Polymorphism in the Rotifer *Asplanchna sieboldi:* Some Chemical Constituents of the Saccate and Campanulate Females

J. R. LITTON, JR., and J. J. GILBERT[1]

Department of Biological Sciences, Dartmouth College, Hanover (New Hampshire 03755, USA), 26 April 1976.

Summary. No detectable differences in total protein, DNA, RNA and glycogen content were found between the morphologically and physiologically distinct saccate and campanulate morphotypes in one clone of the rotifer *Asplanchna sieboldi.*

The large, predatory, ovoviviparous rotifer *Asplanchna sieboldi* can exhibit a pronounced non-genetic polymorphism in female body size and shape. The polymorphism is controlled jointly by dietary tocopherol (vitamin E) and prey-type; these factors also determine the mode of reproduction of the population[2–4]. Females may exist in any of three relatively distinct, basic morphotypes: saccates, cruciforms, and campanulates (see GILBERT[4] for details). Saccates are the smallest, adults ranging from 550 to 750 μm in length, while campanulates may be up to 1250 μm in length; newborn and gravid adult campanulate females have 4.37 and 2.98 times as much dry weight biomass as newborn and gravid adult saccate females, respectively[5]. GILBERT[6] has summarized some anatomical features of these two morphotypes and has compared saccates and campanulates with respect to dry weight biomass, growth, longevity, fecundity, development time, rate of reproduction and mean generation time. WURDAK and GILBERT[7] examined the morphology of the saccate, cruciform and campanulate morphs with light and electron microscopy. Further comparisons of the saccate and campanulate morphotypes are of considerable interest, for while the two morphotypes are comparable in their mode of reproduction (diploid female parthenogenesis) and ultrastructure they present two extremely different morphological expressions of the same species' genotype. Additional information on differences in the two morphotypes may allow further evaluation of the functions and mechanisms responsible for the differentiation of the two morphotypes.

In the present study the saccate and campanulate morphotypes are compared with respect to in toto concentrations of protein, DNA, RNA and glycogen; measurement of static concentrations of these constituents might indicate whether there are morphotypic differences in protein composition, total DNA per individual and ploidy level, static RNA concentration, and energy storage, respectively.

Materials and methods. Procedures for the culture of the saccate and campanulate morphotypes of *Asplanchna sieboldi*, clone 12C1, are described elsewhere[5]. The saccates and campanulates in this experiment were maintained exclusively on *Paramecium aurelia* and *Asplanchna brightwelli*, respectively. Rotifers of all age classes from mass cultures were collected in rotifer medium and washed twice in glass distilled water. Homogenization and dry weight determination procedures are described by LITTON and GILBERT[8]. Homogenates were fractionated by the method of SCHMIDT and THANNHAUSER[9] as reviewed in MUNRO and FLECK[10]. 3 separate aliquots of the homogenates were analyzed with the following analytical methods being substituted for the final determinations of each compound or class of compounds. The above homogenization and analysis procedure was replicated 3 times.

Protein was measured by the HARTREE[11] modification of the LOWRY et al.[12] method. Bovine albumin standard (Sigma Chemical Co., St. Louis, Missouri, USA) was used for calibration. DNA was measured using the fluorometric procedure of KISSANE and ROBBINS[13]. Type I calf thymus

[1] We thank Mrs. MAXINE BEAN for expert technical assistance and Dr. J. D. YAGER and Mr. M. KABAY for careful review of the manuscript. This work was supported by National Science Foundation grant No. GB-31282, Public Health Service research grant No. 1R01 HD 08210 from the National Institute of Child Health and Human Development, and Public Health Service Career Development Award No. K04-GM-70557 from the National Institute of General Medical Sciences to J. J. G.

[2] J. J. GILBERT and G. A. THOMPSON, JR., Science *159*, 734 (1968).

[3] J. J. GILBERT, Oecologia *13*, 135 (1973).

[4] J. J. GILBERT, Arch. Hydrobiol. *75*, 442 (1975).

[5] J. J. GILBERT, Ecology, *57*, 542 (1976).

[6] J. J. GILBERT, Am. J. clin. Nutr. *27*, 1005 (1974).

[7] E. S. WURDAK and J. J. GILBERT, Cell. Tiss. Res., *169* 435 (1976).

[8] J. R. LITTON, JR., and J. J. GILBERT, J. gen. appl. Microbiol. *21*, 345 (1975).

[9] G. SCHMIDT and S. J. THANNHAUSER, J. biol. Chem. *161*, 293 (1945).

[10] H. N. MUNRO and A. FLECK, Meth. Biochem. Analyt. *14*, 113 (1966).

[11] E. F. HARTREE, Analyt. Biochem. *48*, 422 (1972).

[12] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).

[13] J. M. KISSANE and E. ROBBINS, J. biol. Chem. *233*, 184 (1959).

Summary of biochemical determinations in the saccate and campanulate morphotypes of *Asplanchna sieboldi*, clone 12C1

Morphotype	Replicate mean ($n = 3$)	Concentration (mg/g dry weight rotifer)			
		Protein	DNA	RNA	Glycogen
Saccate	a)	560.0	9.3	23.0	8.4
	b)	560.0	9.4	27.0	8.7
	c)	563.0	9.3	24.0	8.6
	mean ± SE	561.0 ± 0.9	9.3 ± 0.2	24.7 ± 0.8	8.6 ± 0.2
Campanulate	a)	563.0	9.3	26.0	8.8
	b)	562.0	9.0	25.0	8.8
	c)	557.0	9.2	29.0	8.6
	mean ± SE	560.7 ± 1.1	9.2 ± 0.2	26.7 ± 0.8	8.7 ± 0.2

DNA (Sigma) served as a standard. Determination of RNA was made with the orcinol method of SCHNEIDER[14]. Type IV calf liver RNA (Sigma) was used for calibration. Glycogen was enzymatically hydrolyzed directly in the homogenate by treatment with amyloglucosidase without any preliminary extraction or purification procedure[15]. The glucose thus released was then determined by the enzymatic-colorimetric glucose oxidase procedure using a Sigma kit[16]. Standards were prepared from pure Type I rabbit liver glycogen (Sigma) as outlined in KOEHRIG and ALLRED[15]. A single-classification analysis of variance[17] was performed to determine the significance of the results.

Results and discussion. The results of the determinations, expressed as mg/g dry weight rotifer, are shown in the Table. The analysis of variance showed that there were no significant differences, at the p 0.05 confidence level, in any of the measured biochemical parameters between the saccate and campanulate morphotypes.

Using mean values for dry weights of gravid adult saccate and campanulate morphs from GILBERT[5], an estimate of the chemical constituents per rotifer can be calculated for the 2 morphs. The saccate values in µg per organism are: protein (1.20), DNA (0.019), RNA (0.053) and glycogen (0.055). The much larger campanulate morph showed an approximately 3-fold increase in constituents per individual: protein (3.57), DNA (0.058), RNA (0.170) and glycogen (0.055). These extrapolations are valid only if chemical constituent concentration does not change appreciably with age.

While campanulates do not show an increased whole body concentration of DNA (or other constituents measured) compared to saccates, the estimated increase of DNA by 3 times on a per individual rotifer basis is worthy of further investigation and comment. WURDAK and GILBERT[7] have shown that the gastric and yolk gland nuclei in the campanulate morph are larger (approximately 25% increase in nuclear dimension) and more numerous (yolk gland nuclei 8% more; gastric gland 27% more) than in the saccate morph. An increase in nuclear size and number also may occur in other campanulate organs and tissues but probably would not account for the 3-fold estimated increase in DNA content of the campanulate morph. Thus there may well be more DNA per nucleus in the campanulate morph. A scanning microspectrophotometric analysis of the relative DNA contents of nuclei in saccate and campanulate morphs is in progress.

While no gross chemical differences between the saccate and campanulate morphs were observed in this study of rotifers of all age classes, we cannot exclude different rates of synthesis and turnover of these compounds in the 2 morphotypes or changes in concentration with age. MEADOW and BARROWS[18], for example, showed increases in fat, ash and protein with age in a bdelloid rotifer while RNA content remained constant.

The chemical constituent measurements may be of direct value, in future ecological investigations of *A. sieboldi*, since standing stock biomass estimates can be converted to individual constituents. Also, these measurements will permit the normalization of rotifer extracts with regard to protein, DNA, RNA and glycogen concentration.

The data obtained correspond well with published accounts of chemical measurements in rotifers. Our protein values for *Asplanchna* (56.10% and 56.07% of the dry weight) are close to those of POURRIOT[19] (57% of the dry weight) for a mixed assemblage of rotifer species, but they are higher than those of ERMAN[20] (41.2% of the dry weight) for *Brachionus calyciflorus*. MEADOW and BARROWS[18], working with the bdelloid rotifer *Philodina acuticornis odiosa* Milne, reported a protein value range of 43–54% of the dry weight. Their range of 1.7–5.6% of the dry weight for RNA includes our mean values of 2.47% and 2.67% of the dry weight. No reference values for DNA or glycogen in rotifers seem to be available.

[14] W. C. SCHNEIDER, in *Methods in Enzymology* (Eds. S. P. COLOWICK and O. KAPLAN; Academic Press, New York 1957), vol. 3, p. 680.
[15] K. L. KOEHRIG and J. B. ALLRED, Analyt. Biochem. *58*, 414 (1974).
[16] Sigma Chemical Co., Technical Bulletin No. 510 (1973).
[17] R. R. SOKAL and F. J. ROHLF, *Biometry* (W. H. Freeman, San Francisco 1969).
[18] N. D. MEADOW and C. H. BARROWS, JR., J. exp. Zool. *176*, 303 (1971).
[19] R. POURRIOT, Ann. Hydrobiol. *1*, 171 (1970).
[20] L. A. ERMAN, Dokl. Akad. Nauk SSSR *144*, 926 (1962).

Correlation of Blood and Brain Amino Acids in Hypoglycemic and Normoglycemic Rats

R. A. WAPNIR[1]

Department of Pediatrics, North Shore University Hospital, Manhasset (New York 11030, USA); and Department of Pediatrics, Cornell University Medical College, New York (N. Y. 10021, USA), 27 April 1976.

Summary. Utilization of gluconeogenic amino acids as a source of energy by brain can occur in starved newborn rats. This capacity is lost later in life as evidenced by changing ratios in blood and brain concentrations between fed and fasted animals.

Brain is considered to be one of the organs or tissues with mandatory requirements for glucose, similarly to blood corpuscles and muscle[2]. Only under pathological conditions, or early in life, alternative sources of energy have been demonstrated to be utilized directly by brain, namely, 'ketone bodies' or gluconeogenic amino acids. This has been shown to occur during starvation[3], in the neonatal period[4,5], or during in vitro experiments when the supply of glucose to the brain is totally excluded[6].

This communication illustrates the correlation between circulating levels of glucose and several gluconeogenic amino acids and their respective concentrations in brain during the nutritional stress induced by prolonged food deprivation in experimental animals at various stages of development.

[1] I thank SUSAN A. MOAK for technical assistance.
[2] R. BALÁZS, in *Handbook of Neurochemistry* (Ed. A. LAJTHA; Plenum Press, New York 1970), vol. 3, p. 1.
[3] O. E. OWEN, A. P. MORGAN, H. G. KAMP, J. M. SULLIVAN, M. G. HERRERA and G. F. CAHILL JR., J. clin. Invest. *46*, 1589 (1967).
[4] Z. DRAHOTA, P. HAHN, J. MOUREK and M. TROJANOVÁ, Physiologia bohemoslov. *14*, 134 (1965).
[5] D. C. DEVIVO, M. P. LECKIE and H. C. AGRAWAL, J. Neurochem. *25*, 161 (1975).
[6] B. MUKHERJI, J. TURINSKY and H. A. SLOVITER, J. Neurochem. *18*, 1783 (1971).