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ON THE COMPARATIVE OECOLOGY OF THE BLOOD-SUCKING DIPTERA

I. THE DOUBLE ROLE OF THE CROP IN THE WATER-ECONOMY OF MOSQUITOS

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1. Statement of the problem.

The crop of the Diptera takes the form of a single sack-like appendage to the esophagus, joined to the latter by a more or less lengthy channel. The role of the crop in blood sucking mosquitos (*Culicidae*) was first correctly explained by Falleroni (1926); its workings were studied in more detail by Dolmatova (1940). As Falleroni showed, the blood ingested by the mosquito from a skin-puncture goes directly to its mid-intestine, while free liquids ingested without the insertion of the proboscis go to the crop. Under natural circumstances, the crop receives either water or sweet plant-juices. The frequent presence of glucose in the contents of the crop of wild-caught *Anopheles maculipennis* females has been established by Ye.N.Nel'zina, research student at the Central Malaria Institute (in litteris).

Falleroni's rule also applies, it seems, to all the other blood-sucking Diptera which have kept the ability to drink free liquids /the *Tabanidae* (Olsuf'yev 1940), *Phlebotomus* (Dolmatova, 1942), *Stomoxys* (Kuzina, 1942)/. In all these groups, liquid taken into the crop is slowly and gradually transferred to the mid-intestine, where it is speedily absorbed. Not the slightest absorption takes place in the crop itself (McGregor, 1930; Dolmatova, 1940; Yaguzhinskaya (in litteris). The slow and gradual passage of water from the crop to the mid-intestine is of great importance in the mosquito. In the overwhelming majority of the periodically feeding blood-suckers, it is characteristic that liquids arriving in the mid-intestine are absorbed with an extraordinary speed, and that there is an equally rapid excretion of excess liquid from the body cavities by way of the Malpighian tubules (insects), coxal glands (ticks), nephridia (leechs) and emunctories in general. These features are obviously related to the enormous quantities of blood ingested by all the periodically feeding blood-suckers, and to the necessity of freeing their bodies as rapidly as possible of the weight of water contained in the blood (Beklemishev, 1940).

While mosquitos under the ordinary conditions of their existence are continually transpiring water, the quantity remaining in the body of the insect after getting rid of the excess is very often not enough to last over the period taken to digest blood (which under the most favorable temperature-conditions will amount to two days). When atmospheric moisture is low, this water will scarcely last for twenty-four hours, and the mosquito will be obliged to make up the loss of water by drinking [findings of Beklemishev and Detinova (1940) for *Anopheles superpictus* in Turkmenistan]. But in the majority of cases the mosquito can fly to seek water only at night. Consequently the water drunk at night must last the insect at least to the following night. If the water went directly to the mid-intestine, it would be absorbed and excreted just as rapidly as the water-content of the ingested blood. The crop with its wall impervious to water, plus the slow transfer of water from the crop to the mid-intestine, will guarantee the insect an economical utilization of the water which it drinks. No less than to the mosquito, an economical consumption of the water intake is essential to the horse-fly too, an insect which flies long distances for water, and also to other diptera which have a dual nutrition.*

However, the oecological significance of the mosquito's crop is not limited to the economical consumption of water; it is possible that the crop has one other vitally important rôle. Such a hypothesis was suggested by the experiments of Lister and Lloyd (1928) on the tsetse fly (Glossina). Tsetse flies feed exclusively on blood: they have completely lost the ability to drink free liquids, including water.

When the tsetse fly sucks blood, this blood goes in part to the crop and is immediately transferred in its entirety to the mid-intestine. If the fly is deceived into ingesting hot water through a membrane, instead of blood, it drops dead. What happens is that in the mid-intestine of the tsetse fly absorption takes place at the same high rate as in the case of other periodically feeding blood-sucking insects, and when the mid-intestine is filled with water, this water passes in very large quantities into the body cavity, causing an acute dilution of the haemolymph, necrosis of the organs, and death.

We might assume that the result would be the same if a large quantity of water was introduced into the blood cavity of a mosquito: the insect would die of haemolymph dilution. If such were indeed the case, then under its ordinary conditions of life the mosquito would be protected from this danger by:

Water swallowed going exclusively to the crop;
The impermeability of the crop wall;
The slow transfer of water from the crop to the mid-intestine.

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* Two mechanisms or sources of nutrition. (Translator.)

This adaptative apparatus would not merely guarantee the economical use of the water imbibed; it would be the very factor making it possible for the insect to drink water and hypotonic solutions. One might say that it is indeed only the pecularities of the functioning of the crop that make the phenomenon of dual nutrition (blood and free liquids) possible in the form in which it is seen in a number of the Diptera. À propros of this, we note that outside the order of Diptera this type of feeding is generally unknown.

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2. Experimental method.

In order to test the assumption of tho lethal effect on the mosquito of a large quantity of water flooding rapidly into the body cavity, we had recourse to an injection procedure. Injection of liquids into the body cavity of the mosquito has never before, so far as we are aware, been tried by anyone. Because of the small size and fragility of the subject of the experiment, the operation presents certain difficulties which, however, we were successful in overcoming.

The injection was performed by means of a syringe with a fine platinum needle. The mosquito was first anaesthetized with sulfuric ether. The needle was inserted into the pleura of the sixth or seventh segment and pushed forward, parallel to the pleural wall of the abdomen or slanting backwards, so as not to damage the intestine. In the experiments, we used young (Stage I on Perry's scale) starved females of *Anopheles maoulipennis* caught in a single-day shelter (for types of shelter, see Shipiçyna, 1934); that is, our experimental material was, as far as possible, uniform. Two liquids were injected: *aqua bidestillata* and Ringer's solution. Before and immediately after the injection, the mosquitos were weighed on a micro-analysis balance; the difference in weight was taken as the amount of the injected liquid. The quantity of liquid which passed into the abdomen showed very great differences: sometimes it exceeded the total weight of the insect, sometimes it was equal thereto, and sometimes it was only a fraction of the body weight (from 1/2 to 1/10 and even less). The mean weight of the females before the injection was 3.9 mg, extremes 2.0 mg and 6.1 mg (in a total of 169 individuals). The maximum weight of liquid injected was 5.1 mg. In unsuccessful injections, the liquid did not get into the body cavity at all; the insects received only a puncture of the pleura. Such individuals were used for comparison with the injected mosquitos. As a second control, we used intact mosquitos.

The mosquitos tested were placed in individual test-tubes with cotton-wool stoppers. They were given no opportunity to drink water. The mean room temperature was 23.6°C; the mean saturation deficiency -8.9 mm.

The test mosquitos and controls were all weighed daily on a micro-balance to determine their gradual loss of weight. We were able to study 104 females injected with aqua bidestillata, 65 injected with Ringer's solution, 13 with the abdominal wall pierced by the needle, and 53 controls (intact).

After injection, some of the mosquitos died almost before they were out of the anaesthesia; others quickly recovered, stood on their legs, and even took flight. These insects were in quite good spirits for the first day, and some continued to crawl about till the third and fourth days. We did not make any observations of differences in the symptoms of incipient death in the mosquitos injected with different liquids.

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3. Experimental results.

To settle the question of the possibility of mosquitos dying from haemolymph dilution, it is of fundamental importance to examine the mortality on the first day of the test: death from haemolymph dilution should of course be somewhat delayed, as in the case of the tsetse fly. If an insect does not die at once, and if its internal organs remain able to function, excretion of water by way of the Malpighian tubules will begin, and the normal osmotic pressure of the haemolymph will soon be restored. Thus we do not think that death during the immediately succeeding days can be ascribed to the osmotic pressure of the injected water. Accordingly, let us first examine the mosquito mortality during the first day of the experiment.

As might be expected, the mortality is found to be least among the control (intact) mosquitos. 7.6% of these died on the first day. Somewhat greater mortalities were observed among the mosquitos with the abdomen pierced and those injected with Ringer's solution (about equal in each of these two cases). The mortality was greatest among those injected with distilled water. However, even among these the mortality on the first day amounted only to 28.3%.

The higher mortality (as compared with the controls) among the mosquitos with the abdomen punctured and those injected with Ringer's solution is obviously due to the trauma caused in each case.

The mosquitos given the Ringer solution died in a somewhat greater percentage on the first day than those which had merely suffered an abdominal puncture, but the difference is within the limits of statistical error for the data. One might suppose that it would depend on the additional mechanical traumatic effect of the liquid introduced, but if so, the greatest mortality should be among those mosquitos which had received the greatest quantity of Ringer's solution. However, as may be seen from Fig.2, this is actually not the case.

The mortality among the mosquitos given distilled water was greater as compared with those given Ringer's solution, yet considered by itself it was still not very great. If the increased mortality of this group was due to the osmotic action of the water, it ought to be more marked in those individuals which had received the greatest quantity of water. Indeed we do find that among those mosquitos which had been injected with a quantity of water exceeding half the body weight, 50% died on the first day (Fig.3). Early mortality in this group was much greater than in any other group in our tests. On the other hand, among the mosquitos given lesser quantities of water, the mortality was on the average only a little greater in the case of the Ringer's solution or the simple puncture.

The early mortality among the mosquitos given Ringer's solution or which had the abdominal puncture amounted to about 15%, while among the controls it was 8%. If similar percentages of natural mortality and mortality from trauma are accepted in the case of the mosquitos given large quantities of distilled water, then the osmotic action of the water will account for the death of 35% of the number of mosquitos in the test. Thus the harmful effect of a large quantity of water flooding rapidly into the body cavity is beyond doubt, but on the other hand we see that *Anopheles maculipennis* is much more resistant as regards haemolymph dilution than Glossina. What may be the reason for this relatively greater resistance? From Vinogradskaya's experiments (1936), we know that the haemolymph pressure in *Anopheles maculipennis* fluctuates over a wide range even under the normal life-conditions of the insect: in the summer season from $\Delta = 0.6^{\circ}$ to $\Delta = -1.42^{\circ}$, in winter, to $\Delta = 2.6^{\circ}$; that is, the maximum observed pressure is several times greater than the minimum. If part of our mosquitos at the moment of their injection had a maximum haemolymph concentration, then the introduction of even a considerable quantity of water might have been insufficient to cause a fatal dilution of the haemolymph. On the other hand, if at the moment of injection a mosquito already had a low haemolymph concentration, the introduction of a large dose of water very likely produced a further lowering of the concentration, which was often fatal. Mosquitos caught during the daytime period present, as regards their haemolymph pressure, quite a varied picture (Vinogradskaya, 1936), and as we might expect from this, among the mosquitos given large injections of water death was the outcome only for certain individuals. Hence it is quite understandable that the injection of small doses of water does not generally result in death from osmotic causes; the reduction of osmotic pressure produced in this way is not harmful to the mosquito, with its fairly great resistance as regards variations in the haemolymph pressure. We note that death was sometimes caused by the injection of quantities of water which from the oecological point of view are not excessively great, since the mosquito is quite capable of drinking similar quantities.

The mortality on the days after the first day of the experiment no longer bore any relation to haemolymph dilution. It was mainly due to loss of water and to some extent trauma. As seen from Fig.1, the control mosquitos had the longest mean lifetime. In this group, the time-distribution curve of mortality was almost symmetrical, the mode being on the third day.

The mosquitos with only the abdominal puncture are, on the first day, indistinguishable from the others. On the second day, they show the maximum mortality, and the last of them die on the third day. The picture is almost the same for the mosquitos injected with very small doses (1/4 of the body weight or less) of water (Fig.3) or Ringer's solution (Fig.2). These groups also show a low mortality on the first day and die out entirely, or almost entirely, on the second day.

The mosquitos injected with Ringer's solution exhibit a simple and clear mathematical relationship: the greater the amount of solution, the longer the mean lifetime and the higher the percentage surviving to the third and fourth days of the test.

The mosquitos injected with water show a more complicated picture. As we go from the minimum doses to higher doses, the lifetimes at first increase, as in the case of the Ringer's solution. Survival until the fourth day of the test is observed to be a maximum for quantities of injected water equal to 1/3 to 1/4 of the body weight. At a dose of 1/2 to 1/3 of the body weight, the percentage of individuals surviving to the fourth day was only a little less. Of the mosquitos receiving the maximum doses (1/2 of the body weight and up), one-half die on the first day, as we have seen, and not one lives to the fourth day; on the third day, however, there is still a considerable number surviving.

Thus we may in general say that the mosquitos given Ringer's solution lived longer than those with simply the abdominal puncture, and the greater the amount injected, the longer they live; the same would be true of the mosquitos injected withwater, if it were not for the harmful action of over-dilution of the haemolymph which occurs with the maximum doses.

The obvious explanation of these facts is that in such tests, at high temperature and at quite low humidity, the mosquitos die of desiccation, and that the desiccation is retarded by the presence of injected water.

This conclusion is confirmed by the results obtained in weighing the insects. All the experimental mosquitos in the test lost water and diminished in weight. The rapid loss of weight in the injected mosquitos was due, on the one hand to transpiration, and on the other hand to excretion of water by way of the Malpighian tubules. Excretion of the injected water through the anal orifice began very soon: ten minutes after the injection, it was in full progress, one of the mosquitos actually splattering water in droplets. Most of all was excreted by those mosquitos which had been given Ringer's solution.

Two-thirds of the mosquitos (both of those given water and of those given Ringer's solution) got rid of the whole of the injected liquid during the very first day, and from the second day on began to lose their original water reserves. As will be seen from the weights quoted, it was only among the mosquitos given the maximum injected doses that there were individuals which had not expended all the injected water by the end of the first day, and thus had not yet begun to use up their reserves (and the greater the dose, the greater the number of such individuals). Some of the mosquitos which had not fully lost their injected water by that time lived to the second or even to the third day. The rest had by this time lost their ordinary reserve of water and perished. Death for the most part occured when the loss of water reached 30-40% of the initial weight of the insect (see Table).

Thus the greater survival power of the mosquitos given the largest injections of liquid is entirely understandable. They have been given a store of water which preserves them for some time from desiccation. However, they expend this store of water in a most extravagant manner: when given large doses, the greater part of it is immediately excreted by way of the Malpighian tubules, and the survival power is a long way from being proportional to the dose injected.

This example once again enables us to form an idea of the importance of the crop in economizing water expenditure.

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4. Conclusions.

1) The malaria mosquito, in its normal life, periodically ingests large doses of liquid, and in the intervals between the ingestions loses water very rapidly; it possesses, as shown by Vinogradskaya, a non-constant body-fluid osmotic pressure, and consequently is able to tolerate sizable fluctuations of the haemolymph concentration. The same thing is found in experiment. The

rapid injection of small doses (not higher than half the body weight) of distilled water into the body cavity does not do the insect any harm. The injection of more massive doses (not exceeding, however, the quantity of water which the insect could drink) is often lethal ... apparently in those cases when the haemolymph concentration is already quite low to begin with.

2) Liquid injected parenterally (or more properly speaking, in any other way than via the crop) is expended by the mosquito at a very extravagant rate: the whole of its excess liquid is rapidly excreted by way of the Malpighi canals, and only slight use made of it to cover expenditures in the normal water-economy of the insect. What happens to the injected water is quite the same as what happens to the water content of ingested blood, which also by-passes the crop to go directly to the mid-intestine, and from there into the body cavity.

3) Thus the crop, with its water-impermeable wall and the very slow transfer of its contents to the mid-intestine, is an adaptation which in the first place guarantees the economical use of imbibed water or other hypotonic liquids, and which in the second place protects the body fluids of the insect from extreme variations of concentration, variations which are not only undesirable but dangerous to it.

The result at which we arrive is that the functioning of the crop enables the blood-sucking Diptera to overcome the extraordinarily rapid absorption of liquid by the walls of the mid-intestine, which is characteristic of all the periodically feeding hematophages, and enables them to make use of a supplementary intake of hypotonic solutions ... water and plant juices. In other words, the crop makes possible that <u>dual nutrition</u> which is so characteristic of the modus vivendi of the blood-sucking Diptera (Beklemishev, 1942) and which has been lost only by the most specialized forms ... the tsetse fly and Puppipara.

In conclusion, I extend may profound thanks to Professor V.N.Beklemishev for his supervision and for a number of valuable hints; also to G.P.Mandryka for assistance in the work.

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* German spelling of Vinogradskaja as used in the text. (Translator.)

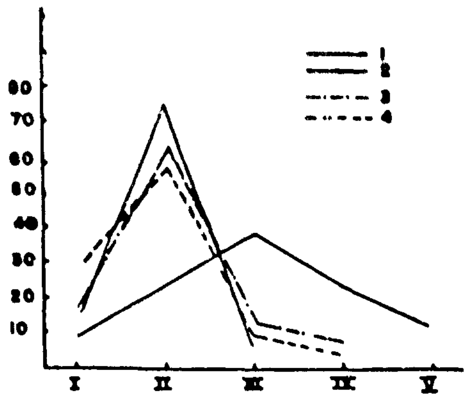

Fig.1. Mortality in injected mosquitos and controls, shown in percent of the initial number of mosquitos in each class. On the horizontal axis, days after the start of the experiment.

1. Controls (intact insects).
2. Insects with abdominal puncture only.
3. With Ringer's solution injected.
4. With distilled water injected.

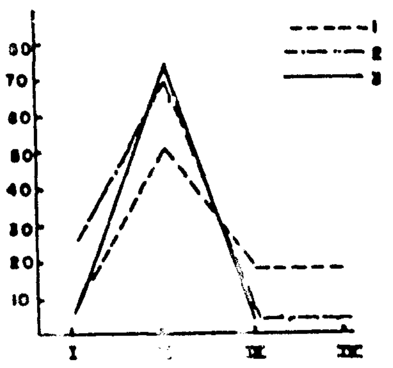

Fig.2. Mortality in mosquitos injected with Ringer's solution. The curves are constructed in the same way in the preceeding figure.

1. Mosquitos with a quantity of Ringer's solution injected exceeding 1/2 of their body weight.
2. Injected with a quantity equal to 1/4 to 1/2 the body weight.
3. Injected with less than 1/4 of the body weight.

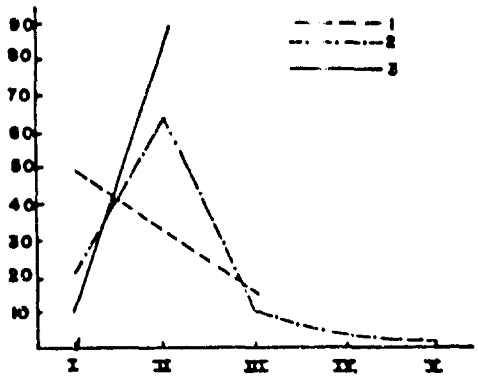

Fig.3. Mortality in mosquitos injected with different doses of distilled water. Curves constructed as in previous figures.

1. Mosquitos injected with a quantity of water exceeding 1/2 the body weight.
2. Injected with 1/4 to 1/2 the body weight.
3. Injected with less than 1/4 of the body weight.

Comparative Loss of Body-weight in Mosquitos, with and without Injection, and with Needle-puncture Alone (in % of Body Weight).

iquid jection	Loss of Body-weight in Mosquitos for Different Amounts of Injected Liquid in Fractions of Body-weight											Arithmetical mean of injections	Mosquitos with needle puncture (no liquid injected)
	Equal or greater than body weight	1 to $\frac{1}{2}$ body weight	$\frac{1}{2}$ - $\frac{1}{3}$	$\frac{1}{3}$ - $\frac{1}{4}$	$\frac{1}{4}$ - $\frac{1}{5}$	$\frac{1}{5}$ - $\frac{1}{6}$	$\frac{1}{6}$ - $\frac{1}{7}$	$\frac{1}{7}$ - $\frac{1}{8}$	$\frac{1}{8}$ - $\frac{1}{9}$	$\frac{1}{9}$ - $\frac{1}{10}$	$\frac{1}{10}$ or less		
stilled water, inger's olution	-	9.4	2.7	10.5	10.6	12.0	6.0	12.5	18.5	11.0	10.0	10.7	17.6
	-	10.4	13.2	14.5	14.0	25.3	18.0	-	7.5	-	19.3	15.0	18.8
"	-	-	24.3	14.5	37.3	25.7	8.5	31.0	39.5	-	-	26.3	-
	14.0	25.5	24.7	20.5	38.0	-	-	-	-	-	-	27	-
"	-	11.1	13.0	27.3	-	-	-	-	-	-	-	23.0	-
	-	-	-	-	-	-	-	-	-	-	-	-	-
"	-	-	31.4	31.3	-	-	-	-	-	-	-	31.4	-
	-	-	-	-	-	-	-	-	-	-	-	-	-

Comparative Loss of Body-weight in Mosquitos, with and without Injection, and with Needle-puncture Alone (in % of Body Weight).

Survival of mosquitos after injection, and time of observation.	Liquid injection	Loss of Body-weight in Mosquitos for Different Amounts of Injected Liquid in Fractions of Body-weight										
		Equal or greater than body weight	1 to $\frac{1}{2}$ body weight	$\frac{1}{2}$ - $\frac{1}{3}$	$\frac{1}{3}$ - $\frac{1}{4}$	$\frac{1}{4}$ - $\frac{1}{5}$	$\frac{1}{5}$ - $\frac{1}{6}$	$\frac{1}{6}$ - $\frac{1}{7}$	$\frac{1}{7}$ - $\frac{1}{8}$	$\frac{1}{8}$ - $\frac{1}{9}$	$\frac{1}{9}$ - $\frac{1}{10}$	$\frac{1}{10}$ or less
1st day (1-10 hrs.)	Distilled water, Ringer's Solution	-	9.4	2.7	10.5	10.6	12.0	6.0	12.5	18.5	11.0	10.0
		-	10.4	13.2	14.5	14.0	25.3	18.0	-	7.5	-	19.3
2nd day (20-35 hrs.)	"	-	-	24.3	14.5	37.3	2[illegible].7	8.5	31.0	39.5	-	-
		14.0	25.5	24.7	20.5	38.0	-	-	-	-	-	-
3rd day (45-57 hrs.)	"	-	11.1	13.0	27.3	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-	-	-	-
4th day (70-80 hrs.)	"	-	-	31.4	31.3	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-	-	-	-