TECHNIQUES TO GIVE BETTER HATCHES OF THE EGGS OF *AEDES AEGYPTI* (L.) (DIPTERA: CULICIDAE) [1]

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If fertilized *Aedes aegypti* eggs remain on a moist substrate for several days after oviposition, the embryos develop to maturity, and then remain dormant until a suitable hatching stimulus is applied. Shannon and Putnam (1934) found that ordinary distilled water would stimulate some of these dormant eggs to hatch, especially if they were less than 10 days old or had been moistened for several hours. Similarly, Granett and Haynes (1944) obtained good hatches in ordinary distilled water of eggs stored up to several weeks under humid conditions.

We found, however, that eggs from our colony of *A. aegypti* hatched very poorly in ordinary distilled water. Of other hatch-promoting stimuli reported in the literature, the reduction of dissolved oxygen in the hatching medium appeared to be one which might be put to good use for hatching eggs in the laboratory. Studies of the relation of egg hatching to low oxygen tensions were first made by Gjullin, Hegarty and Bollen (1941), and later by Borg and Horsfall (1953), Horsfall (1956), and Horsfall, Lum and Henderson (1958). With *A. aegypti*, Gjullin *et al.* found that the same stimulus to hatching was produced regardless of whether oxygen was removed by biological, chemical, or physical processes. They found that eggs 3 to 4 days old hatched about equally well in ordinary distilled water and in deoxygenated distilled water, but that deoxygenated water hatched more of older eggs. Recently, Barr and Al-Azawi (1958) have referred to the use of deoxygenated water to produce larvae of uniform age.

If an egg-hatching stimulus is to be used to best advantage, it is important that eggs be stored so as to maintain a maximum viability. Marshall (1938) stressed that if eggs become too dry the embryos die, thus indicating that humidity could affect the viability of stored eggs. Although Granett and Haynes (1944) recommended that eggs be stored at a high humidity, in practice they are often stored at the ambient humidity of the laboratory. Thus, in addition to a study of the usefulness of reduced dissolved oxygen for hatching *A. aegypti* eggs in laboratory rearing procedures, the following investigation also includes studies of the effects of humidity on the viability of stored eggs.

MATERIALS AND METHODS. The eggs used were obtained from a colony of *A. aegypti* maintained on a 10 percent sucrose solution and allowed to take blood meals from a guinea pig twice weekly. The eggs were laid on filter paper placed on a pad of moist cellucotton in a Petri dish. They were kept moist for 3 days and then stored in the laboratory (except where otherwise stated) at 23–30° C. and 30–60 percent relative humidity.

Eggs were hatched in small glass dishes filled with water and covered with watch glasses, except in experiments on different types of hatching containers. At least 50 eggs were used for each test. With several exceptions, hatches were evaluated on the bases of the time from immersion to commencement of hatching and of the number of eggs hatching during the first hour. The one-hour period was chosen arbitrarily as a suitable period for hatching in any standardized procedure that might be developed.

For the experiments on egg storage and relative humidity, three levels of humidity were used as follows: 90–100 percent,

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obtained by placing the eggs over moist cotton in a closed jar; 30–60 percent, obtained by keeping the eggs at the ambient humidity of the laboratory; and, 0–10 percent, obtained by placing the eggs over calcium chloride in a closed jar.

Dissolved oxygen was removed from distilled water by boiling the water for 30 minutes, then cooling it to 28–29° C. in the absence of air. This reduced the amount of dissolved oxygen in the distilled water from its normal value of 7–8 p.p.m. to less than 2 p.p.m. by weight. Determinations of dissolved oxygen were made by the Winkler method as described by the American Public Health Association (1947, pp. 124–139).

EXPERIMENTS AND RESULTS. A preliminary experiment with 13-day old eggs showed that ordinary distilled water hatched less than one percent of them in 45 minutes, whereas deoxygenated water (1.0 p.p.m. of dissolved oxygen) hatched 29 percent. The hatching of some eggs from 3 to 19 days old was then tested at a number of different levels of dissolved oxygen within the range of 1.0 to 7.9 p.p.m. As the amount of dissolved oxygen remaining in the water was increased, the percentage hatching in one hour generally decreased (Table 1). This was more marked in older eggs, those over six days old failing to hatch at 7 to 7.9 p.p.m. of dissolved oxygen.

As deoxygenated water gave good hatches of eggs from three to 19 days of age, the hatches of eggs in deoxygenated water were determined at intervals over a greater range of age. The best hatches were obtained during the first week, thereafter there was a general decrease in hatch, and by the 86th day eggs no longer hatched when immersed for one hour in deoxygenated water (Figure 1). Also, the time from immersion until commencement of hatching steadily increased during the experiment (Figure 1).

Next, the effect of humidity on the viability of stored eggs was determined. Some eggs from a large sample three days old were tested for hatching in deoxygenated water, and the remainder were divided into three groups which were stored at 90–100 percent, 30–60 percent, and 0–10 percent relative humidity respectively. In subsequent tests, the eggs stored at higher humidities gave better hatches, hatched more quickly and maintained viability longer than those stored at lower humidities (Figure 2). These differences were significant at the five percent level of probability.

Finally, as it was clear that even eggs several months old would give good hatches if they were stored at a high humidity and then immersed in deoxygenated water, tests were made of the efficiency of two types of hatching containers, large enough to hatch at least 500 eggs. The dissolved oxygen content of deoxygenated water poured into open enamel photographic pans, which are often used for mosquito rearing, increased from 1.9 to 8.0 p.p.m. in the one-hour hatching period. By contrast, a corresponding increase of only 0.6 p.p.m. of dissolved

TABLE 1.—Percentages of *A. aegypti* eggs of different ages that hatched in one hour in distilled water containing various amounts of dissolved oxygen, 50 eggs being used per test

Age, days	Dissolved oxygen, p.p.m.					
	1–1.9	2–2.9	3–3.9	4–4.9	5–5.9	7–7.9
3	62	44	50	—	38	38
4	52	62	—	46	44	4
5	74	—	—	10	—	0
6	86	54	38	—	22	6
7	72	60	—	6	—	0
13	56	22	18	—	8	0
19	74	50	2	—	0	0

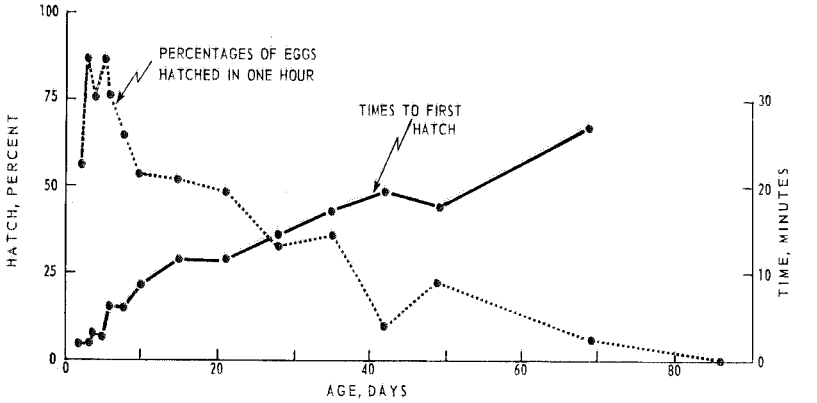

FIG. 1.—Percentages of *A. aegypti* eggs of various ages that hatched in one hour in deoxygenated distilled water (i.e., 0.6 to 1.4 p.p.m. of dissolved oxygen), and times required for hatching to commence, after storage at 30–60 percent relative humidity

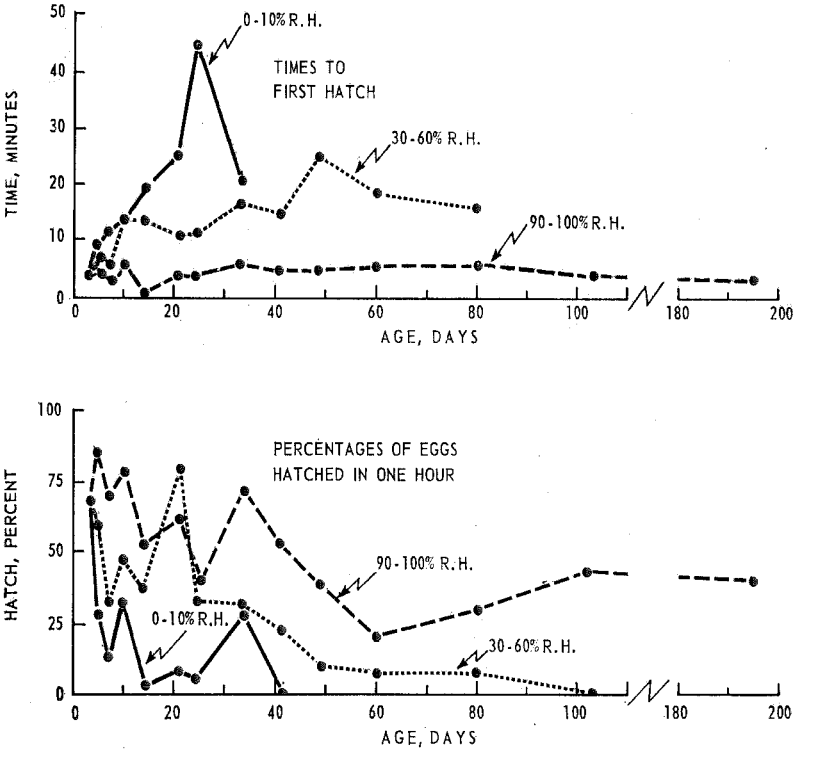

FIG. 2.—Percentages of *A. aegypti* eggs of various ages that hatched in one hour in deoxygenated distilled water (i.e., 0.6 to 1.3 p.p.m. of dissolved oxygen), and times required for hatching to commence, after storage at three different levels of relative humidity

oxygen was found in deoxygenated water in completely filled, unstoppered, 250 cc. reagent bottles. Subsequent tests showed that the bottles hatched about three times as many eggs as the open pans in a one-hour hatching period. The restricted size of the water surface exposed to air in the bottles appeared to have no adverse effect on the newly hatched larvae.

DISCUSSION. The effectiveness of deoxygenated water in stimulating *A. aegypti* eggs to hatch, as first described by Gjullin *et al.* (1941) is wholly supported by the foregoing experiments. In the experiments on maintenance of egg viability, the decrease in hatch with advancing age of eggs stored at the ambient humidity of the laboratory can be attributed partly to drying of the eggs. Other factors must also contribute to this decline in viability as even eggs stored at 90 to 100 percent humidity still showed some decline in viability with advancing age. The longer time required for hatching to begin, of those eggs stored at lower humidities, may be due to drying of the chorion, or to direct effects on the unhatched larvae.

The following procedures were adopted for handling and hatching eggs. Oviposition dishes with eggs were removed from the cages daily, placed in a closed container for 24 hours, and then exposed in the rearing room (approximately 60 percent relative humidity) for the next 48 hours. During the last half of this 48-hour period, the filter paper was loosened slightly from the underlying cellucotton. The filter paper discs, with eggs attached, were then stored over moist cotton in a closed glass jar until used. Hatching was carried out in narrow-necked 250 cc. reagent bottles. The required number of egg papers were rolled up and inserted into a bottle full of freshly boiled distilled water at a temperature of 28–29° C., and with a dissolved oxygen content of approximately 1.2 p.p.m. The bottle was left unstoppered, and the hatched larvae were carefully poured into rearing trays at the end of one hour.

CONCLUSIONS. Immersion in distilled water containing less than 2 p.p.m. of dissolved oxygen was shown to be a very efficient procedure for hatching *A. aegypti* eggs for laboratory rearing. Increasing the humidity at which eggs were stored increased and prolonged their viability and caused them to start hatching more quickly in deoxygenated water. Containers which exposed little surface of deoxygenated water to the air were most efficient for hatching eggs.

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