

# Specific Gravity Increase of *Caenorhabditis briggsae* with Age

B. M. ZUCKERMAN, B. NELSON and M. KISIEL<sup>1</sup>

**Abstract:** The specific gravity of old *Caenorhabditis briggsae* was shown to be greater than that of young nematodes. The possible explanations for this age-associated change are discussed. **Key Words:** aging, specific gravity, methods.

The observation that aging in *Caenorhabditis briggsae* was marked by a decreased ability to withstand osmotic stress (3) led us to postulate that specific gravity increased with age. Specific gravity changes associated with physiologic aging have precedent on a cellular level, for many authors have noted a specific gravity increase with the physiologic aging of red blood cells (2). To test this postulate, we used a method devised by Danon and Marikovsky (2) for determining the density distribution of rabbit erythrocytes, modifying the technique to the extent required to manipulate the nematodes.

## MATERIALS AND METHODS

A set of water nonmiscible fluids of predetermined specific gravity (hereafter called the fluids), consisting of a mixture of di-n-butyl phthalate and methyl phthalate, was used to obtain the specific gravity distribution of *C. briggsae* by age. The specific gravity of the fluids in this set ranged between 1.062 and 1.138, with increments of 0.004 (manufactured by Miles-Yeda Ltd., Kiryat Weizmann, Rehovot, Israel and sold under the name of Gravikit).

Nematodes used in these tests were cultured axenically and allowed to age as described by Zuckerman et al. (3). The tests were set up as follows:

The tip of a capillary, 100- $\mu$ liter capacity and 1.1 mm inside diam, was dipped into the fluid and held there until a 5- to 7-mm column had penetrated. The tip then was placed in a vial containing culture medium (hereafter called the suspending medium) without nematodes until a 5- to 7-mm column of suspending medium was drawn into the tube. Next, the capillary was inserted into a wire holder attached to a dissecting microscope in a

position so that the tip could be viewed, and five nematodes were transferred by needle into the suspending medium. The opposite end of the capillary then was flame-sealed (Fig. 1). The capillary was placed in an adapter fabricated to hold these tubes, then centrifuged. After centrifugation, the tubes were viewed under a dissecting microscope, and the number of nematodes which penetrated the fluid or had not penetrated and remained in the suspending medium was recorded.

Before initiating these experiments, it was necessary to establish parameters for centrifuge speed, time of centrifugation, temperature at which tests were to be run and the solution densities which most closely approximated the specific gravity of *C. briggsae*. Axenic cultures containing nematodes of all ages were used in these preliminary tests. It is of interest that 1.084 is the figure calculated by Andr assy (1) as being the specific gravity for all free-living nematodes. However, our method measured the relative specific gravity of the nematodes in the two age groups and not actual specific gravity, since the centrifugal force to which the nematodes were subjected aided penetration into the fluid. In the final tests, which encompassed 106 trials and observations on

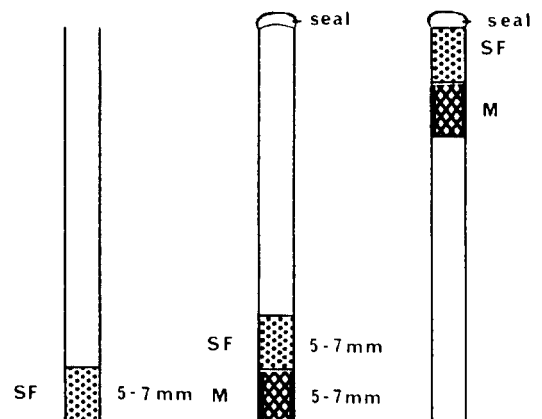


FIG. 1. M = Suspensing medium in which nematodes are placed. SF = water, nonmiscible fluid of known specific gravity.

Received for publication 14 February 1972.

<sup>1</sup> Professor, Research Assistant and Postdoctoral Fellow, respectively. Laboratory of Experimental Biology, University of Massachusetts, East Wareham, Massachusetts 02538.

TABLE 1. Relative specific gravities of young and old *Caenorhabditis briggsae* as measured by their penetration into fluids of different specific gravity<sup>a</sup>.

Specific gravity of the fluid	% Penetration	
	Young	Old
1.062	79	100
1.078	25	100
1.082	24	100
1.086	33	93
1.090	39	97
1.094	5	84
1.098	3	88
1.102	0	88
1.138	0	10

<sup>a</sup>Penetration difference between young and old were all significant at the 1% level.

630 nematodes, centrifugation was at 3200 g for 5 min at 20 C. Since several series of tests were run, the ages of the nematodes varied slightly, the young being 5-7 days and the old, 25-28 days. All nematodes tested were adults, with the young containing eggs in various stages of development; the old generally were without eggs.

## RESULTS

Though tests were run with each of the fluids, the tests with fluids 1.066 through 1.074 and 1.106 through 1.134 are not included, since the data presented adequately illustrate the results (Table 1).

The specific gravity of older nematodes was significantly greater than that of younger nematodes. The most striking differences observed were with fluids of specific gravity 1.094 through 1.102, for here the interaction of the several factors (specific gravity of the fluid, centrifugal force and specific gravity of the nematodes) balanced each other in such a way that the specific gravity differences between young and old nematodes were accentuated.

The results also indicate specific gravity differences between nematodes of the same age. For example, the retention of some young nematodes in the suspending medium in the 1.062 fluid test, and the penetration of some old nematodes into the 1.138 fluid, support this conclusion. In fact, if nematode specific gravity within age groups was almost identical, then at each level of fluid tested most of the

nematodes of the same age should have either penetrated the fluid or been retained in the suspending medium. Our conclusions are not in agreement with those of Andr assy (1) that free-living nematodes, regardless of their species and size, have almost the same specific gravity.

## DISCUSSION

The results of these experiments may explain the decrease in ability of *C. briggsae* to withstand osmotic stress as physiologic aging progresses, for an increase in specific gravity could accompany an increase in the solute concentration in the pseudocoelom. This would lead to increased water uptake, thus explaining the observations by Zuckerman et al. (3) that old nematodes burst when exposed to an osmotic pressure which did not affect young nematodes. On the other hand, decreased ability to osmoregulate may be related to a greater extent to age-related changes in the collagen fibrils and hence in the permeation characteristics of the cuticle, or changes in the active transport mechanisms of the hypodermis. In either case, the excretory function would be directly affected, leading to a buildup of solutes within the body cavity. Breakdown of storage materials (glycogen or fats) would also increase osmotic pressure. The reduced ability of older nematodes to withstand osmotic stress could be related to factors other than the solute concentration of the pseudocoelomic fluid. For example, the collagen macromolecule in vertebrates is known to manifest changes with age, such as increased physical aggregation, crystallization of the molecules, or cross-linking between the molecules. Assuming that nematode collagen senesces in a similar way, these changes would lead to loss of elasticity of the cuticle in older nematodes, hence a greater susceptibility to hypotonic shock.

Experiments are in progress to gather further information on age-associated changes in *C. briggsae*.

## LITERATURE CITED

1. ANDR ASSY, I. 1956. The determination of volume and weight of nematodes. *Acta Zool. (Hungarian Acad. Sci.)* 2:1-15 (in German).
2. DANON, D. and Y. MARIKOVSKY. 1964. Determination of density distribution of red cell population. *J. Lab. Clin. Med.* 64:668-674.
3. ZUCKERMAN, B. M., S. HIMMELHOCH, B. NELSON, J. EPSTEIN and M. KISIEL. 1971. Aging in *Caenorhabditis briggsae*. *Nematologica* 17:478-487.