tillering and elongation of shoots (4–5 cm long) (figure 1c).

The callus containing embryoids was transferred to a medium with K (3 mg/l) + IAA (0.5 mg/l), where it formed plantlets in 5 weeks.

The initial callus formed on media with both auxin and cytokinin showed higher frequency of regeneration compared to the callus formed on a medium containing auxin alone. Regeneration was also obtained on other media containing 2,4-D (1 mg/l) + K (0.2 mg/l) or BAP (0.2 mg/l) + NAA (1 mg/l) or NAA (5 mg/l). But in all these media the number of regenerated shoots was low.

For rooting, the *in vitro* shoots were isolated and transferred to filter paper bridges in test tubes with liquid MS medium or to agar media containing IAA (5 mg/l), IBA (3 mg/l), NAA (5 mg/l), or MS medium without growth regulators. The percentage of shoots forming roots on the above media was 71, 77, 84 and 25 respectively. Although roots were formed on all these media, in terms of frequency, number and type of roots formed, the best media were those containing NAA (5 mg/l) or IBA (3 mg/l). Roots emerged directly from the base of shoots 2 weeks after transfer, resulting in the establishment of complete plantlets (figure 2a). It was observed that for best rooting the length of shoots should be about 4–5 cm. Plantlets thus formed were transferred to pots containing a 3:1 mixture of sterilized garden soil and vermiculite. Pots were kept covered with a glass jar in the culture chamber. After 12–15 days of acclimatization in the growth room at 26 ± 2°C, the plants were transferred to soil and maintained to maturity. Plants flowered and set seeds normally (figure 2b). Seeds were slightly shrunken but viable. Immature embryos from the regenerated plants were also cultured and could be regenerated.

In the present report plant regeneration was observed through embryogenesis as well as through shoot-bud formation, in contrast to a previous report which describes only embryogenesis[3]. Another important observation is that callus formed from immature embryos on medium containing auxin in combination with cytokinin was compact, nodulated, green and highly regenerating, whereas callus initiated on medium containing only auxin was yellow, hard and less regenerative. This has also been reported for callus formed from immature and mature embryos[3] and leaves[6] of grain *Sorghum*. A second subculture was necessary for good tillering and elongation of shoots (4–5 cm long).

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1. Rangan, T. S. and Vasil, I. K., In: *Handbook of plant cell culture*, (eds) P. V. Ammirato, D. A. Evans, W. R. Sharp and Y. Yamada, Collier Macmillan Publishers, London, 1984, Vol. 3, p. 126.
2. Strongnov, B. P., Komizerko, E. I. and Butenko, R. G., *Sov. Plant Physiol.*, 1968, **15**, 173.
3. Mackinnon, C., Glenys, G. and Nabors, M. W., *Plant Cell Rep.*, 1986, **5**, 349.
4. Thomas, E., King, P. J. and Potrykus, I., *Naturwissenschaften*, 1977, **64**, 587.
5. Gamborg, O. L. Shyluk, J. P., Brar, D. S. and Constabel, F., *Plant Sci.*, 1977, **10**, 67.
6. Wernicke, W. and Brettel, R., *Nature (London)*, 1980, **287**, 138.
7. Brettel, R. I. S., Wernicke, W. and Thomas, E., *Protoplasma*, 1980, **104**, 141.
8. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 473.

EFFECT OF AESTIVATION ON FOOD UTILIZATION IN THE FRESHWATER SNAIL *PILA GLOBOSA* (SWAINSON)

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OUR previous publications concerned with the effects of food quantity[1–3], quality[4,5], crowding[6], water level[6] and starvation[7,8] on food utilization in the freshwater snails. The present paper reports the effect of aestivation on energy budget in the freshwater snail *Pila globosa*.

Snails of *P. globosa* were collected from pond Idumban (Palni, Tamil Nadu) and acclimated to laboratory conditions. Snails of 12 ± 2 g live weight were recruited from the stock and buried in mud troughs at 7.5 cm depth (temperature: 32°C; moisture: 8%) and forced to aestivate for 5 months. Fifty aestivated snails were selected and fed on *Chara fragilis* at chosen feeding levels (10 to 100%) for one month period. Every day a control sample of 5 g

food plant was dried at 80°C and weighed to measure the initial water content. The scheme of energy budget followed here is the slightly modified IBP formula[9] C (consumption) = P (growth) + R (metabolism) + F (faeces). Unconsumed plant material and faecal pellets were collected daily and dried at 80°C. The sacrifice method[10] was followed to estimate the initial and final dry weight (inclusive of shell) of the test snails. Following the same procedure, energy budget was estimated for normal *P. globosa* and was compared with that of the post-aestivated individuals.

Survival was 86% for one month aestivated snail and it decreased to 58% for the 5 months aestivated individual. The total mortality during the tenure increased from 14% in the 1st month to 42% in the 5th month and mean mortality calculated was 31% (table 1). When compared with mortality values of 1 or 2 months aestivated *P. globosa* (14%), those aestivated for a longer duration (3 to 5 months) showed lower values (4 to 6%). In the field *P. globosa* is capable of aestivating for more than 5 years[11]. Hence it is possible to suggest that acclimation could be the reason for the lower mortality values of the long-term aestivated snails.

Haniffa[11] suggested that high temperature, insufficient soil moisture and inadequate energy-releasing substances resulted in mortality of aestivating *P. globosa.* Weight loss (including shell) via metabolism accounted to 1.11 mg dry weight/g live snail/day for one month aestivated *P. globosa* and it decreased to 0.74 mg/g/day for those aestivated for 5 months (table 1). An average dry weight of 0.89 mg/g/day was lost on metabolism. *Acatina fulica* aestivated for one year[12] and *Trachia vittata*[13] aestivated for one month lost 60% and 25% initial weight respectively. It is a well known fact that aestivating snail minimizes metabolism[14,15] and utilizes the energy resources from the body[16].

Feeding rates of normal and post-aestivated *P. globosa* increased from 2 to 18 mg and 3 to 32 mg/g/day respectively. Aestivation caused a two-fold increase in food consumption and absorption. Arokiam[13] reported 2.5 times increase in the feeding rate of *T. vittata* as a function of one month aestivation. Maximum growth rate was 2.2 mg/g/day for the normal snail and it slightly increased to 2.8 mg/g/day for the post-aestivated ones. Similar enhancement in the conversion rate was reported for the freshwater snails *Viviparus variatus*[7] and *P. globosa*[8] as a function of starvation and in *T. vittata*[13] as a function of aestivation. Metabolic level of the post-aestivated snails was lowered to 1/18th of that of the normal snail and similar observations have also been made in *P. virens*[14] and *P. ovata*[15]. An average dry weight of 0.89 mg/g/day (including shell) was lost by aestivating snails and on the whole, the snail lost 136 mg/g live snail during the 5 months aestivation period. The recovery periods to attain the original weight (loss of body weight/growth rate) were calculated as 257, 162, 110, 58 and 48 days at 12, 14, 16, 18 and 20% respectively (table 2).

Simple regression, Student's *t* test and correlation coefficient analyses confirmed that (i) increase in the rates of feeding and conversion as a function of ration level was statistically significant ($P < 0.001$) in both normal and post-aestivated snails; (ii) enhancement in the rates of absorption, metabolism and conversion due to increased ration levels was also significant ($P < 0.001$), and (iii) decrease in absorption efficiency and increase in conversion efficiency due to increase in feeding rate were statistically significant ($P < 0.001$ and 0.05) in the normal snail but in the post-aestivated individuals, increase in conversion efficiency was statistically insignificant ($P > 0.1$).

Analysis of variance was attempted after Zar[17] and *F* values were separately derived for the effects of ration and aestivation. Except for the conversion rate, increase in the rates of feeding ($P < 0.05$), absorption and metabolism ($P < 0.05$) was statistically significant as a function of aestivation. When compared with aestivation effect, ration produced a significant enhancement only in the rates of feeding and conversion.

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Table 1 *Influence of aestivation on survival, mortality and weight loss in P. globosa*

Month	Survival (%)	Mortality per month (%)	Total mortality (%)	Weight loss (mg dry weight/g live snail/day)
1	86 ± 6	14	14	1.11 ± 0.25
2	72 ± 5	14	28	0.98 ± 0.15
3	68 ± 3	4	32	0.84 ± 0.25
4	62 ± 4	6	38	0.78 ± 0.20
5	58 ± 3	4	42	0.74 ± 0.23
Mean				0.89 ± 0.22

Each value represents the average performance of three observations; ± indicates the standard deviation.

Table 2 *Effects of ration level and aestivation on the energy budget of P. globosa*

Ration level (%)	FR	AR (mg/g/day)	CR	MR	AE (%)	CE (%)	RP (day)
Normal *P. globosa*							
10	2.35	1.96	−2.60	4.56	83.4		
20	4.41	3.64	−3.26	6.90	82.5		
30	6.78	5.71	−2.43	8.14	84.2		
40	9.99	7.98	−1.84	9.82	79.9		
50	12.93	10.19	0.72	9.47	78.8	7.07	
60	14.11	10.82	0.90	9.92	76.7	8.32	
70	14.70	11.05	1.18	9.87	75.2	10.68	
80	14.26	10.82	0.92	9.90	75.9	8.50	
90	15.87	11.81	1.53	10.00	74.4	12.96	
100	17.64	13.09	1.58	10.96	74.2	12.07	
Post-aestivated *P. globosa*							
10	4.12	3.60	−2.37	5.97	87.3		
20	6.76	5.65	−2.18	7.83	83.5		
30	12.35	10.29	−1.13	11.42	83.3		
40	11.17	9.39	−0.84	10.23	84.1		
50	13.52	11.09	−1.85	12.94	82.0		
60	14.70	11.60	0.53	11.07	78.9	4.6	257
70	15.88	13.29	0.84	12.40	83.6	6.3	162
80	19.40	15.19	1.24	13.95	78.2	8.2	110
90	25.28	18.83	2.34	16.49	74.5	12.4	58
100	32.34	22.74	2.84	19.90	70.3	12.5	48
Analysis of variance							
Ration effect	$P<0.05$	>0.05	<0.05	>0.05			
Aestivation effect	$P<0.05$	>0.05	>0.05	<0.05			

Each value represents the average performance of 3 observations; FR, Feeding rate; AR, Absorption rate; CR, Conversion rate; MR, Metabolic rate; AE, Absorption efficiency; CE, Conversion efficiency; RP, Recovery period to attain original body weight.

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1. Haniffa, M. A. and Pandian, T. J., *Veliger*, 1974, **16**, 415.
2. Vivekanandan, E., Haniffa, M. A., Pandian, T. J. and Raghuraman, R., *Freshwater Biol.*, 1974, **4**, 275.
3. Haniffa, M. A., *Hydrobiologia*, 1981, **97**, 141.
4. Haniffa, M. A., Amaladoss, A. J., Murugesan, A. G. and Isai Arasu, L., *Indian J. Exp. Biol.*, 1984, **22**, 482.
5. Haniffa, M. A. and Sethuramalingam, T. A., *Indian J. Comp. Anim. Physiol.*, 1985, **3**, 56.
6. Haniffa, M. A., *Indian J. Exp. Biol.*, 1980, **18**, 71.
7. Haniffa, M. A. and Mullainathan, P., *Int. J. Ecol. Environ. Sci.*, 1982, **8**, 187.
8. Haniffa, M. A., *Proc. Indian Acad. Sci.*, (*Anim. Sci.*), 1987, **96**, 135.
9. Petrusewicz, K. and Macfadyen, A., *I.B.P. Hand Book No. 13*, Oxford Blackwell Scientific Publications, 1970, p. 190.
10. Maynard, A. L. and Loosli, K. J., *Animal nutrition*, McGraw Hill, New York, 1962, p. 533.
11. Haniffa, M. A., *Hydrobiologia*, 1978, **61**, 169.
12. Warburg., *Proc. Malacol. Soc.*, 1965, **36**, 297.
13. Arokiam, J. P., Ph.D. thesis, Madurai Kamaraj University, Madurai, 1984, p. 93.
14. Visser, S. A., *West Afr. J. Biol. Appl. Chem.*, 1965, **8**, 41.
15. Meenakshi, V. R., *Comp. Biochem. Physiol.*, 1964, **11**, 365.

16. Nielsen, K. S., Taylor, C. R. and Shkolnik, A., *J. Exp. Biol.*, 1971, **55**, 385.
17. Zar, J. H., *Biostatistical analysis*, Prentice Hall, USA, 1974.

FLUORESCENCE MICROSCOPIC STUDIES ON RNA OF CYTOPLASMIC POLYHEDROSIS VIRUS OF TASAR SILKWORM, *ANTHERAEA MYLITTA* (DRURY)

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POLYHEDRAL inclusion bodies (PIBs) of occluded insect viruses are important for identification of the viruses and their strains[1-10]. PIBs have also been used as source for extraction of cytoplasmic polyhedrosis virus (CPV) virions and their RNA. In order to characterize the viral RNA, several methods have been used, including X-ray diffraction, melting point analysis, base composition analysis and electron microscopy[11-13]. The use of acridine orange staining to visualize RNA of *Antheraea mylitta* CPV virions occluded within polyhedra is reported here.

Samples were taken in the form of thin tissue smears from highly infected midgut epithelium of larvae that had been fed orally 120 h earlier with a lethal dose of purified polyhedra (0.02 ml of 5×10^6 polyhedra/ml). Larvae were cooled on ice before samples were taken. Slides were fan-dried, washed gently in basal salt solution, then stained with acridine orange (G) without fixing or after fixing with Carnoy's acid alcohol following the methods of Lea[14] and Primose and Dimmock[15]. The slides were examined immediately under short-wave UV light (source, Philips HOB-202 mercury lamp) in a Zeiss phase fluorescence microscope fitted with dark-field condenser.

Polyhedra in unfixed samples gave yellowish-orange fluorescence while those in fixed samples gave green fluorescence, suggesting the high molecular weight of the viral RNA[14,15]. The green fluorescence of the fixed polyhedra faded out, however, on molybdic acid treatment (figure 1a, b). This suggests double-stranded nature of the viral RNA[15]. The polyhedra in all cases did not give any fluorescence without the dark-field condenser, in transmitted or in incident light. This suggests that

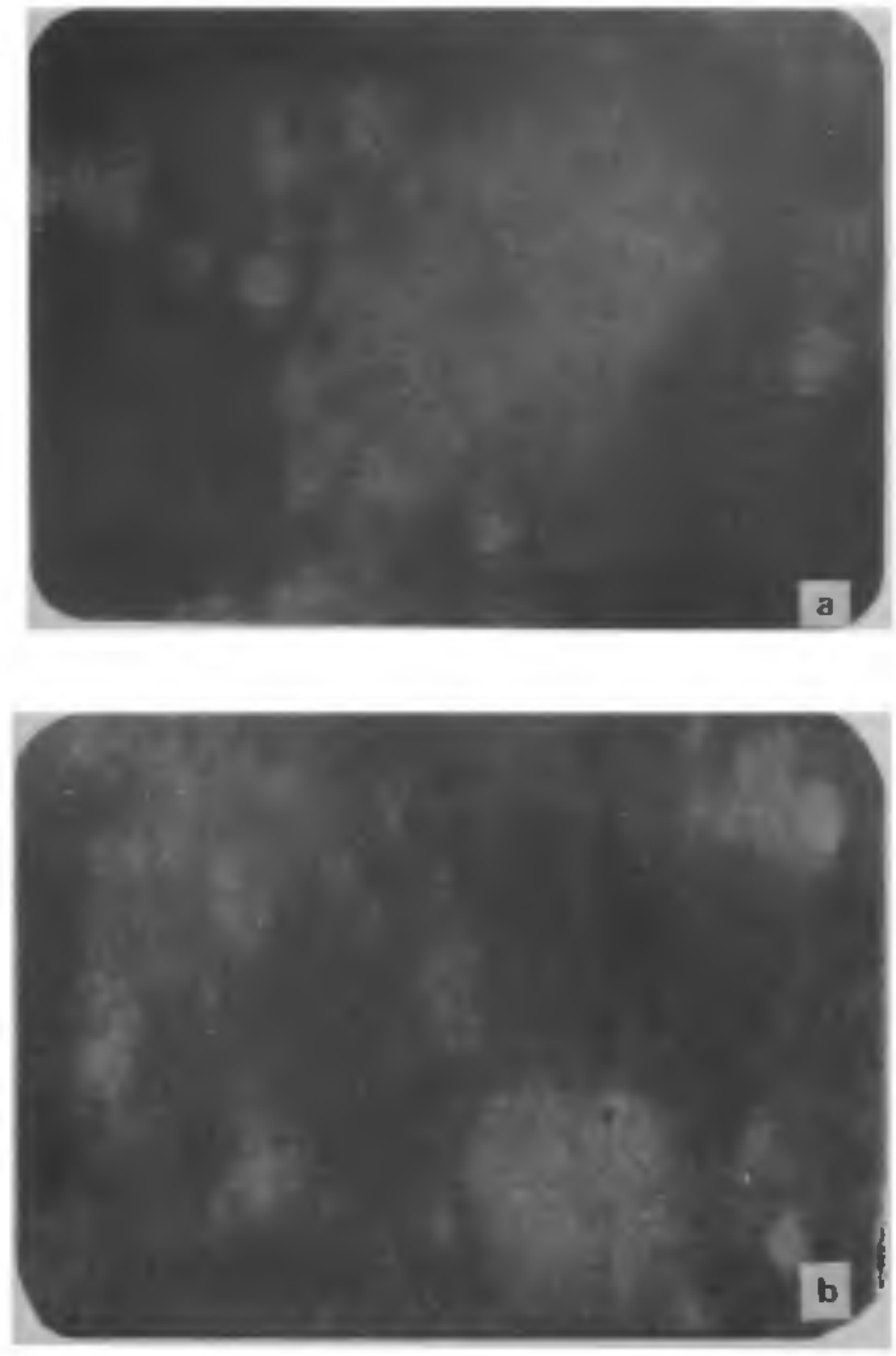

Figure 1a, b. **a.** Phase-fluorescence micrograph of polyhedra of CPV from highly infected midgut epithelium of *Antheraea mylitta*, fixed with acid alcohol and stained with acridine orange (× 800); **b.** Phase-fluorescence micrograph of polyhedra as in **a** on molybdic acid treatment, showing faded fluorescence of the polyhedra (× 800).

the polyhedra were fluorescing because of the presence of naked virions on the surface of the polyhedra.

The direct fluorescence technique has been used extensively for visualizing nucleic acids of the naked viruses[14-17]. The fluorescence of CPV polyhedra under dark-field and transmitted UV light clearly indicates that naked virions are present on the surface of polyhedra. The presence of naked virions on the surface of CPV polyhedra was also reported by other workers[18-22]. They further suggested that the virions of CPV undergo maturation (maturation of viral nucleic acid) before occlusion within PIBs.

This report of high molecular weight double-stranded RNA as the nucleic acid of the CPV of