

Table), which has two negative charges. Furthermore, the hydrolysis of imide linkages under mild alkaline conditions might be more selective in copolymers of aspartic acid, such as proteinoids<sup>8</sup>. Here the reactivity of the aspartoyl residues would be differentially influenced by the neighboring amino acid residue as well as by the number of free carboxylate groups. The esterase activity of a histidine-rich proteinoid has been found to be imide structure-dependent<sup>9</sup>.

Imides are also of interest in other areas. A role for an aspartoyl group in an enzyme mechanism of action that involves active site acylation of an adjacent serine residue has been proposed<sup>10,11</sup>. Aspartoyl groups can be produced under certain relatively mild conditions during peptide synthesis<sup>12,13</sup>.

Finally, the great reactivity of the phthalimide ring of phthaloyl-DL-aspartoyl-β-alanine is further evidence that the biological activity of Thalidomide resides in the acylating capability of its phthalimide ring<sup>4</sup>. Furthermore, we have been able to acylate methylamine, lysine, or glycine at pH 9.5 to 10.0 with the imide form of polyaspartic acid in water at room temperature. In these cases the amino groups compete with hydroxide ions for reaction with imide linkages. Coupling of amino compounds with the polyaspartic acid was significant, as judged by the following molar ratios calculated after exhaustive dialysis and amino acid analysis: 1. Methylamine:aspartic acid = 5:6; 2. Lysine:aspartic acid = 1:9; 3. Glycine:aspartic acid = 1:11. The reactivity of polyimides with amino compounds in water suggests that Thalidomide may interfere with embryo development through analogous acylation of amino groups of proteins, particularly histones.

**Zusammenfassung.** Nachweis, dass die alkalische Hydrolyse des Phthalimids durch eine N-Succinimidgruppe erhöht wird. Während der alkalischen Hydrolyse des Polysuccinimids fällt die Reaktionsgeschwindigkeit in folge Auftretens negativer Ladungen ab.

P. D. HOAGLAND<sup>14</sup> and S. W. FOX<sup>15</sup>

*Eastern Regional Research Laboratory,  
Agricultural Research Service, U.S. Department of  
Agriculture, Philadelphia (Pennsylvania 19118, USA),  
and Institute for Molecular and Cellular Evolution,  
521 Anastasia, Coral Gables (Florida 33134, USA),  
17 January 1973.*

<sup>8</sup> S. W. FOX and K. HARADA, J. Am. chem. Soc. 82, 3745 (1960).

<sup>9</sup> D. L. ROHLFING and S. W. FOX, Archs Biochem. Biophys. 118, 127 (1967).

<sup>10</sup> S. A. BERNHARD, A. BERGER, J. H. CARTER, E. KATCHALSKY, M. SELA and Y. SHALITIN, J. Am. chem. Soc. 84, 2421 (1962).

<sup>11</sup> Y. SHALITIN and S. A. BERNHARD, J. Am. chem. Soc. 88, 4711 (1966).

<sup>12</sup> D. F. DETAR, M. GOUGE, W. HONSBURG and U. HONSBURG, J. Am. chem. Soc. 89, 988 (1967).

<sup>13</sup> D. F. DETAR and T. VAJDA, J. Am. chem. Soc. 89, 998 (1967).

<sup>14</sup> Eastern Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Philadelphia (Pennsylvania 19118, USA).

<sup>15</sup> Institute for Molecular and Cellular Evolution, University of Miami, Coral Gables (Florida 33134, USA).

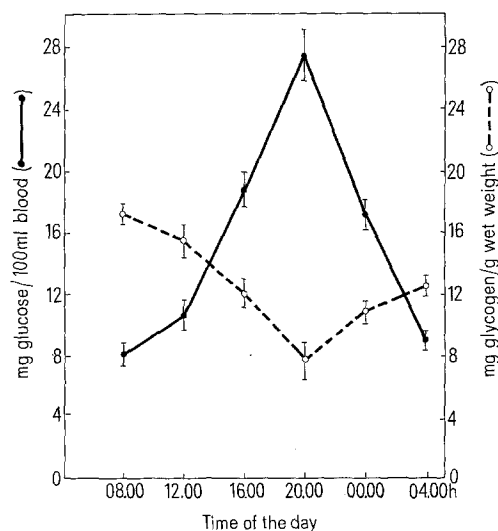
## Circadian Rhythm in Blood Glucose and Liver Glycogen Levels of Scorpion, *Heterometrus fulvipes*

DEVARAJULU NAIDU<sup>1</sup> reported that the heart rate of scorpion, *Heterometrus fulvipes*, was shown to follow a regular circadian rhythm with the maximum rate at 20.00 h and the minimum at 08.00 h. The studies of VENKATACHARI and DEVARAJULU NAIDU<sup>2</sup> on choline esterase activity of the heart muscle of the scorpion,

*Heterometrus fulvipes*, revealed that the maximum enzyme activity was with the maximum rate of heart beat and vice versa. Similar findings were also made on the rhythmic change of choline esterase activity in the ventral nerve cord of scorpion<sup>3</sup>.

The above investigation suggests that the biological constituents (metabolites and enzymes) vary in a rhythmic manner during 24 h time. Among the metabolites that vary rhythmically are liver glycogen, blood glucose<sup>4</sup> and plasma free fatty acids<sup>5</sup>. But in the previous studies no attempt was made to analyse the rhythmicity of the metabolites like glycogen and glucose. This prompted us to study these constituents in scorpion blood and hepatopancreas (liver in chordates) at different intervals during 24 h day.

**Material and methods.** The commonly available South Indian Scorpion, *Heterometrus fulvipes*, was used during the present study. The animals were starved for 24 h preceding the estimations. The hepatopancreas (liver in chordates) were isolated at different times of the day in cold scorpion Ringer<sup>6</sup> and kept for 5 min for recovery. The blood from individual specimens was drawn with a



Circadian rhythm in relation to the levels of blood glucose and liver glycogen of Scorpion, *Heterometrus fulvipes*.

<sup>1</sup> V. DEVARAJULU NAIDU, Experientia 25, 1274 (1969).

<sup>2</sup> S. A. T. VENKATACHARI and V. DEVARAJULU NAIDU, Experientia 25, 821 (1969).

<sup>3</sup> S. A. T. VENKATACHARI and M. KRISHNA DASS, Life Sci. 7, 617 (1968).

<sup>4</sup> A. SOLIBERGER, Ann. N.Y. Acad. Sci. 117, 519 (1964).

<sup>5</sup> A. M. BARRETT, Br. J. Pharmac. 22, 577 (1964).

<sup>6</sup> B. PADMANABHA NAIDU, Nature, Lond. 213, 410 (1967).

hypodermic syringe through the arthrodial membrane of the chelate leg. Six different timings were chosen to cover the 24 h period of the day choosing 3 specimens for each time. The experiment was repeated for 3 consecutive days to see whether the pattern of activity levels remained the same in all 3 days. The levels of blood glucose and hepatopancreatic glycogen was assayed using the methods of MENDEL, KEMP and MYERS<sup>7</sup> and KEMP and HEIJNINGER<sup>8</sup> respectively.

**Results and discussion.** The mean  $\pm$  standard deviation values of the blood glucose and hepatopancreatic glycogen levels at different times of the day are shown in the figure (Figure). The amount of the blood glucose ranges from  $7.99 \pm 0.48$  (08.00 h) to  $27.32 \pm 0.63$  mg. (20.00 h) of glucose/100 ml of blood and hepatic glycogen from  $7.84 \pm 0.56$  (20.00 h) to  $17.21 \pm 0.37$  mg (08.00 h). From the Figure it is evident that through out the light period and into the early part of the dark period, glycogen stores decline to a minimum at 20.00 h. The blood glucose levels rose steadily during that time to reach a maximum at 20.00 h. As glycogen stores then increased (20.00 h to 04.00 h), blood glucose levels fell. This pattern of activity was seen on all the 3 days proving clearly the existence of diurnal rhythm in the levels of metabolites like glucose and glycogen.

A rhythmic pattern has been shown to occur in various activities like locomotion, poison secretion<sup>9</sup> and neuro-secretion<sup>10</sup> besides the rate of heart beat<sup>1</sup> and choline esterase activity on the heart muscle<sup>2</sup> in the scorpion. In the present study, as evident from the results, the variations in the hepatic glycogen bear an inverse relation to the corresponding variations in the blood glucose level, while glycogen declines to a minimum (20.00 h) the blood glucose level shoots up to a maximum (20.00 h) and vice-versa. A similar trend was also found in the rate of heart beat<sup>1</sup> and acetylcholine activity<sup>2</sup> in the heart muscle.

The variations noticed in the blood glucose of *Heterometrus fulvipes* at different intervals of the day indicate variation in its glucose utilisation rate and hence in its metabolic rate during those periods. These variations in its metabolic rate is directly proportional to the variations in the internal physiological activities like synthesis of

enzymes and regulatory mechanism of different organs in the body. The high amount of choline esterase activity in the heart muscle<sup>2</sup> of the scorpion at 20.00 h and the maximum rate of heart beat<sup>1</sup> of the scorpion at the same time, from the earlier findings suggested the need for high amount of energy during those hours of the day. The necessary energy is perhaps made available through increased metabolic degradation of blood glucose.

That hepatic glycogenolysis is the predominant source of blood glucose is evidenced by the trend of variation of hepatopancreatic glycogen relating to blood glucose (Figure). The following observations provide further evidence for this. As the scorpions were starved prior to these estimations, dietary carbohydrates could not have been the source of blood glucose.

From these findings it is tempting to suggest the difference in the levels of blood glucose in our findings reflect its varying levels of utilization to meet the energetic and synthetic demands, such as synthesis of acetylcholine, in accordance with their pattern of activity, thus showing a regular circadian rhythm like that of heart beat<sup>1</sup> and choline esterase activity<sup>2</sup>.

**Zusammenfassung.** Experimenteller Nachweis einer reziproken Tagesrhythmik von Blutzucker und Leberglykogen beim Skorpion, *Heterometrus fulvipes*.

D. CHENGAL RAJU, M. D. BASHAMOHIDEEN and C. NARASIMHAM

Department of Zoology, S. V. Arts College, Tirupati (A. P. India); and Department of Zoology, S. V. University, Tirupati (A. P. India), 1 September 1972.

<sup>7</sup> B. MENDEL, A. KEMP and D. K. MYERS, Biochem. J. 56, 639 (1954).

<sup>8</sup> A. KEMP and HEIJNINGEN A. J. M. KITSVAN, Biochem. J. 56, 646 (1954).

<sup>9</sup> T. GOPALAKIRHNSNA REDDY, Dissertation submitted to Sri Venkateswara University, Tirupati (1966).

<sup>10</sup> Md. HABIBULLAH, Dissertation submitted to Sri Venkateswara University, Tirupati (1962).

## Evidence for Actinomycin D Inhibition of Transcription of Carotenoid Loci in *Neurospora*

Most micro-organisms that produce carotenoids do so in response to light induction<sup>1-3</sup>. Compounds such as  $\beta$ -ionone (4-(2,6,6-trimethyl-2-cylo, hexen-1-yl) 3 buten-2-one)<sup>4</sup> or diphenylamine<sup>5</sup> inhibit carotenogenesis at the protein level while cycloheximide<sup>6</sup> and chloramphenicol<sup>7</sup> have been found to inhibit at the level of translation in eukaryotes and prokaryotes respectively. To date no evidence has been produced to demonstrate that induction proceeds by some photo-induced mechanism resulting in the specific transcription of hitherto repressed or low level constitutive genes that are responsible for the production of the carotenogenic proteins. This paper presents evidence for the existence of a photosensitive control molecule (either a repressor or inducer) which mediates the specific transcription of carotenogenic cistrons.

The action spectrum for carotenoid induction in *Neurospora*, plateaus at 450-480 nm<sup>8</sup> and tails off towards both ends of the visible spectrum although one recent report has shown some induction at 254 nm<sup>9</sup>. Using a *Neurospora* wild type strain<sup>10</sup> we observed that the carotenoid induction potential was greatest in the late log or

stationary phase of growth and that induction is stoichiometrically dose dependent i.e. dark conditions subsequent to light induction do not repress carotenogenesis and the amount of carotenoid formed is proportional to the amount of incident light. The constitutively synthesized dark phase carotenoids were kept at a constant low level by the addition of Tween 80 (15-20 ppm) or some other wetting agent.

<sup>1</sup> H. C. RILLING, Biochem. biophys. Acta 60, 548 (1962).

<sup>2</sup> W. RAU and C. ZEHENDER, Arch. Mikrobiol. 32, 423 (1959).

<sup>3</sup> C. D. CHICHESTER, P. S. WONG and G. MACKINNEY, Pl. Physiol. 29, 238 (1954).

<sup>4</sup> G. MACKINNEY, T. NAKAYAMA, C. BUS and C. CHICHESTER, J. Am. Chem. Soc. 74, 3456 (1952).

<sup>5</sup> G. TURIAN, Physiologia Pl. 10, 667 (1957).

<sup>6</sup> R. W. HARDING, Archs Biochem. Biophys. 128, 814 (1968).

<sup>7</sup> H. HOWES and P. BATRA, Archs Biochem. Biophys. 137, 175 (1970).

<sup>8</sup> M. ZALOKAR, Archs Biochem. Biophys. 56, 318 (1955).

<sup>9</sup> E. DE FABO, personal communication.

<sup>10</sup> Isolate No. 74A-OR23-1A gratefully obtained from Fungal Genetics Stock Centre Humboldt College, California, USA.