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

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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).

Table I. Relationship between fed and fasted rat blood and brain metabolites

	Mean fed/fasting ratios							
	Newborn (12 h)[a]		Newborn (24 h)		Sucklings (48 h)		Adults (120 h)	
Blood glucose[b]	1.95[d]	3.72 ± 0.33 (12) 1.91 ± 0.34 (17)	3.74[e]	3.37 ± 0.29 (18) 0.90 ± 0.19 (16)	1.59[d]	4.76 ± 0.31 (22) 3.00 ± 0.23 (25)	1.74[c]	6.77 ± 0.52 (6) 3.88 ± 0.21 (9)
Blood alanine	5.14[e]	1.13 ± 0.16 (12) 0.22 ± 0.05 (17)	3.13[e]	0.72 ± 0.10 (18) 0.23 ± 0.07 (16)	2.09[e]	0.73 ± 0.05 (22) 0.35 ± 0.04 (24)	0.78	0.43 ± 0.02 (6) 0.55 ± 0.06 (9)
Brain alanine	3.33[d]	0.70 ± 0.15 (8) 0.21 ± 0.02 (7)	5.17[e]	0.62 ± 0.08 (10) 0.12 ± 0.03 (11)	1.34[d]	0.39 ± 0.02 (27) 0.29 ± 0.03 (25)	0.85	0.23 ± 0.01 (5) 0.27 ± 0.01 (10)
Blood glutamine/-ate	1.97[d]	1.79 ± 0.15 (12) 0.91 ± 0.18 (17)	1.41[c]	1.48 ± 0.15 (18) 1.05 ± 0.11 (16)	1.33[c]	1.38 ± 0.04 (22) 1.04 ± 0.06 (24)	1.09	1.02 ± 0.04 (6) 0.94 ± 0.08 (9)
Brain glutamate/-ine	1.49	2.96 ± 0.43 (8) 1.99 ± 0.26 (7)	1.96[d]	3.57 ± 0.20 (10) 1.82 ± 0.20 (11)	1.03	5.32 ± 0.33 (23) 5.19 ± 0.44 (22)	0.73[c]	5.10 ± 0.20 (5) 6.97 ± 0.83 (10)

[a] Fasting period. [b] Values tabulated represent the ratios between the concentrations of blood and brain metabolites of comparable fed and fasting rats. All concentrations in blood are expressed in mM and in brain as μmoles/mg DNA. In brackets are the means ± SEM of fed (numerator) and fasted (denominator) animals. Number of rats in parenthesis. [c] $p < 0.05$; [d] $p < 0.01$; [e] $p < 0.001$.

For experiments in the perinatal period, Wistar-derived pregnant rats (Carworth, New City, N.Y.) were fed a complete commercial ration (Purina Lab Chow, Ralston Purina Co., St. Louis, Mo.) ad libitum and maintained in a controlled environment throughout gestation and lactation. After delivery, litters were reduced, if necessary, to 12 pups. Broods smaller than 8 were excluded. Immediately after birth, half of each litter, regardless of sex, was taken away from the dams for 12 or 24 h and maintained at a temperature of 29–31 °C. The remaining pups were left with the dams during the same time. Weight losses were replaced with i.p. injections of 0.04 M NaCl thrice daily. In other experiments, sucklings 2 to 21 days old were fasted for 48 h. Adult males were starved for 120 h. Water was offered to these animals at will. At the end of the fasting period, both these rats and their fed counterparts were placed under light ether anesthesia and the heads severed into liquid nitrogen. Blood was collected from the trunks. Analytical methods were described in a previous publication[7].

Starved newborn animals suffered a marked hypoglycemia, with mean glucose levels falling below 1.0 mM (18 mg/100 ml) in the survivors of the stress. A mortality of 33% was recorded after 24 h of fasting in the newborn group. However, sucklings withstood 48 h of food deprivation and adults up to 120 h of fasting with a marked weight loss but without effects on their survival. Blood glucose levels of both suckling and adult rats declined sharply after either 48 or 120 h of fasting, but remained above the 2.8 mM (50 mg/100 ml) mark.

The ratios between blood concentrations in fed and fasted animals for glucose, alanine and glutamate plus glutamine, and brain levels of these amino acids under the same conditions are shown in Table I. It was apparent that in newborn rats, the decline of free amino acids in circulation was concomitant with a fall in brain alanine after 12 h of fasting. Following 24 h of food withdrawal, the depletion of brain alanine was even more marked, and a comparable, but less sharp change occurred for glutamine/-ate and its brain stores. In sucklings 2 to 21 days old, only brain alanine suffered a decline comparable to that in blood, while the decrease in brain glutamate/-ine was insignificant. Furthermore, adult rats, even when starved for 5 days, were capable of maintaining lower, but adequate, levels of blood glucose. The stress was insufficient to affect either circulating levels of alanine or brain deposits of this amino acid. The same was true for blood glutamine plus glutamate in the adult rat, while the brain concentrations of these amino acids were actually increased.

The interrelationship between circulating and brain metabolites was assessed in terms of the correlation coefficients between blood versus brain concentrations (Table II). Glucose level alterations, namely, changes due to hypoglycemia secondary to starvation, showed no correlation with brain alanine and glutamate in newborn and suckling rats. In adult rats, the accumulation of

[7] R. A. WAPNIR, Brain Res. *57*, 187 (1973).

Table II. Correlation coefficients between glucose, alanine and glutamine plus glutamate in blood and brain of fed and starved rats

	Brain alanine		Brain glutamate/-ine	
	2-21 days old	Adults	2-21 days old	Adults
Blood glucose	0.249 (28)	− 0.633[a] (12)	0.246 (28)	− 0.647[b] (12)
Blood alanine	0.714[c] (29)	0.107 (12)	− 0.053 (29)	− 0.249 (12)
Blood glutamine + glutamate	0.652[c] (29)	− 0.472 (12)	0.247 (29)	− 0.607 (12)

Number of animals in brackets. [a] $p < 0.05$; [b] $p < 0.02$; [c] $p < 0.001$.

these two amino acids corresponded with a significant, negative, regression coefficient in regard to blood glucose. However, changes in circulating levels of alanine and glutamine plus glutamate were a good index for the concentration of alanine in the brain of newborn and suckling rats. In addition, there was a negative correlation between free glutamate plus glutamine in the blood and the stores of these two amino acids in the brain of adult animals. In contrast, the concentration of brain glutamate plus the lesser amounts of glutamine present in that organ could not be related to changes in the circulation of either glucose, alanine or glutamine-glutamate in juvenile rats, regardless of their nutritional status. These correlations, when significant, do not imply a causal relationship in the two variables, but serve to point out complementary physiological effects.

The analysis of the figures presented here provide an additional understanding of the potential sources for gluconeogenesis during starvation and of the possible alterations in brain metabolites which may occur at various stages of development in the rat. The sharp declines in blood alanine, with a concomitant reduction of brain levels of this amino acid in young animals are a consequence of the limited mobilization of protein-derived amino acids in growing animals. This phenomenon becomes fully operative in adult rats[8,9]; hence, it is understandable the lack of correlation between circulating alanine and the level of this amino acid, as well as that of glutamate, in the brain of older rats. This metabolite actually accumulated in the brain of adult, starved, animals. Its depletion occurred only during extreme deprivation in immature animals, as observed in the present and previous studies[10–12].

The severe hypoglycemia attained in the newborn rat when no feedings were given after birth seems to be an in vivo experimental condition comparable to the one achieved by in vitro perfusion of brain with glucose-free buffers[6]. The larger stores of brain glutamate and their reduction in cases of severe hypoglycemia exemplify the concept that direct utilization of such an ancillary metabolite through the tricarboxylic acid cycle is a possible alternative to provide energy for the brain when the availability of glucose and other primary fuels, such as glycolytic intermediates or 'ketone bodies' is diminished[4,5,13].

[8] P. Felig, Metabolism *22*, 179 (1973).
[9] P. T. Ozand, J. T. Tildon, R. A. Wapnir and M. Cornblath, Biochem. biophys. Res. Comm. *53*, 251 (1973).
[10] R. M. C. Dawson, Biochem. J. *47*, 386 (1950).
[11] J. Mourek, H. C. Agrawal, J. M. Davis and W. A. Himwich, Brain Res. *19*, 229 (1970).
[12] B. S. Reddy, J. R. Pleasants and B. S. Wostmann, Proc. Soc. exp. Biol. Med. *136*, 949 (1971).
[13] M. K. Roach, J. Corbin and W. Perrington, J. Neurochem. *22*, 521 (1974).

Studies on the Survival Time of the Eupyrene and Oligopyrene Spermatozoa of the Prosobranch, *Vivipara bengalensis* (Lamarck)

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Summary. Vivipara bengalensis, like many other gastropods, produces two types of spermatozoa viz., eupyrene (normal) and oligopyrene (abnormal). The eupyrene ones are comparatively small and uniflagellated, whereas the oligopyrene ones are much larger, worm-like and each with a tuft of tail flagella. Eupyrene and oligopyrene spermatozoa reveal in vitro differential survival characteristics; the eupyrene spermatozoa exhibit considerably shorter survival as compared with their oligopyrene counterparts.

Vivipara bengalensis, like many other gastropods, exhibits sperm dimorphism[1–6]. The two types of spermatozoa, viz. 1. normal or eupyrene, and 2. abnormal or oligopyrene, are markedly different morphologically. The mature eupyrene spermatozoon is about 45 µm long, a flagellated cell with screwy head comprizing spirally twisted nucleus carrying a pointed triangular acrosome at its tip. The middle-piece region is characterized by a distinct cytoplasmic wrapping, rich in mitochondria, around the axial filament. This is followed by a sufficiently long vibratile tail. The abnormal or the oligopyrene spermatozoon, on the other hand, is cylindrical, more voluminous and reveals caterpillar-like movements under the phase-contrast microscope. The oligopyrene spermatozoon comprizes 3 distinct regions, viz. 1. the anteriormost, 3–5 µm head region which is represented by a diminutive degenerating nucleus and is apparently devoid of any acrosome, 2. a long tubular and bulky middle-piece followed by 3. a tufted tail comprizing a large number of flagella which arise from near the nucleus and travel all through the cylindrical middle-piece region, from the posterior extremity of which they pierce out. The number of flagella comprizing the tail tuft varies from 10 to 15.

Time (min)	Living eupyrene spermatozoa (%)	Living oligopyrene spermatozoa (%)
0	53.33 ± 2.149	95.38 ± 0.856
15	12.82 ± 0.643	67.65 ± 0.686
30	11.36 ± 0.071	66.13 ± 0.417
45	11.36 ± 0.007	50.88 ± 1.952
60	0	49.76 ± 3.026
75	–	21.23 ± 0.325
90	–	0

[1] J. B. Gatenby, Q. Jl microsc. Sci. *63*, 401 (1919).
[2] J. Hanson, J. T. Randall and S. T. Bayley, Expl Cell Res. *13*, 65 (1952).
[3] A. Franzen, Zool. Bidr. Upps. *30*, 399 (1955).
[4] G. Yasuzumi and H. Tanaka, J. biophys. biochem. Cytol. *4*, 621 (1958).
[5] G. P. Sharma, B. L. Gupta and O. P. Mittal, Cytologia *24*, 423 (1959).
[6] J. G. Gall, J. biophys. biochem. Cytol. *10*, 163 (1961).