with 2,6-lutidene. Two areas of growth were observed. One area compared favorably with synthetic material while the other area indicated a faster moving active principle.

No effort was made in this experiment to obtain maximum release of folinic acid either from the cells or from whatever bound form in which the factor may exist.

References

1. BOND, T. J., *et al. J. Am. Chem. Soc.*, **71**, 3852 (1949).
2. WILLIAMS, R. J., *et al. The Biochemistry of the B Vitamins.* New York: Reinhold, 575 (1950).
3. KLINE, I. T., and DORFMAN, R. I. *Proc. Soc. Exptl. Biol. Med.*, **76**, 203 (1951).
4. NICHOL, C. A., and WELCH, A. D. *Ibid.*, **74**, 52 (1950).
5. FLYNN, E. H., *et al. J. Am. Chem. Soc.*, **73**, 1979 (1951).
6. SHIVE, W., *et al. Ibid.*, **72**, 2817 (1950).
7. MAY, M., *et al. Ibid.*, **73**, 3067 (1951).
8. ROGERS, L. L., and SHIVE, W. *J. Biol. Chem.*, **172**, 751 (1948).
9. SNELL, E. E., GUIRARD, B. M., and WILLIAMS, R. J. *Ibid.*, **143**, 519 (1942).
10. BARDOS, T. J., *et al. J. Am. Chem. Soc.*, **71**, 3852 (1949).

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The Life History of *Echinoparyphium flexum* (Linton 1892) (Dietz 1910) (Trematoda: Echinostomidae)

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Linton (*1*) described the trematode *Distomum flexum* found in the intestine of the black scoter, *Odemia americana,* from Yellowstone National Park. Dietz (*2*) incorporated the species in the genus *Echinoparyphium.* McCoy (*3*) obtained the adult experimentally by feeding chicks metacercariae from the snail, *Planorbis?* (= *Helisoma*) *trivolvis,* collected from Romana Lake, Missouri. Although his attempts to hatch the trematode egg were unsuccessful, he was able to obtain cercariae in 9 weeks by placing eggs in a small aquarium wth laboratory-reared *Physa integra.* Najarian (*4*) found the metacercaria in the kidneys of several species of frogs in the vicinity of Ann Arbor, Michigan.

The life cycle of the worm has been experimentally established in this laboratory, and all the stages have been studied. The chick was used as the experimental definitive host. The natural definitive host was found to be the blue-winged teal, *Anas discors.* The small intestine of two of the eight ducks shot in a woodspool area six miles west of Ann Arbor, Michigan, contained the adults of *E. flexum.*

The natural snail host in the area studied was *Lymnaea palustris.* Of the 3755 specimens collected and individually isolated, 83, or 2.2%, showed infection with the cercaria. The percentage of infected snails was small and ranged from 1.6 to 2.4 from April through October 1952. A single infected snail sheds 900–1300 cercariae within a 24-hr period. The bulk of shedding takes place between 1:00 P.M. and 4:00 P.M.

The metacercaria was found in nature both in the kidneys of frogs and tadpoles and in the kidney and heart of *Lymnaea palustris.* The cysts were found in the following species of frogs: 108 *Rana sylvatica,* 75% infected; 9 *Hyla crucifer,* 88% infected; 8 *Pseudacris migrita triseriata,* 25% infected; 7 *Rana pipiens,* 42% infected; 41 *Rana clamitans,* 14% infected.

Young adults of *R. pipiens* and *R. sylvatica,* reared in the laboratory from the egg stage, could not be infected by exposure to the cercariae. Tadpoles of the same species were easily infected. The metacercaria found in the kidneys of frogs in nature is apparently the result of the cercaria entering the tadpole and remaining in the kidney until metamorphosis is completed.

Experimentally, the cercaria encysts in the snails *Lymnaea palustris, Gyraulus parvus,* and *Physa gyrina.* In all cases the cysts are infective within 24 hr.

The feeding of the infective metacercariae to chicks was shown to give only a 1.1–1.6 yield of adult worms of *E. flexum.*

The eggs leave the uterus of the worm in an uncleaved condition. In the laboratory they were incubated in aerated tap water at room temperature from May through August 1952 and were shown to hatch in 10–14 days. The ciliated epidermal plates of the miracidium were studied by the silver nitrate technique and were found to have the formula 6–6–4–2 =18 plates. The two plates of the fourth tier are lateral and not dorso-ventral as in the genus *Echinostoma.* This feature is apparently characteristic for the genus *Echinoparyphium.*

The miracidium penetrates young specimens of *L. palustris* and within 7 hr is found within the heart of the snail. There, within 24 hr, the miracidium transforms into a sporocyst stage. Johnson (*5*) believed that the miracidium of *E. revolutum* metamorphoses directly into a redia. This study supports the results of Mathias (*6*), Rasin (*7*), and Churchill (*8*), all of whom observed sporocysts in their echinostome studies.

Mother rediae developed from the sporocysts and were first seen in the snail heart at 9 days. At 10 days they were found in the lumen of the heart and were extremely variable in shape. They were shown to be the migratory stage of the mother rediae. In no cases were daughter rediae observed in the heart of the snail.

The mother rediae migrate, apparently, via the snail's circulatory system. They were found at 10–12 days in the digestive gland and ovotestis, where they produce large numbers of daughter rediae.

The daughter rediae are avid eaters, and in both natural and experimental infections the gut was found to be filled with orange-colored material of the snail's

[1] Contribution from the Department of Zoology, University of Michigan, under the direction of Dr. A. E. Woodhead.

digestive gland. In many snails of natural infection the digestive gland was found to be reduced to about one-fifth of its usual mass. In one specimen, which died shortly after being brought into the laboratory, 1647 daughter rediae, by actual count, were removed from the snail.

Recognizable cercarial embryos were first seen within the daughter rediae at 33 days. They are fully formed when they leave via the birth pore of the redia. After entering the lung cavity, the cercariae emerge from the snail via the respiratory aperture. The total time from exposure of the snails to the miracidium to the first emergence of the cercariae from the respiratory aperture was 40–46 days.

Details of the life cycle with systematic considerations will be published later.

References

1. LINTON, E. *Proc. U. S. Natl. Museum*, **15**, 87 (1892).
2. DIETZ, E. *Zool.; Jahrb., Suppl.*, **12**, 265 (1910).
3. MCCOY, O. R. *J. Parasitol.*, **14**, 207 (1928).
4. NAJARIAN, H. H. *Ibid.*, **38**, (4), Suppl. 38 (1952).
5. JOHNSON, J. C. *Univ. Calif. (Berkeley) Publ. Zöol.*, **19**, 335 (1920).
6. MATHIAS, P. *Ann. sci. nat., Botan. et Zool.*, **10**, 289 (1927).
7. RASIN, K. *Spisy Vysoke. Skoly. Zserol. Brno. C.S.R.*, **19**, 1 (1933).
8. CHURCHILL, H. M. *J. Parasitol.*, **36**, (6), Suppl. 27 (1950).

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The Cultivation of Hydra Under Controlled Conditions

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Despite the classical studies that have been carried out on the regenerative capacities of hydra, as well as their extensive use in teaching courses, no reliable method has been available for their continued culture under controlled conditions, a fact that may explain their having been neglected to date as experimental animals.

Previous methods of culturing hydra have required the use of such variable and uncontrolled media as pond (*1–5*) and aquarium (*6*) water and, in addition, have needed subcultures of the water-flea *Daphnia* or other crustacean to provide the living food needed by all species of hydra. Unfortunately, present methods of culturing *Daphnia* are as unsatisfactory as those for culturing hydra; one recent publication has stated (*7*): "So far as we have been able to determine by experimentation, there has been no 'sure-fire' method discovered of keeping a culture of *Daphnia* permanently in the laboratory." The usual technique is to maintain four or five cultures in large wooden tubs, or barrels, so that there is a good chance that at least one culture will contain numerous *Daphnia* at any given time.

Even with an adequate supply of living food available, culturing hydra has been difficult. Hyman has stated (*4*): "The great difficulty in the continuous

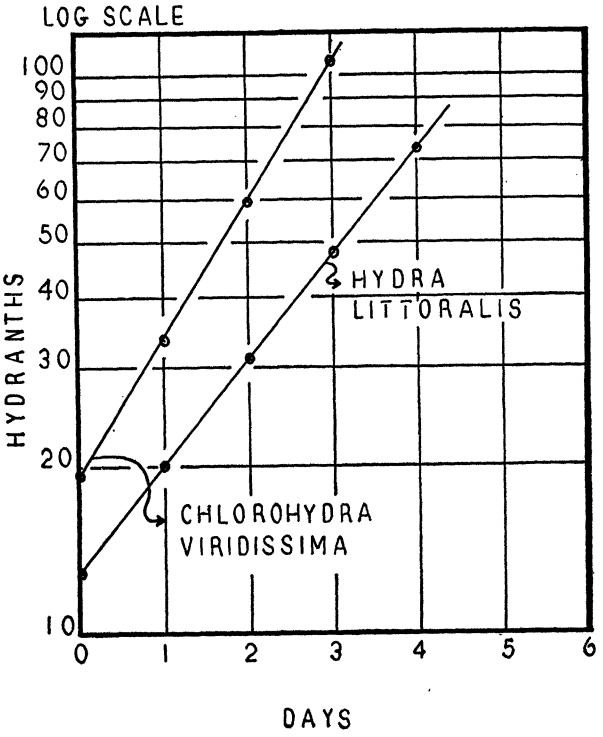

FIG. 1. Logarithmic increase of hydranths in two species of hydra grown under controlled conditions at 20° ± 0.5° C (provisional identification of hydra Littoralis).

culture of hydra is the occurrence of the phenomena of 'depression.' In spite of every care, hydra will pass into this state at intervals and, unless prompt measures are taken, will die out. In depression, column and tentacles fail to expand, the animal ceases to feed, shortens to a stumpy appearance, and finally disintegrates from the tips of the tentacles aborally."

The method of culturing hydra described here avoids depression by controlling both the medium and the food supply of the hydra. In place of pond water, a chemically defined solution is utilized. In place of *Daphnia* cultures, the dried and stable eggs of the brine shrimp *Artemia* are used as a source of living crustacea. As these dried eggs are viable for years, they may be hatched on schedule in reproducible batches of any desired size.

By utilizing the technique described below, rapid logarithmic reproduction (asexual) has been observed (Fig. 1), and thousands of hydra obtained daily with a minimum of effort. All the species studied to date have grown well under these conditions,[1] and depression has been entirely avoided.

1) Brine shrimp eggs[2] are hatched serially at room temperature on a 48-hr schedule, the daily routine

[1] Purchased hydra are often received in a state of depression. On receipt they should be placed singly in test tubes and fed and changed daily until actively budding. After a clone of 10–20 has been formed, they may be transferred to larger vessels.

[2] Brine shrimp eggs are available in quantity from aquarium stock companies.