free and those that had received vitamin-containing food.

For this purpose, we prepared, by means of extraction by ether, the fat extract of the roaches in each group. The extract was dissolved in a small quantity of cod liver oil which contained no vitamin E and tested for its vitamin E content by the following tests.
Five female rats received, shortly after their weaning, synthetic foods (without vitamin E) of the same composition as the food fed to the roaches. After they reached puberty, they were tested to see if they suffered from E - avitaminosis. The females were paired with pubescent males who had received ordinary feeding. All of the females became pregnant, but after a certain time, a resorption of the embryos occurred and the sexual cycle of the female was again restored. Since, we convinced ourselves, in this way, that E - avitaminosis existed in the females concerned, we sought to restore their fertility. For this purpose, we used the extract taken from test and control roaches, "which was administered to pregnant females who were ill with E - avitaminosis at some time." In the first place, we tested the extract taken from 700 roaches of the first group. These were the ones fed with synthetic food which did not contain vitamin E. Before the beginning of pregnancy, this extract, in doses of 0.5-0.7 cc, was administered twice daily during a five day course; in addition, it was administered some time during the pregnancy. Nevertheless, on the 12th or 13th day of pregnancy, the palpate embryos were resorbed. Afterwards, in the same way, the fat extract of the second and third groups (which had been fed vitamin - containing food) was introduced into the pregnant females ill with E - avitaminosis. In this case also, it resulted in resorption of the embryos.

The testing of other extractions, from roaches, the vitamin fed ones as well as those fed foods without vitamins, also shows that in none of these cases, is the introduced extract capable of reproducing fertility in females suffering from E - avitaminosis. This either.
indicates that vitamin E is lacking in the extracts taken from the roaches of all groups or that the supply of vitamin E in the organism of the roach is very limited. Therefore, it is to be assumed that the maintenance of the vitality of the spermatozoa in roaches, fed foods without vitamin E for eight months, cannot be conditioned by a greater supply of this substance in the organism of the animals concerned. It may also be assumed that in contrast to the males, the vitality of mature spermatozoa in the sexual ducts of the male roaches is independent of vitamin E.

Summary:

1. The lack of vitamin E in the food, for a long period of time (4-8 months), in no way influences the vitality of the mature spermatozoa of male roaches.

2. Extracts taken from a large number of roaches, which have either received vitamin containing, or non-vitamin foods, are not able to restore the fertility of females suffering from E-vitaminosis.

3. It was shown, by biological tests, that the supply of vitamin E, in the organism of the roach, is either very limited or entirely lacking.