cholesterol levels rose to a maximum value in 4 to 8 months. The greatest value obtained to date is 880 mg per 100 cm^3. In a number of animals, subsequent to the period of maximum level of serum cholesterol, which was maintained for several months, the value fell precipitously along with decrease in body weight. In many cases this was followed by death of the animals, in which general emaciation or evidence of pulmonary hemorrhagic edema, or both, was observed.

We are firmly convinced that this species will become a useful laboratory animal. To date there have been no successful matings, however. This may be due to the failure to separate pairs. We would appreciate any information which other investigators may have on the matter of propagation of this species (*8*).

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2. This was a concensus obtained after consultation with personnel of several zoological gardens and individuals who obtain and sell animals from South America.
3. A. H. Schultz ["Postembryonic age changes," in *Handbuch der Primatologia*, H. Hofer *et al.*, Eds. (Karger, Basel, Switzerland, 1956), vol. 1, pp. 887–964], gives the following "rough averages of unpublished data by the writer, and data from reports of zoological gardens and other sources": lemur, 14-year life span; macaque, 24 years; gibbon, 30 years; orangutan, 30 years; chimpanzee, 35 years. Unfortunately, no adequate data for any New World monkey seem to be available; hence, whether the pygmy marmoset is typical of New World monkeys in general, or whether it is aberrant, cannot be said.
4. We are grateful to Dr. J. R. Hendrickson, University of Malaya, for supplying this information.
5. The vitamin mixture contained vitamin C, 250 mg; vitamin B_1, 4 mg; vitamin B_2, 5 mg; vitamin B_6, 20 mg; niacin, 20 mg; pantothenol, 20 mg; *p*-aminobenzoic acid, 20 mg; inositol, 1 gm; choline, 1.2 gm.
6. The salt mixture contained KCl, 24.4 gm; $FeSO_4 \cdot 7\,H_2O$, 13.6 gm; $MgSO_4 \cdot 7H_2O$, 14.4 gm; $CuCl_2 \cdot 2\,H_2O$, 0.42 gm; $ZnSO_4 \cdot 7\,H_2O$, 0.7 gm; $CoCl_2 \cdot 6\,H_2O$, 0.4 gm; $MnSO_4$, 0.2 gm; KI, 0.2 gm, in a volume of 125 ml.
7. The procedure of J. J. Carr and I. J. Drekter [*Clin. Chem.* **2**, 353 (1956)] was modified so that tests could be carried out on 0.025 ml of serum.
8. This work was supported by grants from the U.S. Atomic Energy Commission and the U.S. Public Health Service.

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Synxenic and Attempted Axenic Cultivation of Rotifers

Abstract. Three species of rotifers have now been grown synxenically and, to a limited extent, axenically. *Brachionus variabilis* thrives in suitable media containing *Chlorella pyrenoidosa* and a bacterial species. *Lecane inermis* and *Philodina acuticornis* var. *odiosa* are bacteriophagous, the former doing best with two bacterial species (dixenically), the latter doing well with *Escherichia coli* alone (monoxenically).

Relatively few species of invertebrate metazoa have been cultured synxenically [that is, such that a species is grown in the presence of a known number (one or more) of other species or of their living cells (*1*)]. Even fewer have been grown indefinitely under axenic conditions (*2*). During the past 2½ years several species of the aschelminth class Rotifera have been maintained in xenic culture (harboring a mixed, undetermined microbial flora) in our laboratory (*3, 4*). Certain of these have been established synxenically and numerous efforts to initiate axenic cultures have been made (*4–6*).

Our work has been done principally with two species of monogonont rotifers, *Brachionus variabilis* and *Lecane inermis* [the latter of which was erroneously referred to as a tiny, unidentified "bdelloid" in recent notes (*3, 6*)], and with a large bdelloid, *Philodina acuticornis* var. *odiosa (7).*

Xenic cultures of *B. variabilis* were first maintained in Petri dishes containing Seitz-filtered pond water, to which were added enough packed cells of axenic *Chlorella pyrenoidosa* (from a mineral medium) to give a semitransparent green turbidity apparent on inspection under the dissecting microscope. To initiate a culture in this medium one or more egg-bearing females, accompanied by a mixed microbial flora, were inoculated. More recently, *B. variabilis* has been grown well in 13-mm outside diameter, screw-capped test tubes containing the same basal medium except that the chlorellae are better derived from a mineral-glucose medium. *Lecane inermis* flourishes in Petri dishes of 0.1-percent Horlick's malted milk in distilled water. *Philodina acuticornis* grows vigorously in Petri dishes of distilled or pond water containing a ground-up Longlife infusoria table (*8*).

By various techniques the foregoing rotifers have been put into dixenic or monoxenic cultures with certain protists. This has generally been accomplished by first axenizing eggs with hypochlorite followed by antibiotic (penicillin and streptomycin) treatment (*6*) or by antibiotic treatment alone (*6, 9*). More detailed information is being published elsewhere (*4*).

Brachionids so far studied under synxenic conditions (*5, 8–10*) are typically algivorous. Until our culture became contaminated, we grew *B. variabilis* well dixenically for several months with serial subculturing in the presence of *C. pyrenoidosa* plus an unidentified species of gram-negative, rod-shaped bacteria. The basal medium was a 19:1 mixture of Seitz-filtered pond water and concentrated *C. pyrenoidosa* from a mineral-glucose medium. With *E. coli*, instead, as the sole bacterial species, the rotifers died out quickly.

By contrast, sustained monoxenic cultivation of *B. variabilis* has yet to be accomplished. Nathan and Laderman (*9*) have reported limited growth of this species in monoxenic association with *C. pyrenoidosa* in unsupplemented Seitz-filtered pond water; this has also been our finding. However, supplementation with traces of yeast extract and cyanocobalamine has led to flourishing cultures, although conditions permitting successful subculturing from these have not yet been worked out. Bazire (*10*) reported culturing *Epiphanes* [syn. *Hydatina*] *senta* monoxenically or dixenically, with one or two species of algal flagellates.

Our results with *Lecane inermis* and *Philodina acuticornis* indicate that both can live as bacterium-feeders.

Lecane inermis grows well in 0.05-percent Horlick's malted milk (in distilled water and Seitz-filtered pond water, 1:1) in dixenic association with *E. coli* and an unidentified species of gram-negative, rod-shaped bacteria, or with the latter plus a second unidentified species of similar morphology. With either of these unidentified species it also grows well monoxenically. It survives for a while, but does not reproduce in the company of *E. coli* alone. [After our recent report (*5*) of successfully growing *L. inermis* monoxenically with *E. coli*, we found that the second organism had been overlooked earlier because of its initially very slow growth in test media.]

Unlike *L. inermis, P. acuticornis* grows well in the presence of *E. coli* as the sole other living species. The best medium known at present is an empirically developed autoclaved extract of Longlife infusoria tablets.

Our efforts to culture rotifers axenically have so far met with very limited success, despite intensive effort.

The best axenic growth so far observed for *B. variabilis* has been in Seitz-filtered pond water to which were added low levels of whole human blood (about 1 percent), chick embryo ex-

tract (about 1 percent), yeast extract (about 0.001 percent), and cyanocobalamine (about 0.005 μg/ml (5); in this medium an immature female matured and produced an egg which did not hatch.

Lecane inermis has been tested in modifications of the liver medium used for the cultivation of axenic stocks of the nematode *Caenorhabditis briggsae* (*11*). In liver medium diluted to ¼ strength with autoclaved 0.1-percent malted-milk solution, lecanes have survived for at least a week and in a few cases have laid an egg, which has hatched. Various supplementations with vitamin mixes or glucose or both have not improved growth or survival. Our experience with *Philodina acuticornis* has been similar.

The rotifers we have so far worked with, whether algivorous brachioni or bacteriophagous lecanes and bdelloids, are clearly less tolerant of high levels of organic substances, and also of antibiotics (except possibly *P. acuticornis*), than are the rhabditid nematodes studied for several years in this laboratory (*11*). Nevertheless, it seems likely that, since *L. inermis and P. acuticornis* utilize gram-negative bacteria as food, they, at least, have nutritional patterns somewhat like such bacteriophagous nematodes as *Caenorhabditis briggsae*. When one or more rotifer species are tamed to axenic culture, it will be a logical next step to develop chemically defined [holidic (*2*)] media for their permanent maintenance. Like their aschelminth cousins, the nematodes, they promise to be useful tools in the study of comparative nutrition of the lower Metazoa and, most importantly perhaps, for fundamental studies in metazoan physiology and biochemistry (*12*).

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5. Theretofore unpublished results cited in Table 3 (p. 34) of (*2*); values given in that table for supplements to Seitz-filtered pond water medium are too high—see text of present paper for correct approximate values.
6. E. C. Dougherty and Bjørn Solberg, *Anat. Record* **134**, 555, Note II, (1959).
7. *Brachionus variabilis* was initially isolated in October 1957 from a sewage oxidation pond near Concord, California. *Lecane inermis* was obtained in December 1958 from Carolina Biological Supply Company, along with the gastrotrich *Lepidodermella squamata*, in a commercial supply of the latter. *Philodina acuticornis* var. *odiosa* was found in August 1959 in a laboratory culture originating from a fish pond in the backyard of one of us (L.G.H.). We are indebted to Dr. Elbert H. Ahlstrom of the U.S. Fish and Wildlife Service, San Diego, California, for verifying identification of *B. variabilis*, to Dr. John J. Gallagher of Pocatello, Idaho, for identifying *L. inermis*, and to Dr. Josef Donner of Mautern, Steiermark, Austria, for identifying *P. acuticornis*.
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Regulation of Reproductive Rate by Intra-uterine Mortality in the Deer Mouse

Abstract. Under crowded conditions (four mating pairs per cage), pregnant female deer mice of a partially domesticated strain, *Peromyscus maniculatus bairdii*, showed increased resorption of implanted embryos, and therefore a 60 percent reduction in effective natality, as compared with control females living at a population density of one pair per cage. No significant difference was found in the incidence of pregnancy or in the number of embryos implanted, but the adrenal glands of crowded females were enlarged by an average of 17 percent (by weight).

Population biology has become increasingly concerned with the study of fluctuations in the number of offspring produced by populations of animals. The problem of population cycles and crashes is related to the number of viable young produced by females in a population. Recent investigations (*1–3*) indicate that lower reproductive performance may be the result of stress induced by high population densities. The hypothesis that high densities create a stressed condition in members of such a population has been demonstrated by Chitty (*4*), Christian (*5–7*), Clarke (*8*), Louch (*2*), and others, while the effects of stress on endocrine responses have been extensively documented by Selye (*9*), comprising his "general adaptation syndrome."

Intra-uterine mortality (embryonic resorption) has been noted in many wild populations of mammals (*1, 10, 11*). It has been proposed that increased intra-uterine mortality may be the result of stress induced by high population densities and that it is a possible mechanism for the regulation of population size under natural conditions (*11*). Christian (*7*) demonstrated that crowding of albino mice resulted in adrenal hypertrophy, an increase of intra-uterine mortality, and disturbance of lactation.

In the light of such research the effects of crowding on reproductive performance were investigated in the JAX strain of the deer mouse, *Peromyscus maniculatus bairdii* (*12*). A pilot study was conducted to ascertain the feasibility of employing a widely used tranquilizer, reserpine, to vary stress levels in crowded mouse populations. Reserpine has a demonstrated ability to reduce aggressive behavior (*3*) and the incidence of fighting in crowded groups of mice (*5*). It was found, however, that effective dosages of reserpine caused significant increases in intra-uterine mortality and disruptions of the oestrus cycle, thus duplicating the postulated effects of stress on reproduction. As a result, reserpine studies on crowded populations were discontinued.

A standard procedure was used in all crowding experiments. Four male and four female *P. maniculatus bairdii* were placed in a 12- by 4- by 3-in. cage for 3 weeks. An excess of food and water was present at all times. A temperature of 74°F was maintained in the animal room and the room was constantly illuminated. Daily vaginal smears were taken of the females to ascertain the state of the oestrus cycle and the time of impregnation. Five such mating colonies were maintained. Additional mating pairs of the same stock were kept isolated in individual cages under the same conditions, as controls.

At the end of the 3-week period all female experimental and control animals were sacrificed. The animals were dissected, and the reproductive tracts were examined for resorbing and normally developing embryos and implantation scars. The mean paired weights of the adrenals of all experimental and control animals were calculated.

The paired adrenal weights of the crowded females averaged 5.12 ± 0.64 gm, while those of the isolated controls averaged 4.35 ± 0.14 gm. The increase of 17 percent in the crowded animals

Table 1. Reproductive performance of crowded and of isolated *Peromyscus*. Numbers given are means in each category.

Implantation sites (*N*)	Developing embryos (*N*)	Intra-uterine mortalities (*N*)
	Crowded animals (*20*)	
6.4	2.1 ± 0.96	4.3
	Control animals (*10*)	
8.3	5.2 ± 0.81	2.4