

structure throughout the estrual cycles. This is shown also by the differences in its weight (table 2) which shows a statistically larger weight in the phase of estrus than in that of diestrus.

The action of the adrenal glands in the sexual cycle of the hamster does not show any statistically significant differences from the point of view of its oxidative metabolism. It has to be pointed out that it is possible that the effects of the adrenal cortex remain hidden because of

the medula, because if we take into account the total weight of these glands (table 2) in the phase of estrus, they reach a very high value compared to that reached in the phase of diestrus, which indicates a higher activity in this gland at that moment. Finally, in table 2, the values of glucemia are shown. As can be seen, statistically significant differences do not exist, which seems to indicate that the level of glucose in the blood does not experience changes during the sexual cycle.

Rhythmicity of aminotransferase in the cockroach, *Periplaneta americana*

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Summary. Aspartate (AAT) and alanine aminotransferase (AlAT) activities in nervous system and coxal leg muscle of the cockroach showed circadian variations with maximal activity around midnight, alternating with minimal activity at 12.00 noon of the solar day. The enhanced activity levels of the enzymes observed during night dark hours may be related to higher energy requirements during increased locomotor activity of the animals.

Studies on circadian rhythmicity in enzymatic activities have been gaining momentum in the recent years²⁻⁵. Diurnal variations in aminotransferases in vertebrates have been reported⁶⁻⁸. But such reports are lacking in invertebrates. Aminotransferases play an important role in transamination of amino acids to their respective keto acids and constitute a junction between the metabolism of protein and that of carbohydrates and lipids. The present study demonstrates the existence of cyclic variations in aspartate (AAT) and alanine aminotransferase (AlAT) activities in the nervous system and coxal leg muscle of the cockroach, *Periplaneta americana*.

Methods. Adult male cockroaches collected in Tirupati were acclimatized for 1 month to the laboratory conditions ($27 \pm 3^\circ\text{C}$; $75 \pm 5\%$ RH). The animals were fed daily with bread pieces. 6-time-periods, viz., 8.00, 12.00, 16.00, 20.00, 0.00 and 4.00 h, were selected for experimentation to cover the 24 h period of the day. Nervous system, including all the ganglia and coxal leg muscle, were isolated and pooled each time from a minimum of

3 animals. The tissues were preserved in ice-cold glass tubes till experimentation. Each experiment was repeated 5 times.

AAT and AlAT activities were estimated by the method of Reitman and Frankel⁹ as given by Bergmeyer¹⁰. The incubation mixture contained 100 μmoles of phosphate buffer (pH 7.2), 2.5 μmoles of α -ketoglutarate, 50 μmoles of L-aspartic acid (AAT), 50 μmoles of DL-alanine (AlAT) and 0.2 ml of clear supernatant fraction of 1% tissue homogenates prepared in 0.25 M ice-cold sucrose solution. The contents were thoroughly mixed and incubated for 1 h for AAT and 30 min for AlAT at 37°C , as they represent initial velocities. The reaction was stopped by the addition of 1.0 ml of 2,4-dinitrophenyl hydrazine (ketone reagent) in 0.1 N HCl. 10.0 ml of 0.4 N sodium hydroxide solution were added and the colour developed was read at 546 nm in Bausch and Lomb Spectronic 20. The enzyme activity was expressed as μmoles of pyruvate formed/mg protein/h.

Results and discussion. AAT and AlAT activities in nerve and muscle tissue showed cyclic variations with maximal activity at 0.00 h for AAT and 20.00 h for AlAT, alternating with minimal activity at 12.00 noon for both the enzymes (table 1). During the 24 h period, the levels of the enzymes were higher during dark h (20.00 to 4.00 h) than during light h (8.00 to 16.00 h) (table 1). From De Ritis quotient, it is observed that the tissues were pyruvate preponderant (table 2).

Table 1. Rhythmicity in aminotransferases in *Periplaneta americana*

	Time of day in h								
	8.00	12.00	16.00	20.00	0.00	4.00	MEL	A	B
Aspartate aminotransferase									
NS	3.43	2.25	2.66	4.35	4.81	3.9	3.57	2.78	4.36
	\pm	\pm	\pm	\pm	\pm	\pm			
	0.21	0.36	0.27	0.57	0.33	0.42			
MS	4.26	2.5	3.06	5.21	5.65	4.62	4.22	3.27	5.16
	\pm	\pm	\pm	\pm	\pm	\pm			
	0.35	0.47	0.32	0.64	0.49	0.22			
Alanine aminotransferase									
NS	7.58	4.41	5.51	11.23	9.55	7.82	7.67	5.83	9.53
	\pm	\pm	\pm	\pm	\pm	\pm			
	0.29	0.66	0.44	0.37	0.5	0.6			
MS	9.55	6.27	6.94	12.22	11.19	9.97	9.35	7.58	11.12
	\pm	\pm	\pm	\pm	\pm	\pm			
	0.74	0.79	0.66	0.46	0.84	0.98			

Enzyme activity is expressed as μmoles of pyruvate formed/mg protein/h.

NS, Nervous system; MS, coxal leg muscle; \pm indicates SD; MEL, mean enzyme level of 6 periods; A, average enzyme level during 8.00–16.00 h; B, average enzyme level during 20.00–4.00 h.

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Rhythmic variations in total amino acids and AAT and AlAT activities in the slug, *Laevicaulis alte*, were correlated to the animal's locomotor activity⁵. In the present work, AlAT activity is 2fold higher than AAT in the nervous system and coxal leg muscle homogenates of the cockroach. This suggests that transamination of alanine is greater in the tissues. Besides, AAT and AlAT activities were higher in muscle than in the nervous system. Higher

levels of aminotransferases in the muscle suggest that, in association with motor activity, the tissue may show facultative energy metabolism. It is probable that in cockroaches the higher levels in AlAT activity are coupled with energy metabolism of the animal.

The feeding of amino acids into carbohydrate and lipid oxidation is mobilized by aminotransferases. Accelerated AAT and AlAT activities during night apparently reflect accelerated biological oxidations leading to energy supply. Associated with the activity phase of the animals¹¹, accelerated TCA cycle enzyme activities^{12,13} were shown for scorpions. Hence, in cockroaches the enhanced AAT and AlAT activities during night dark hours probably relate to energy supply for overt locomotor activity of the animal.

Table 2. De Ritis Quotient (AAT/AlAT)

	Time of day in h						Mean AAT/ mean AlAT
	8.00	12.00	16.00	20.00	0.00	4.00	
NS	0.39	0.51	0.48	0.35	0.51	0.499	0.46
MS	0.45	0.4	0.44	0.43	0.51	0.46	0.45

NS, Nervous system; MS, coxal leg muscle.

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Biotin as a regulator of some haematic parameters and of DNA-content of the liver of old rats

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Summary. Biotin administration to old rats (28 months) causes in the blood an increase of ATP, glucose, triglycerides, alkaline phosphatase and a decrease of cholesterol and acid phosphatase; in the liver DNA and electrostatic interactions between DNA and histones are increased. Such parameters come within the values shown by adult rats.

In the whole blood of old rats, the ATP^{1,2}-content is decreased; serum cholesterol^{2,4} and alkaline phosphatase² are respectively increased and decreased; α - and γ -globulins are increased; for albumin the same amount both in old and young rats sera is observed. The accumulation of total globulins is not the consequence of a different rate of synthesis, but of a decreased rate of degradation⁵. The increase of the amount of α - and γ -globulins has been reported also by Veibel et al.^{6,7} and Horne et al.⁸. Moreover the liver of aged rats shows a marked decrease in the ratio of arginine-rich to arginine-poor histone fractions. There is no significant age-associated change of total histone content of the liver^{9,10}. The DNA of the old rat liver is decreased².

In view of the effect of biotin on protein synthesis, lipogenesis, glucose metabolism and oxidative phosphorylation¹¹⁻²³, we have investigated the action of biotin on some haematic parameters (ATP, glucose, protein levels, total lipids, triglycerides, cholesterol, acid and alkaline phosphatase) and on the nucleic acid content and the electrostatic interactions between histone proteins and DNA of old rat liver.

Materials and methods. Female Sprague-Dawley rats aged 10 and 28 months were used. A group of old rats was treated every second day during with an aqueous solution of biotin (200 μ g/100 g b.wt). Control groups of adult and old rats received an equal volume of saline.

24 h following the final injection and 12 h fasting, the animals were sacrificed by bleeding. The determinations of blood ATP and glucose were effected using respectively

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