Table 1 gives these data against *Trichoderma viride* USDA T-1.

These data show the *cis* compound to be considerably more fungistatic than the corresponding *trans* compound. No conclusive reason for this behavior is now available.

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Preliminary Observation on a Hatching Stimulus for *Aedes* Eggs (*Culicidae*)

W. E. Beckel[1]

Department of Zoology, State University of Iowa, Iowa City, and *Defence Research Northern Laboratory, Fort Churchill, Manitoba, Canada*

Many aedine mosquitoes have a diapause in the egg stage. This diapause may be an obligate one or one brought on solely by adverse conditions. Diapause may be broken in various ways but often an additional stimulus is necessary for hatching. In experimental work it is important that hatching of the eggs occurs whenever desired. Moreover, ecological studies on such problems as succession of species require a knowledge of the factors which influence hatching. For example, why do the eggs of *Aedes campestris* Dyar and Knab hatch in the field in the region of Churchill, Manitoba, so much later than do those of *Aedes hexodontus* Dyar? These considerations make an understanding of hatching stimuli of interest.

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Recent experiences with the hatching of *A. campestris* eggs and with *A. hexodontus* eggs have led to the conclusion that there may be some factor in an infusion of decaying mosquito bodies that stimulates hatching of these eggs at an appropriate temperature. Table 1 describes the experiments carried out and the results obtained.

Gjuillin *et al.* (*1*) found low dissolved oxygen of the hatching medium to be a factor stimulating *A. vexans* (Meig.) eggs to hatch. Therefore, it was decided to try a medium of low dissolved oxygen content with *A. campestris* eggs. In our experiments there was no hatch in distilled water at normal pressure, in distilled water subjected to reduced pressure to remove oxygen, or in distilled water over pyrogallol. Abdel-Malek (*2*) and Horsfall (*3*) found plant hormones to be a factor in the hatching of *Aedes* eggs; but for us a solution of 1:10 or 1:100 of indoleacetic acid in water gave no results with *A. campestris.*

It is interesting that Mail (*4*) kept *A. campestris* eggs in pond water for 20 months at a temperature between 0 and 10° C and when he returned them to 22° C he got a 25% hatch. There may have been some substance in the pond water similar to that which is present in the bacteria and mold infusion.

A. hexodontus eggs will hatch just above 0° C,

TABLE 1

HATCHING RESULTS WITH EGGS OF *Aedes campestris* AND *Aedes hexodontus* IN AN INFUSION OF DECAYING MOSQUITOES

Expt. No.	Species	Number of eggs and history	Treatment	Results	Comments
1	*A. hexodontus* *A. excrucians* *A. campestris*	Unknown number stored together on filter paper with decaying mosquitoes. 15 days old.	Distilled water added.	First instar larvae of *A. campestris* appeared on 3rd day. No eggs of other species hatched.	No attempt was made to determine % of *A. campestris* that hatched since the eggs of the 3 species overlap in size.
2	*A. campestris*	150 eggs 15 days old, stored on clean filter paper.	Placed in infusion of decaying adult mosquitoes.	30% of the viable eggs hatched over a 3-day period.	A control of 150 eggs in distilled water gave a hatch of one larva. On dissection, the embryos were viable.
3	*A. campestris*	50 eggs stored 4 months in distilled water at between 4 and 10° C.	Placed in infusion of decaying first instar larvae.	90% of the viable eggs hatched within 24 hr at 25° C.	A control of 25 eggs in distilled water gave no hatch in 15 days at 25° C. On dissection, the embryos were viable.
4	*A. hexodontus*	250 eggs exposed to cold for 6 months to break diapause were placed in distilled water at 20° C for 5 days. No hatch resulted.	Half the eggs were then placed in an infusion of decaying mosquitoes.	In the infusion over 50% of the eggs hatched.	The exact % was not recorded. In the control there was no hatch. On dissection the embryos were viable.

whereas *A. campestris* eggs will do so only at considerably higher temperatures. It was originally thought that temperature alone was responsible for the delay in hatch of *A. campestris* in the field. However, it may be that temperature is only a limiting factor in the action of a hatching stimulus from decaying organic matter.

It is possible that an infusion of bacteria and molds growing on decaying mosquito bodies will stimulate eggs of other species of *Aedes* to hatch. Many references in the literature suggest that microorganisms often play a part in the hatching of *Aedes* eggs (*5*). Bacteria and molds are now being isolated from decomposing mosquitoes, and further experiments on the effect of these microorganisms and extracts from them are in progress.

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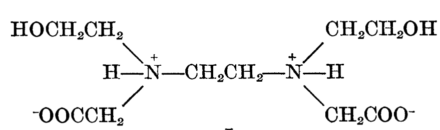
New Chelating Agents for Trivalent Iron

Stanley Chaberek, Jr., and F. C. Bersworth

Bersworth Chemical Company, Framingham, Massachusetts

There have been developed in these laboratories two metal ion chelating agents that show promise as iron carriers for the treatment of iron chlorosis in plants (*1*). These compounds are N,N′-dihydroxyethylethylenediaminediacetic and N-hydroxyethylethylenediaminetriacetic acids.[1] The amino acids, having the structures I and II, are new compounds which have been prepared and



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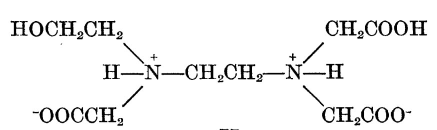

II

purified by methods to be reported subsequently (*2*).

[1] F. C. Bersworth, patents pending.

The acid dissociation constants and the interactions of the various acid species with ferric ions were determined for both I and II by potentiometric measurements of pH in a manner similar to that described for N,N-dihydroxyethylglycine (*3*). The reactions investigated and the corresponding equilibrium constants may be summarized as follows.

N,N′-Dihydroxyethylethylenediaminediacetic Acid (I)

$H_2A(OH)_2 \rightleftarrows HA(OH)_2^- + H^+$	$pK_1 = 4.7$
$HA(OH)_2^- \rightleftarrows A(OH)_2^{-2} + H^+$	$pK_2 = 8.6$
$Fe^{+3} + A(OH)_2^{-2} \rightleftarrows FeA(OH)_2^+$	$\log K_1 \leq 15$
$FeA(OH)_2^+ \rightleftarrows FeAO(OH) + H^+$	$pK_2 = 2.2$
$FeAO(OH) \rightleftarrows FeAO_2^- + H^+$	$pK_3 = 5.5$

N-Hydroxyethylethylenediaminetriacetic Acid (II)

$H_3A(OH) \rightleftarrows H_2A(OH)^- + H^+$	$pK_1 = 2.6$
$H_2A(OH)^- \rightleftarrows HA(OH)^{-2} + H^+$	$pK_2 = 5.1$
$HA(OH)^{-2} \rightleftarrows A(OH)^{-3} + H^+$	$pK_3 = 8.3$
$Fe^{+3} + A(OH)^{-3} \rightleftarrows FeA(OH)$	$\log K_1 \leq 21$
$FeA(OH) \rightleftarrows FeAO^- + H^+$	$pK_2 = 3.8$

The most important property of these chelating agents is their ability to form 1:1 ferric chelates of sufficient strength so that the complexed trivalent iron is stabilized against hydrolysis even in strong alkaline solutions. Thus, while the ferric ethylenediaminetetraacetate complex is decomposed in alkaline medium to ferric hydroxide, the corresponding ferric chelates of these hydroxyethyl derivatives are completely stable against hydroxide precipitation. The resistance of these ferric complexes to hydrolytic decomposition results from the fact that 1:1 chelate formation is accompanied by the simultaneous ionization of the weakly acidic ethanolic protons of the hydroxyethyl groups.

Both amino acids function as hexadentate chelating agents in the presence of ferric ions. The hexacoordinated ferric ion is bound to the ligand through the two nitrogen and four oxygen atoms.

The development of N,N′-dihydroxyethylethylenediaminediacetic and N-hydroxyethylethylenediaminetriacetic acids makes possible the study and treatment of chlorosis conditions in calcareous soils as well as other studies in alkaline medium. Further investigation of the properties of these chelating agents is in progress and will be reported soon.

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