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].

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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

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The eosin test, originally employed by BURGOS and DI PALO[7] to differentiate between dead and living mammalian spermatozoa, was used to determine the survival characteristics of both eupyrene and oligopyrene spermatozoa in vitro. This test is based upon the fact that eosin in aqueous solution stains dead cells only, leaving living cells unstained. The eosin test works well on gastropod spermatozoa and stains the dead spermatozoa – both oligopyrene and eupyrene types.

The testes were teased in Ringer solution (NaCl 0.65 g; KCl 0.042 g; $CaCl_2$ 0.025 g; H_2O 100 ml) and testicular squashes studied at room temperature (30 ± 2 °C) employing phase-contrast microscope. Percentage of living and dead spermatozoa of both categories was determined after every 15 min. These observations were supplemented and confirmed when such squash preparations were made in 0.5% eosin in Ringer solution and percentage of stained (eosinophilic) and unstained (eosinophobic) spermatozoa carefully counted. The data has been recorded in the Table.

Survival characteristics of the two types of spermatozoa vary a good deal. Percentage motility at 0 h in case of eupyrene spermatozoa was only 53.33 ± 2.149%, whereas for oligopyrene spermatozoa it was high, viz. 95.38 ± 0.856%. The percentage of motility for both categories of spermatozoa sharply declined in the first 15 min, whereafter the fall in percentage motility became gradual; whereas all eupyrene spermatozoa died within 60 min, a good percentage of oligopyrene spermatozoa (over 21%) showed motility up till 75 min. However, after 90 min, all oligopyrene spermatozoa were also killed.

All this shows that eupyrene spermatozoa in vitro have a shorter survival span as compared with the oligopyrene ones which survived much longer.

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Neurochemical Correlates of Alloxan Diabetes: Protein and Ribonucleic Acid Levels in the Different Regions of the Rat Brain

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Summary. The levels of protein and ribonucleic acid in the cerebrum, cerebellum, optic lobes and medulla oblongata of normal and alloxan-diabetic rats were measured. In general, the protein content and levels of ribonucleic acid in the broad compartments of the brain of rat decreased during diabetes.

Abnormalities of protein metabolism in various tissues of mammals during diabetes have been extensively studied[2–9]. BUCK et al.[4] demonstrated a decrease in the rate of nuclear protein biosynthesis in rat liver; while hepatic ribosomal protein synthesis in rats has been shown to decrease during experimental diabetes[5]. SAYUK[8] reported a decrease in protein and nucleic acid content in tissues from rats with diabetes. However, no information is available on the regional distribution and changes in the levels of protein and nucleic acids in the brain of rat during alloxan diabetes. The paper presents information on the changes in the distribution of protein and ribonucleic acid in different regions of rat brain during diabetes.

Materials and methods. Immature albino rats (Wistar strain) of both sexes, weighing 60–85 g were used. They were maintained in cages at room temperature (25 ± 2 °C) on a commercial diet (Hindustan Lever Ltd., Bombay, India). Water was available ad libitum.

Diabetes was induced by i.v. injection of alloxan monohydrate, as described earlier[10].

Rats were decapitated and the brain was removed from the ventral side. The adhering blood vessels were removed and different regions of the brain, viz., cerebrum, cerebellum, optic lobes and medulla oblongata, were separated with a sterilized scalpel by keeping the brain in mammalian Ringer[11] at 0 °C. The tissues were weighed in an electric balance with Ringer and immediately used for analysis.

Proteins were estimated by the method of LOWRY et al.[12] The levels of ribonucleic acid were measured following the method described by SCHMIDT-THANNHAUSER and SCHNEIDER[13]. Blood glucose was measured by the method of Folin[14].

Results. The Table summarizes the results obtained. Blood glucose levels showed 108% increase over controls during diabetes. In general, the protein content decreased

Changes in the levels of protein and ribonucleic acid in different regions of the brain of alloxan-diabetic rats

	Cerebrum		Cerebellum		Optic lobes		Medulla	
	Control	Test	Control	Test	Control	Test	Control	Test
Protein[a]	125.34 ± 6.8	99.5 ± 4.1	96.3 ± 4.0	79.5 ± 2.3	84.0 ± 4.0	68.2 ± 1.8	87.6 ± 5.2	66.2 ± 3.0
	– 20.65[c] $p > 0.01$		– 17.5[c] $p > 0.01$		– 18.8[c] $p > 0.01$		– 24.5[c] $p > 0.01$	
RNA[b]	6.7 ± 0.9	4.7 ± 0.8	5.9 ± 0.6	4.2 ± 0.3	6.3 ± 0.4	4.6 ± 0.7	7.3 ± 0.2	5.04 ± 0.6
	– 30.1[c] $p > 0.05$		– 29.4[c] $p > 0.01$		– 27.7[c] $p > 0.01$		– 31.3[c] $p > 0.01$	

[a] Mean ± SD of 5 observations expressed as mg protein per g wet tissue.
[b] Mean ± SD of 5 observations expressed as mg RNA/g wet tissue.
[c] Percentage change; sign (–) indicates a decrease in the levels of protein and RNA, over controls.