DISCUSSION

Weathersby and McCall (1967) successfully demonstrated that the infectivity of Plasmodium gallinaceum in Aedes aegypti, frozen in liquid nitrogen, was retained for more than two years. They stated that freezing in liquid nitrogen was one of the best methods for storage of pathogenic protozoa.

Minter and Goedbloed (1971) stated that preservation and storage of parasitic protozoa in whole insect hosts by liquid nitrogen freezing was useful as a field technique, because of its simplicity, rapidity, the long storage times permitted and good retention of infectivity. The results of the experiments reported here substantiated the above assertions.

It appeared that, as stated by Moffett and Wilson (1971), protozoan spores are not killed by cold.

Comparison of infectivity of lyophilized materials after four weeks of storage suggested that host tissues provided better protection to infectivity than the additives used, since some infectivity was retained by classes V and X but more by class XI. Probably, the loss of infectivity after lyophilization was due to drying, since freezing without drying had little effect on infectivity. Ohshima (1964) commented that protozoan spores were sensitive to freeze-drying. He also explained that, because of the visco-elastic nature of the filament of the microsporidan, Nosema bombycis, it was coagulated by the drying process of lyophilization and lost the ability to evaginate.

Lewis and Lynch (1974) tried to preserve the microsporidan, Nosema pyrausta, in infected Ostrinia nubilalis by lyophilization and by vacuum drying. The results were that viability and infectivity of vacuum dried material was better than that of lyophilized material. Comparison of the results in class IV with those of classes V and X indicate that lyophilization is more harmful to Helicosporidium spores than is vacuum drying. The detrimental effect of lyophilization on protozoan spores was also observed by Pilley (1976). Although lyophilization is a useful method for long term storage of bacteria and other microorganisms, the failures to preserve pathogenic protozoa by this method observed here and by several other workers suggest that spores of protozoa should not be desiccated for storage.

Collins and Jeffery (1963) and Walker and Ashwood-Smith (1961) suggested that dimethyl sulfoxide be used as an alternative to glycerol as a freezing protectant, because of the lower toxicity to both the protozoa being preserved and to hosts in which they were subsequently placed and because of its greater ability to penetrate cell membranes.

Aberrant changes in the IC_{50}'s observed during storage probably were the effects of uncontrollable variation in methods. These variations could have affected both hosts and pathogens. The most conspicuous variations in methods resulted from inability to control the temperature in the insectary in which the pre-exposure larvae were reared, the room in which exposure to the pathogen was done and the insectary in which the larvae were reared after

exposure. This study required that infectivity of a pathogen be tested against different generations of the host. This inevitably introduced variations in the experimental hosts (Hoskins and Craig, 1962), which, ideally, should have been minimized by producing those hosts under as precisely the same conditions as possible. In regard to temperature, it is commonly observed in pathobiology that younger (smaller) hosts are more susceptible to disease agents. Hembree (personal communication) has shown that a marked difference exists in the infectivity of A. aegypti of different ages to Helicosporidium. Younger (smaller) larvae were more susceptible, while older (larger) larvae were so much less susceptible that third stage larvae were almost refractory to infection. Thus, larvae incubated for 48 hr at lower temperatures before exposure would be smaller and more susceptible to infection. The high correlation found between mean incubation temperature and IC_{50} strongly suggested this as an explanation for the unexpected reductions in IC_{50} with increasing storage that occurred in some tests.

Hurpin (1968) believed that temperature changes influenced not only the host but also the infective organism. It seemed to act as an "activator" of the pathogen and as a "depressor" of the host. This "activator" function was one of the stress phenomena involved in environmental effects on an insect disease.

The low percent mortality in the control groups reflected good rearing methods. The author expected the log-dose mortality curve to parallel the log-dose infection curve. The

percent mortality should have increased with an increase of dosage, but some higher dosages appeared to produce very low percent mortality. It must be considered that the mortality data collected was not total mortality but the mortality occurring by a particular time, the time of appearance of pupae. Most mortality was observed to occur in the late fourth stage of larval development. Development to that stage was delayed in heavily infected individuals and experimental groups, thus mortality appeared to be lower. The low mean rate of mortality in experimental groups was related to infection with the pathogen. That portion of mortality occurring soon after exposure appeared to be caused by septimia, since few Helicosporidium developmental stages were found. This would be difficult to confirm, however, because bacterial decomposition begins rapidly in dead mosquito larvae (Figure 13). The body cavaties of dead fourth stage larvae were almost invariably packed with Helicosporidium spores and developmental stages. The mortality among these might be derived from septimia, from mechanical damage or from the diversion of essential nutrients.