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 Theretofore unpublished results cited in Table 3 (p. 34) of (*2*); values given in that table for supplements to Seitz-filtered pond water medium are too high—see text of present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.
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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

is significant at the 5 percent level. This agrees with findings of adrenal hyperactivity (*5–7, 13*) in crowded *Mus* populations and supports the conclusion that a stressed condition was induced in the crowded colonies.

The reproductive behavior of the crowded and isolated animals is summarized in Table 1. The results indicate that the crowded populations of deer mice show a decreased reproductive potential. Implantation scars showed that all but one of the crowded females became pregnant, while the number of scars, though somewhat smaller in the average member of this group, was not significantly reduced as it might have been under more extreme crowding.

Intra-uterine mortality was found in both the experimental and control animals, as indicated by the greater number of implantation scars than of developing embryos. There was, however, a much higher percentage of intra-uterine mortality in the crowded mice. The number of viable fetuses in the crowded group represents an effective reproduction rate only 40 percent of the rate for the uncrowded mice, and the difference (F ratio = 4.30) is significant at the 2 percent level of confidence.

It is therefore concluded that prenatal mortality is probably one of the means of lowering reproductive performance in dense populations of breeding mice. The inference that hormones of the adrenal cortex induce fetal resorption, though reasonable, requires further experimental proof, whereas ecologists will be interested in field confirmation of the suggestion that moderate crowding can act as a stressor in Selye's sense (*14*).

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Studies on the Regulation of Fatty Acid and Cholesterol Synthesis in Avian Liver

Abstract. Homogenates of pigeon liver have been incubated with acetate-C^{14} in excess. Simultaneously, substrate for glycolysis was provided as glucose-6-phosphate. The incorporation of carbon-14 into cholesterol was maximal at low levels of glycolysis, whereas fatty acid turnover was maximum at higher glycolytic levels. A regulatory mechanism is proposed to explain the differential synthesis of cholesterol and fatty acids.

An interesting problem in the control of metabolic pathways is presented by the differential synthesis of fat and cholesterol under various pathologic conditions. For example, diabetic liver can synthesize cholesterol, but cannot synthesize fat normally. Supplementation with large amounts of glucose or fructose in vivo improves the rate of incorporation of acetate into fat by diabetic liver (*1*).

The carbon substrate for the synthesis of both fat and cholesterol is acetyl coenzyme A (acetyl CoA), and synthesis of both requires a hydrogen source. In each case the hydrogen source probably derives from the glycolytic pathway (*2, 3*).

A simple, but perhaps not altogether trivial, hypothesis for the selective control of the pathway of incorporation of acetate into lipide is derived from an analysis of the over-all balance of the carbon and hydrogen requirements for the utilization of equivalent amounts of acetyl CoA for cholesterol or fat synthesis.

$$18\ \text{Acetyl CoA} + 8\ \text{H} + \text{HOH} \longrightarrow \text{cholesterol} + 9\ \text{CO}_2 + 18\ \text{CoA}^{-\text{SH}}$$

$$18\ \text{Acetyl CoA} + 64\ \text{H} \longrightarrow 2\ \text{stearic acid} + 18\ \text{CoA}^{-\text{SH}} + 14\ \text{HOH}$$

This balance shows that the net molar hydrogen requirement for fat synthesis is about eightfold greater than the hydrogen requirement for cholesterol synthesis. It may be assumed that the diabetic animal in ketosis has a plethora of acetyl CoA, so that the controlling substrate factor might be the other requisite, the hydrogen source. Restoring glycolysis by the administration of glucose in vivo enhances the incorporation of acetate into fat in experimental animals (*1*) and relieves ketosis in the human diabetic (*4*). We therefore propose that the selections of pathways for utilization of acetate depend upon the total available reduced coenzmye, which is a function of the rate of glycolysis. To test this hypothesis, homogenates of pigeon liver were prepared and incubated with acetate-1-C^{14} in excess. Substrate for glycolysis was provided as glucose 6-phosphate.

Ten-percent homogenates of pigeon liver in 0.1M potassium phosphate (pH 6.5) were dialyzed against ten volumes of this buffer at 4°C for 1 hour to unmask a dependence of acetate incorporation on added glycolytic substrate. Longer dialysis resulted in an inactivation of acetate incorporation which could not be restored by the addition of nucleotides or coenzyme A. Preincubation with beta-amylase to destroy glycogen is not required. The dialyzed homogenate was supplemented with diphosphopyridine nucleotide (DPN), triphosphopyridine nucleotide (TPN) (*5*), Mg^{++}, Mn^{++} (*6*), coenzyme A, and acetate-1-C^{14} in excess. Two milliliters of this suspension were added to tubes containing glucose-6-phosphate and $KHCO_3$ (*7*), and the resulting solution was made to a final volume of 3.0 ml with 0.15M KCL and incubated with vigorous shaking for 1 hour at 37°C. Two milliliters of saturated KOH were added, and the mixture was saponified at 100°C for 2 hours. Mixtures of fatty acid and cholesterol were isolated according to the method of Hotta *et al.* (*8*) and counted for radioactivity.

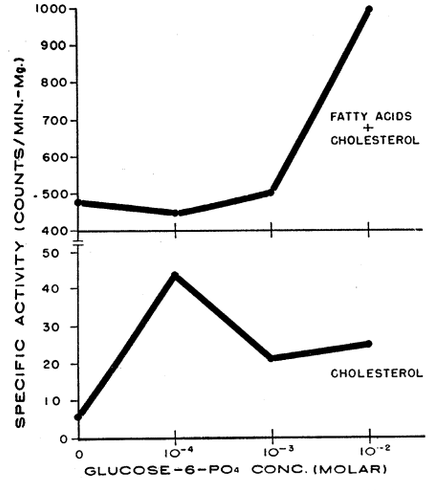

Fig. 1 Incorporation of acetate-1-C^{14} into fatty acids and cholesterol. Each tube contained $1.5 \times 10^{-4}M$ DPN, $1.5 \times 10^{-4}M$ TPN, $3.7 \times 10^{-3}M$ $MgSO_4$, $2.3 \times 10^{-4}M$ $MnCl_2$, $3.3 \times 10^{-3}M$ $KHCO_3$, 0.45 μg CoA, and $1.5 \times 10^{-3}M$ NaOAc-C^{14} (15 μc).