STUDIES IN MOSQUITO BIONOMICS.

By MAJOR C. H. H. HAROLD.
Royal Army Medical Corps.

(Continued from p. 94.)

LARVICIDAL ACTION OF CRESOL.

Early in 1925 a drum of cresol was received for an examination of its efficiency as a larvicide. As regards its chemical and physical conditions and germicidal action it conformed to specification. It had been stated that on account of its non-effective character the anti-mosquito work in a particular Crown colony was disorganized and that it was inferior to a similar preparation manufactured by a rival firm. It should be pointed out that the use of cresol in the Army is normally restricted to germicidal requirements and the specification is based on these. As medical and entomological journals frequently contain details regarding the efficiency of cresols, it is thought that the insertion of the following will not add appreciably to existing confusion.

On the face of it, it would appear a simple matter to mix a little cresol in water, drop a few larvae into the solution and note the effects, but at the same time, from consideration of the literature, some good reasons must exist to account for the wide divergence in the results obtained.

The biological tests for these disinfectants are germicidal ones, and it is also claimed that many of these preparations are non-toxic to animals. As the mosquito belongs to the animal kingdom, it would appear inadvisable to base any speculations regarding their utility on the results of such tests. In addition the majority of these disinfectants are compounded with emulsifying agents, olein, resin, castor-oil made into soaps, and stabilizers such as glue and dextrin are also used. The complex which maintains a fine homogeneous emulsion in the presence of such substances as salt or urine is of particular value in promotion of germicidal action.

The next point is the character of the chemical in suspension. Undoubtedly in the past O, M, and P cresols were components of such fluids, but I have been informed that few of them contain anything but the merest trace of these. In their manufacture use is made of the higher di-methyl and tri-methyl phenoloids which possess a lower toxicity and a higher germicidal power, and some are credited with a R.W. co-efficient of at least 40. Consequently smaller amounts of such compounds maintained in a fine state of division are more effective as germicides than larger quantities of lower grade phenols.

It is possible that crude cresol preparations used for general disinfection purposes occasionally contain other bodies, e.g., naphthalene and crude
phenol, well-known larvicides. Nowadays, in the manufacture of coal-gas and the distillation of tar, the by-products are of increasing value and less possibility of their inclusion arises, and for the compounding of the present-day higher grade disinfectants the active principles are chiefly derived from blast furnaces or from distillations carried out at carefully controlled temperatures. It is therefore obvious that saponified cresol is not a suspension of a particular chemical entity, but is a trade name covering fluids of a dissimilar composition which satisfy particular standards.

In this instance it was thought that it might be of interest to carry out a comparative examination of certain disinfectants which have been recommended or reported on in other places, and two samples of cresol of good quality, a phenolic preparation sold as a specific larvicide and a high grade germicide were procured.

At this time approaching maturity in the natural breeding places were two species of larve, a culicine *A. punctor* and an anopheline *A. bifurcatus*, and both were in an extremely vigorous condition. It was anticipated that these tests would not take up much time, but they were of necessity extended until these broods had emerged. As the young and immature larva readily succumbs to cresol, the plan of making up well-balanced representative collections for each dilution was adhered to throughout. In addition, mortality is markedly affected by the treatment to which the larva has been subjected prior to such tests, e.g., exposed to sunlight, shaken violently in bottles during carriage to the laboratory or kept under unsuitable conditions for considerable periods. To eliminate these fallacies the stocks of larve for each group of tests were collected as required and used as a rule within half an hour of removal from their natural environment.

**Effects of Temperature.**

The temperatures employed were within the normal limits for water in small pools in England, and when a variation of 20° F. was permitted larvicidal action was improved and very much accelerated at higher temperatures—e.g., at 48° F., using 1 in 1,000 dilution, *A. punctor* apparently died in fifteen minutes, at 68° F. in two minutes.

*When is a Mosquito Larva Dead?*

This is an important and often difficult point to decide, seeing that cresol induces a condition of torpor which causes the larva to be immobilized at the bottom in a condition analogous to that described by Klein when investigating the effects of phenol on bacterial spores. He emphasizes the fact that the spores are only “stunned” and not killed, and on inoculation into an animal they rapidly recover and cause death. The torpor affecting the larva may last for a day or more, and on several occasions larve which have been in a condition of suspended animation for over forty-eight hours have eventually come to the surface and pupated in the usual way. If
a very careful watch is kept this moribund condition may be seen to be broken by very infrequent tremors and occasional shudders. In the early stages movement may be elicited by lightly touching the fin hairs or mouth brushes with a bristle, and a slight touch will frequently succeed where a more definite one would fail. In non-lethal dilutions, particularly at higher temperatures, larvae may rapidly fall into a condition of torpor, give no response to stimulation and at first sight be certified dead; still at the end of seventy-two hours they may all be happily swimming about and pupate and emerge in the normal way. Such a state of affairs is very misleading and may easily account for some of the conclusions arrived at. In addition, larvae of *A. punctor*, which have been in lethal dilutions (1 in 10,000) and shown no sign of life for over two hours, if placed in well-aerated water may eventually resuscitate and be found swimming about unaffected on the following morning.

Apart from the absence of movement on stimulation which may not be a positive sign of death, one of the best indications is a delicate change of hue of the larva which tends to lose its greenish coloration and takes on a greyer tone.

The possible recovery of the larva when transferred to untreated water is noteworthy, seeing that during periods of heavy rain (monsoon), particularly in the tropics, moribund larvae may be washed into larger collections of water and revive.

**Resistance of Larvae to Cresol.**

This can be enhanced by placing larvae in non-lethal dilutions overnight and transferring them to lethal ones next day, and this point should be taken into consideration when collections of water are intermittently treated. In addition, cresol is frequently recommended for use in pools in the tropics where a minimum lethal dose is subject to enormous and sudden dilution by tropical downpours.

**Effects of Organic Matter (Silt).**

Apart from the effects of temperature, substances in solution and suspension, the organic silt lining of a pool may play an important part. In the later tests silt was obtained from mosquito pools and at least one inch of this placed in the bottom of all test vessels. The residual water was then drained off and the cresolized water of the required strength poured over this. The effect of the inclusion of silt appeared primarily to lead to a reduction in larvicidal power, and to a large extent the effects of increased temperature were annulled. Larvicidal action was retarded, it became more regular and the results were better differentiated.

**Effects of Other Constituents of Water.**

1. **Acid.**—Control tests demonstrated that *A. punctor* could live for days in waters containing the higher dilutions of mineral acid, but readily
succeeded in a dilution of 1 in 1,000. The conclusion arrived at was that non-lethal dilutions of acid did enhance the action of cresol, but that the amount of acidity requisite to give rise to such improvement was far in excess of the natural content of the most acid type of water.

(2) Salt.—In control tests, using plain tap water (acid type), *A. punctor* bred out in twelve days. An addition of NaCl 0.3 per cent. caused the larvae to die off one by one after exhibiting cannibalism. Extremely large adults were bred out in the same water containing both salt and silt, and on the addition of cresol larvicidal action was considerably improved by the inclusion of salt.

_Germicidal and Larvicidal Action Compared._

1. With the phenols an acceleration of the velocity of germicidal action occurs with higher temperatures, and in this the larvicidal action is in agreement.

2. The presence of acids in excess of the amounts obtaining in nature gives rise to improved larvicidal action, and in this again the larvicidal and germicidal effects correspond.

3. An analogous state of affairs also arises in the presence of common salt.

4. Preparations which maintain superior emulsions and contain special stabilizers other than saponaceous bases are more effective as germicides than as larvicides.

**RESULTS OBTAINED WITH THE VARIOUS PREPARATIONS.**

It appeared from the indefinite information volunteered and from chemical analyses that all of these disinfecting fluids contained similar compounds, i.e., higher homologues of phenol, and that their germicidal efficiency was satisfactory.

The particular cresol under suspicion in the presence of organic silt was effective against *A. punctor* in a 1 in 20,000 dilution, and *A. bifurcatus* in 1 in 10,000.

In 1 in 10,000 dilution of the second sample of cresol all anophelines were fit and one pupated during the test.

In the case of the specific larvicide at the end of thirty hours, in a 1 in 10,000 dilution, all anophelines were alive and well, a few culicines were dead and one culicine succeeded in pupating.

In a dilution of 1 in 10,000 of the high-grade germicide all culicines and anophelines were fit and pupating after twenty-two hours.

In all the above non-effective solutions the larvae pupated and healthy adults eventually emerged.

The following record illustrates the deceptive behaviour of larvae under the influence of cresol:
Studies in Mosquito Bionomics

Dilution—1 in 30,000. Temperature of water—62° F. Time—10 a.m.

<table>
<thead>
<tr>
<th>12 a.m. (2 hours)</th>
<th>1 p.m. (3 hours)</th>
<th>4 p.m. (6 hours)</th>
<th>6 p.m. (8 hours)</th>
<th>10 a.m. (24 hours)</th>
<th>After 30 hours</th>
<th>After 40 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anopheles and culicines all fit at top of the water</td>
<td>Anopheles fit. Some culicines sick but majority on surface of water</td>
<td>Anopheles fit. One culicine at top</td>
<td>All anopheles alive. One pupa emerged (male), A. bifurcatus. One culicine alive and active at bottom</td>
<td>Anopheles fit. Culicines at bottom. Some dead; others move briskly when touched</td>
<td>As before</td>
<td>Recovery. All anopheles fit. Culicines with one or two exceptions all coming to the surface</td>
</tr>
</tbody>
</table>

The readings show that eight hours after the commencement of the experiment, i.e., 6 p.m., it is possible that with one exception all culicines might have been certified dead and the experiment terminated. Still at the end of thirty-five hours they commenced to recover, and after forty hours were swimming to the surface.

When considering the action of these larvicides, the following should be borne in mind:

(1) The pupa is relatively immune to the action of cresol.
(2) This particular culicine is more susceptible than the anopheline.
(3) That under certain conditions larvae certified as dead may make a complete recovery and the normal emergence of the adult ensue.

Although it is difficult to obtain definite knowledge regarding the exact composition of these disinfectants, it would appear that they all contain higher phenoloids and decrease in toxicity results. The high germicidal power and low toxicity of one preparation is undoubted, and this particular one is the least effective as a larvicide. The phenol content of both preparations containing special stabilizers was very much in excess of the amounts present in the simple cresols and to these they were markedly inferior. This inferior larvicidal action may not be entirely due to the presence of less toxic compounds, but other factors may also assist. In the two samples of cresol which were relatively more effective, soap was the emulsifying basis, and they also contained the higher homologues of phenol only. The differences in larvicidal action obtained by people using similar preparations can be largely attributed to varying phenoloid content, but here again the type of phenoloid present is of some importance.

The pupa which is resistant to these larvicides depends for air upon the two air trumpets on its thorax. It does not eat or ingest water and has no anal gills. The only means by which it can be affected is by absorption through the skin, and as the contained mosquito approaches maturity it is protected by a double covering or integument. When 4th instar larvae pupate they shed a skin and are temporarily more vulnerable, and an opinion regarding the efficiency of these larvicides can be quickly formed if the pupating larva is unaffected at this time.

It has also been noted that these and other abnormal solutions may...
provide a stimulus to this act, and that pupation which may occasionally be a defensive measure in nature, under these conditions, frequently brings about the death of the larva.

Larvae which are capable of maintaining themselves at the surface and are left undisturbed have a better chance of escaping death. In the early stages of distress they repeatedly pass their syphon or tail end of the body through their mouth brushes, and the convulsive tremors are reminiscent of the results of central poisoning seen in decerebrate animals after infusion with certain phenolic derivatives.

It is noteworthy that poisoned larvae frequently make a rapid recovery when transferred to well-aerated water, and if the poison were being absorbed from the alimentary tract this should not be the case, seeing that it would take some little time for peptisation of such a dose.

In the lower dilutions of cresol the sides of the vessels frequently become covered with bubbles, and this diminution in the content of dissolved gases should have an effect upon the well-being of larvae, particularly upon those which are accustomed to make considerable use of their gills.

The difference between the resistance of this culicine larva and the anopheline is striking, one being twice as susceptible as the other. It is reasonable to suggest that both should absorb about the same amount of fluid by the mouth and through the skin. The anopheline, on the one hand, has small papilliform gills and remains on the surface of the water, and this is very noticeable in those solutions which are deficient in gases in solution. A. punctor, on the other hand, has well-marked gills and normally spends long periods below the surface, and it is possible that in this its vulnerability lies. If these larvae are interfered with when in cresol solutions they dive below and readily succumb, whereas if they are permitted to remain at the surface they are more resistant. Their gills are large and retractile, and when at the surface there is less demand for their employment and the absorption of cresol is limited.

As regards the other factors, the relative inefficiency of larvicides containing special stabilizers other than soaps, it is doubtful if saponaceous suspensions owe their superiority entirely to the fact that with special stabilizers, such as glue, there is an increased adsorption of stabilizer and chemical by organic matter of a particulate nature. To the bacterium which absorbs nutriment via its cell envelope a coating of colloid and finely divided disinfectant should be very lethal, but from consideration of the vitality of the pupa this should not lead to an improved larvicidal action, seeing that the non-pupating larva absorbs a negligible quantity of larvicide via the skin. The cells covering the gills are undoubtedly highly specialized, selective in action, and saponaceous bases and fats are frequently selected as vehicles of chemicals on account of their permeative properties.

As suggested by Rideal, salt leads to an alteration in the lipoid-water partition co-efficient, and increased fugacity of phenol, and the vital
respiratory membrane covering the gills by osmosis should theoretically reject colloid stabilizers.

In regard to the remarkable recovery of larvae in certain solutions which rival representatives have attributed to instability of the emulsion and precipitation of larvicide. If this were the case, then the moribund larva at the bottom would be in contact with a layer of solution relatively richer in larvicide and there should be still less chance of its recovery. This is not borne out by the fact that with care larvae, after immersion in weaker solutions, can be made to withstand lethal ones, hence the recovery of the larva is most likely due to an induced tolerance.

In conclusion, it may not be considered out of place to mention certain other larvicides. The use of petroleum has many drawbacks, and according to some it owes its efficiency very largely to the contained volatile hydrocarbons which act via the respiratory tract. It is equally effective against culicine and anopheline larvae.

Paris green for the purpose of film formation is normally incorporated with dust or with the exhausts from grain cleansing plant, and certain observers who employ this larvicide almost exclusively are very insistent that it should be scattered directly over groups of larvae and preferably by hand. In addition, they state that although very effective against anophelines, culicines frequently escape. In this instance it may be inferred that the path of entry of the poison is via the alimentary tract, and that the anopheline feeding on the surface ingests a higher concentration of poison than the culicine which draws its nourishment from a lower level.

Apart from all question of larvicidal efficacy, it is impossible to evade the financial aspect, and having in mind the conditions obtaining in India and similar countries, it is evident that there is still scope for the production and development of new larvicides.

I am indebted to Dr. A. Balfour, C.B., C.M.G., Director, London School of Hygiene, for his encouragement and suggestion regarding the hydrogen ion concentration; to Lieutenant-Colonel S. P. James, I.M.S. (Retired), and Mr. P. G. Shute, of the Ministry of Health, for a supply of fed mosquitoes; to F. W. Edwards, Esq., of the British Museum (Natural History), for his interest and for confirming the identification of numerous larvae; to Lieutenant-Colonel W. W. Browne, O.B.E., R.A.M.C., O.C., Army School of Hygiene, for the facilities afforded; to Major S. M. Hattersly, M.C., R.A.M.C., for the chemical analysis of several disinfectants; and to Sergt. H. W. Watson, R.A.M.C., Laboratory Assistant, for his help.

Note.—With three exceptions all observations were made prior to June, and the last series of experiments concluded in August, 1925, when the rough draft of the sections was prepared. The gist of the paper has been communicated verbally to various interested people, but owing to pressure of work the publication of the final draft is overdue. This delay accounts for the inclusion of certain minor details which in the light of recent
writings may now be considered superfluous, and for the absence of allusion to recent important publications, notably, "British Mosquitoes and their Control," by F. W. Edwards and Lieutenant-Colonel S. P. James, M.D., and "Physical Factors in Mosquito Ecology," by Ronald Senior White.

LITERATURE.