vittatus. Field personnel on the Aedes aegypti Eradication Program submitted thousands of eggs for identification or confirmation, and hatched "problem" eggs to help confirm identifications using larval characters. The photomicrographs were made by Jack Gust of the National Medical Audiovisual Center.

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A COMPARATIVE STUDY OF EGG HATCHING TECHNIQUES FOR *AEDES AEGYPTI* (L.)[1]

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The existence of a relationship between the concentration of dissolved oxygen and the percent hatch of mosquito eggs has been established (Gjullin *et al.*, 1941; Borg and Horsfall, 1953 and Horsfall, 1956). Lowering the concentration of oxygen stimulates hatching. Borg and Horsfall (1953) and Burgess (1959) adequately reviewed previous work on hatching stimulants.

A great deal of work has been performed on different types of oxygen removers, and tests have been performed to determine the effectiveness of these methods as possible hatching agents. These include vegetable extracts, acids, sugars, vitamins, salts, microorganisms, gases and evacuation. A great diversity in the efficiency of hatching agents has been reported. Percent hatch and the time required for complete hatch using a particular agent have been rated differently by various workers. But evaluations and comparisons are difficult to make because of the incompleteness of the data reported in many studies. Time required for complete hatch was not always mentioned. Other workers state only the time elapsed before initiation of hatch. Borg and Horsfall (1953) suggest that the diversity in percent hatch and hatching time reported by various workers was due to improper conditioning of eggs.

Burgess (1959), Judson (1960), Gander (1951) and others failed to mention the total time required for hatch. In addition, a high percentage hatch was achieved in only a few of the above studies. The agents used in these studies varied greatly. Among the substances tried were boiled distilled water (Burgess, 1959), nitrogen gas and ascorbic acid (Judson, 1960) and wheat germ (Gander, 1951). In some cases other authors have reported hatch time. Borg and Horsfall (1953) indi-

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cated that, using hydrogen gas, 89 percent hatch was completed in 30 minutes. Using nitrogen gas, 100 percent hatch was achieved. However, Gjullin *et al.*, (1941) reported an average of 25 percent hatch in 2 hours using hydrogen gas. Borg and Horsfall (1953) attributed this to poor conditioning.

We reviewed the literature dealing with egg hatching in mosquitoes because we required a hatching method that was rapid, easy and gave high percent hatch. Using these as criteria we evaluated the three basic methods of lowering the oxygen tension; physical, chemical and biological.

Gjullin *et al.*, (1941) studied some chemical reducing agents. Glutathione, cysteine, iron powder and thioglycollic acid were all found to cause some hatch in 10 minutes, but for most, maximal hatch occurred in about 12 hours. Horsfall (1949) worked on single and multiple hatching agents. These substances included extract of canned corn, coconut milk, glucose, KCL and indoleacetic acid, acetic acid, yeast, and others. This group typically gave high percent hatch (74, 76, and 77 percent) with most larvae hatching within 0–4 hours. Most of the other single or multiple hatching agents tested yielded hatch within 5–24 hours. Representative maximal percentages in this group were 64, 71 and 81 percent. Mulla and Chaudhury (1968) recently achieved 90 percent hatch within 24 hours, with low larval mortality, using 0.01 percent ascorbic acid.

In studying biological means of inducing hatch, Thomas (1943) demonstrated that bacteria and other microorganisms give a high percent hatch. In tests using bacteria, yeasts and molds the average time for complete hatch was about 24 hours. Gjullin *et al.* (1941) tested 36 species of bacteria representing 33 genera and found that all initiated hatch within 6 hours and completed it within 12. Several species of yeasts and molds proved slower but still effective. Borg and Horsfall (1953) also reported hatch in a bacterial suspension but in 60 minutes.

A plant infusion of blue grass gave 50–70 percent hatch in 30 minutes (Abdel-Malek, 1948). Similarly, Gjullin, Yates and Stage (1939) used a willow leaf infusion which was found to reach its most effective hatching strength in 1–2 hours. Gjullin *et al.*, (1941) reported that sterile carrot tissue caused hatching in 1½ hours. We thus find a great disparity in the percent hatch and in the time required for initiation or completion of hatch, ranging from 10 minutes to 60 hours (Gjullin *et al.*, 1941), when biological or chemical methods are employed.

The studies that mention evacuation as a hatching method lack information on time required for complete hatch or percent hatch that can be achieved. Perhaps the earliest reported use of evacuation was by Gjullin, Hegarty and Bollen (1941). They indicated that hatching was accomplished as quickly as with other reducing agents. The use of evacuation as a hatching agent was reported by Barr and Al-Azawi (1958) and Weissman-Strum and Kindler (1963). Hazard *et al.*, (1967) indicated recently that he used the vacuum hatching method in his experiments, attaining hatch in 15 minutes.

This paper will attempt to demonstrate the effectiveness of evacuation as compared to two other methods. These methods were picked to represent each of the other types of hatching agent. Thus we were comparing a chemical, a biological and a physical hatching agent. It is also our purpose here to emphasize the ease, efficiency and speed with which vacuum hatching can be used. We have found this to be true over many years of use in our laboratories and feel that this method should be more widely used.

MATERIALS AND METHODS. Eggs for all experiments were produced by the *A. aegypti* culture on one moist filter paper cone over a 2-day interval. The eggs were stored at a constant 90 percent R.H. (maintained by a supersaturated solution of KNO_3). All tests were conducted with three replicates of 50 eggs each. The eggs used were at least 3 weeks old. Successful

hatch has also been achieved in our daily laboratory routine using older eggs. Three petri dishes, 100 x 20 mm, with 50 ml. of medium per dish were used for each agent tested. Percent hatches were recorded at designated times.

Three methods of hatching were used. The first was vacuum hatching. Eggs were placed in 50 ml. of distilled water and hatched under 25 p.s.i. of vacuum, with a Gast vacuum pump. The second agent tested was a 0.01 percent solution of ascorbic acid, (Mulla and Chaudhury, 1968), prepared with distilled water. The third was a mixture of 0.2 gm yeast and 0.2 gm finely ground dog food in 1000 ml. of distilled water (Hayes and Morlan, 1957). This solution was held for 24 hours to increase its effectiveness (Hayes and Morlan, 1957).

TABLE 1.—Percent hatch of *A. aegypti* eggs with various hatching stimuli over time.

	25 p.s.i. Vacuum Rep				1:1 Dogfood-Yeast Mixture Rep				0.01% Ascorbic Acid Rep			
	1	2	3	$\bar{x}$	1	2	3	$\bar{x}$	1	2	3	$\bar{x}$
10 min.	6	12	24	14.	8	18	8	11.3	4	2	2	2.6
20 min.	56	54	46	52.	16	32	22	23.3	6	4	4	4.6
30 min.	82	78	80	80.	22	34	22	26.0	6	4	4	4.6
60 min.	92	88	88	89.3	26	34	22	27.3	6	4	4	4.6
17 hrs.	..	..	..	..	40	44	54	46.0	6	4	4	4.6

RESULTS AND DISCUSSION. Table 1 shows that vacuum hatching was the most effective of the three methods tested for hatching of *A. aegypti* (L.).

As we have illustrated, the vacuum hatching technique is efficient, producing uniform and rapid hatching. However, we are surprised and distressed that so few people are aware of this technique. At 30 minutes the percent hatch with vacuum averaged 80 percent, with little variability, while the dogfood-yeast mixture was only up to an average of 26 percent, with wide variability. The ascorbic acid technique compared very poorly with either the vacuum hatching method or the dogfood-yeast technique. Even after 17 hours the percent hatch averaged only 4.6 percent. In all fairness, we must point out that the ascorbic acid, a fairly sensitive chemical, may have lost some of its potency due to age. The majority of the larvae hatching under vacuum did so within the first half hour. Only an additional 9 percent hatch was achieved after a full hour.

We have compared vacuum hatching with only two other techniques. As hatching agents, the ones picked here for comparison are relatively simple. Yet, they are still more inconvenient to use than vacuum hatching. The dogfood-yeast solution must be prepared at least 24 hours before use to insure its effectiveness. The ascorbic acid, as well as the dogfood-yeast mixture must be prepared each time larvae are to be hatched. Other agents that might have been selected, such as plant infusions, extracts and gases are much more inconvenient to use than the methods selected. They require more time and effort to use.

Vacuum hatching has been shown to be faster and to provide higher percentages and more uniform hatches. Of even more importance is the assurance of having uniformly aged larvae. In the use of techniques requiring many hours, larvae may range from a few minutes old to almost 24 hours old. This is as much time as may be required for the first instar (Chevone and Peters 1969; Hazard *et al.*, 1967). For all these reasons we can not see why the vacuum hatching technique is not more widely used or better known.

Borg and Horsfall's (1953) emphasis on the need for proper conditioning of eggs

seems to be very important. In our preliminary experiments indications were that conditioning can affect the efficiency of hatch. With optimal conditioning of eggs, vacuum hatching can very well give even higher percentages than those reported here. Further aspects of conditioning as it affects the percentages achieved by vacuum hatching will be dealt with in future research.

SUMMARY. Vacuum hatching was experimentally compared with two other hatching stimulants. These stimulants were representative of a biological agent and a purely chemical agent. Results indicate that vacuum hatching is not only faster but produces more uniform and higher percent hatches.

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