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Biochemical changes during embryogenesis in *Atractomorpha crenulata* (Fab) (Orthoptera: Insecta)

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Abstract. Quantitative changes in the total proteins, carbohydrates and lipids during embryogenesis of *Atractomorpha crenulata* (Fab) are provided. The protein and carbohydrate contents of the egg increase after 4 days of incubation until just prior to hatching when there is a slight fall. Lipid content shows a steep increase on the 6th day of incubation. Qualitative gel electrophoretic analysis revealed as many as 14 protein fractions at various stages of embryogenesis. These observations are discussed based on densitometric scanning studies.

Keywords. *Atractomorpha crenulata*; biochemical changes; embryogenesis.

1. Introduction

Morphological changes during embryogenesis are closely associated with various aspects of the protein metabolism which involves mainly the break-down of pre-existing yolk reserves and the conversion of these into tissue- and organ-specific proteins (Chen 1966). Hill (1943) studied the carbohydrate metabolism in the isolated grasshopper embryo and reported largest variation in the extra-embryonic part of the egg. There is a steady consumption of both polysaccharides and fats during embryogenesis with the level of glucose lower and more stable than that of glycogen. Qualitative and quantitative changes in the carbohydrates of the eggs of grasshopper *Aulocara ellioti* (Thomas) at various stages of development was investigated by Quickenden (1970). Slifer (1930) observed significant change in the saturation of fatty acids during development of grasshopper eggs. The present communication attempts to study the biochemical changes with reference to the protein, carbohydrates and lipid contents during embryogenesis of *Atractomorpha crenulata* (Fabricius) where no diapause is seen during the egg stage.

2. Materials and methods

Newly moulted adults of *A. crenulata* were separated from the stock culture of acridids reared in wooden cages measuring 25 × 25 × 30 cm and fed *ad libitum* on leaves of *Ricinus communis*. Moist soil was provided in plastic containers for oviposition and observations made daily for ovipositional spots which were tagged. Eggs were collected every 48 h after oviposition until 20 days of incubation i.e. just prior to hatching and stored in deep freeze for analytical studies. Care was taken to use only the first batch of eggs laid by the newly moulted female adults for all biochemical estimations since maternal age has been reported to effect the biochemical composition of the acridid egg (Quickenden and Roemhild 1969). Quantitative biochemical estimation was carried out for the total proteins (Lowry *et al* 1951), carbohydrates (Dubois *et al* 1956) and lipids (Folch *et al* 1957). For the

qualitative profile of the proteins, polyacrylamide gel electrophoresis (PAGE) was performed according to the method of Davis (1964). 7·5% tube gels of length 11 cm were prepared and electrophoresis carried out by adjusting the current to give 2·5 to 3 mA per tube for about 2½ h until the tracer dye migrated to a distance of about 90–95 mm into the running gel. Electrophoresis was performed at 5–10°C. After electrophoresis the proteins were stained with 0·02% Coomassie brilliant blue in a mixture of methanol acetic acid and water (25:7:68). The stained gels were scanned using LKB 2202 ultrascan laser densitometer.

3. Results

Females of *A. crenulata* fed with *R. communis* lay egg pods in soil which hatch within 20 days under experimental conditions of 30°C and 75% relative humidity. Figure 1 provides the relative changes in the total protein, carbohydrate and lipid contents of the egg during embryogenesis. There is a decrease in the egg weight during the first 4 days of incubation after which there is a progressive increase in weight till day 10. From the 12th day of embryogenesis till nymphal eclosion the weight remains more or less constant. Analysis of the changes in the total protein and carbohydrates indicate a similar trend in that there is no appreciable change in their contents during the early incubation period i.e. until 4th day beyond which there is a steady increase in the total content until just prior to hatching when the protein and carbohydrate levels fall. It is interesting to observe that this increase in the protein and carbohydrate levels correspond to the period when the weight of the egg also increases. The total lipid content shows significant increase at the 6th day of incubation but declines to a low level by the 10th day. Further changes in the lipid content follow a trend similar to that shown by carbohydrates and proteins.

Qualitative analysis of the various proteins that occur during embryonic development of *A. crenulata* revealed interesting patterns of protein metabolism. As many as 14 protein bands were identified through PAGE studies using densitometric scanning techniques (figure 2). Gels were run through a distance of 9·3 cm from the origin and the electrophoretic mobility of the various proteins were measured which enabled

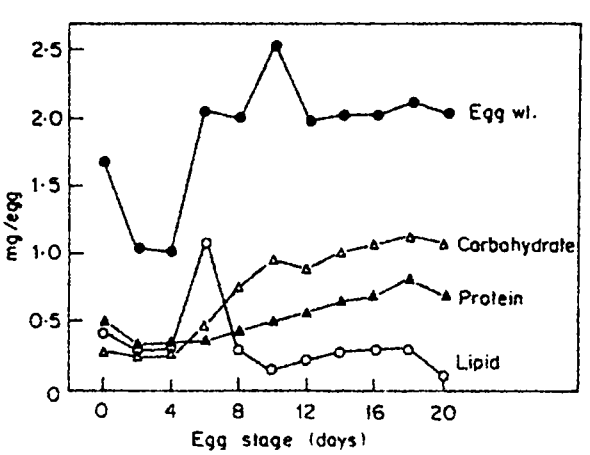

Figure 1. Relative changes in the total proteins, carbohydrates and lipids during embryogenesis.

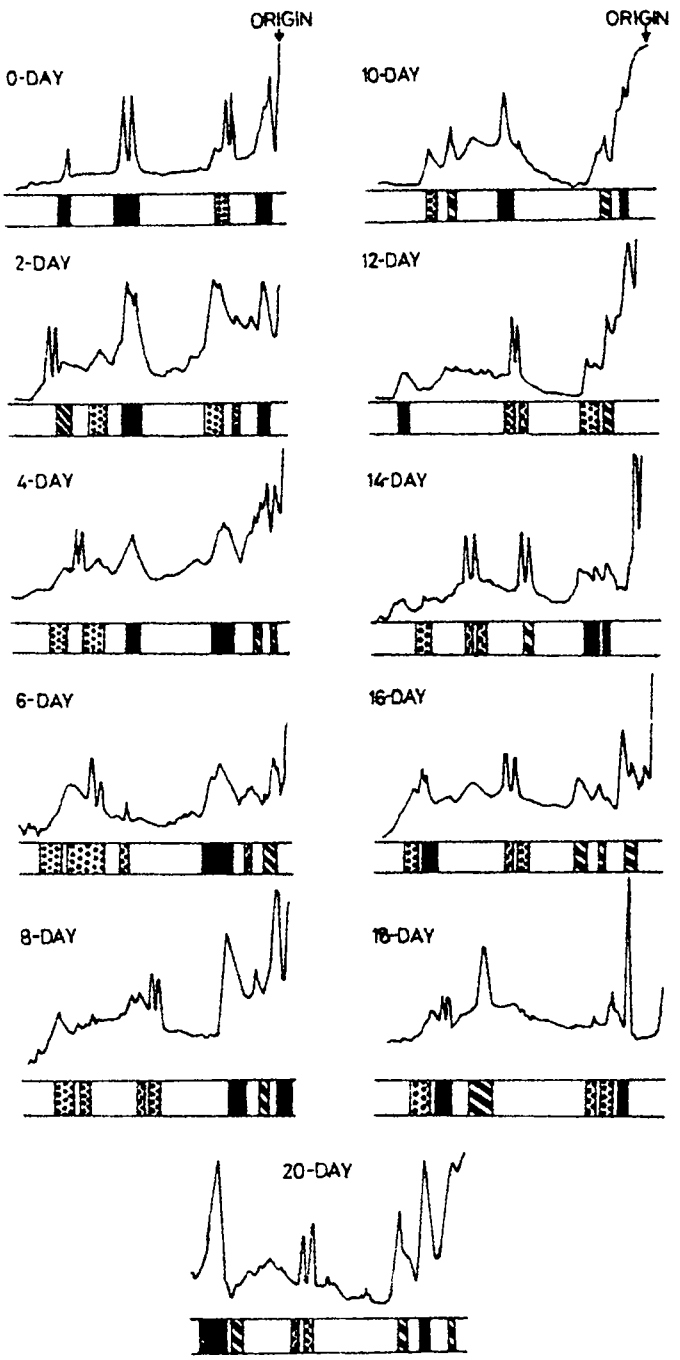

Figure 2. Densitometric scan of protein fractions at various stages of embryogenesis.

comparison of the various fractions through embryonic developmental stages (table 1). Eggs that were just oviposited (0-day eggs) showed 4 distinct bands that had

Table 1. Electrophoretic analysis of the egg proteins of *A. crenulata* during embryogenesis

Egg stage (day)	Protein fractions													
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Rm	(0·075)	(0·129)	(0·172)	(0·258)	(0·322)	(0·473)	(0·505)	(0·559)	(0·580)	(0·634)	(0·731)	(0·752)	(0·838)	(0·935)
0	44·353	—	—	2·049	—	—	—	32·724	—	—	—	—	20·874	—
	(0·326)			(0·020)				(0·292)					(0·253)	
2	28·793	—	3·550	—	5·221	—	—	45·412	—	—	3·477	—	13·545	—
	(0·258)		(0·050)		(0·250)			(0·339)			(0·080)		(0·127)	
4	16·307	13·938	—	35·708	—	—	—	26·855	—	—	3·221	—	3·968	—
	(0·202)	(0·364)		(0·703)				(0·479)			(0·065)		(0·074)	
6	12·799	—	7·736	—	55·810	—	—	9·027	—	—	7·449	—	—	7·175
	(0·202)		(0·116)		(0·706)			(0·099)			(0·095)			(0·076)
8	35·979	12·503	—	32·069	—	—	—	2·542	4·574	—	—	—	2·574	9·755
	(0·394)	(0·333)		(0·541)				(0·092)	(0·151)				(0·116)	(0·729)
10	36·372	—	19·032	—	—	—	—	30·497	—	—	13·721	—	0·375	—
	(0·192)		(0·192)					(0·168)			(0·124)		(0·004)	
12	—	—	12·387	1·259	—	4·827	8·332	—	—	—	—	—	—	73·193
			(0·145)	(0·031)		(0·042)	(0·059)							(0·725)
14	—	—	—	39·702	30·509	14·256	—	—	—	—	5·121	3·937	—	6·472
				(0·117)	(0·466)	(0·122)					(0·055)	(0·042)		(0·057)
16	—	18·187	—	18·747	19·403	—	4·566	3·635	—	—	—	—	27·276	8·191
		(0·114)		(0·254)	(0·369)		(0·064)	(0·069)					(0·566)	(0·133)
18	—	—	45·820	7·743	1·361	—	—	—	—	—	18·715	—	26·102	1·405
			(0·088)	(0·117)	(0·089)						(0·148)		(0·446)	(0·075)
20	17·208	—	22·869	20·500	—	—	—	—	1·819	1·777	—	—	11·274	24·548
	(0·188)		(0·185)	(0·438)					(0·067)	(0·082)			(0·634)	(0·782)

Values represent area percentage.
Values in parentheses represent area/height.

significantly different electrophoretic mobility namely A-band (0·075 Rm), D-band (0·258 Rm), H-band (0·559 Rm) and M-band (0·838 Rm). Newer protein bands were evident as development of the embryo proceeds. The 2-day old egg showed 6 protein bands of which 3 bands (C, E and K) appear to be formed new. The A-protein band was observed through successive stages of egg development until the 10th day. A critical analysis of the protein bands during the first 8 days of egg development reveal a synchronized occurrence of the D as well as C and E protein bands in every two day succession. It is interesting to note the occurrence of D-protein band consistently from the 12 days egg stage to nymphal eclosion. Of equal significance is the observation of the K-protein band which was evident until the 6th day old stage after which they appear only in the 10th, 14th and 18th day egg stages. It could be generalised that the H and M protein fractions which were originally evident in the 0-day egg do not undergo drastic modification and they appear in almost all the egg stages. However, the occurrence of the N-band during the later stages of embryogenesis is a feature of probable significance in egg development. Proteins corresponding to F, G, I, J and K-bands were only observed as light bands in the gel.

4. Discussion

Morphogenesis and metamorphosis in insects are processes that involve cellular differentiation as well as the assembly of cells into organs, both being influenced by insect hormones. However, cellular differentiation is characterized by the appearance of an end product which is a structural protein (Ilan and Ilan 1973). Therefore fluctuations in the protein profile during embryogenesis is an anticipated fact. The present study on the total proteins, during embryogenesis of *A. crenulata* reveals a tendency to show an increasing trend of variation from the 4th day of incubation which corresponds to a period of 25% incubation. Stay and Coop (1973) observed a similar trend in their study where the proteins were recorded to show a linear increase in developing oocytes upto oviposition. After oviposition, there is no increase in protein until 25% incubation time which is coincident with the onset of increase in dry weight. Protein then continues to rise throughout incubation until just prior to hatching when there is a slight decrease in the rate. A similar trend was also observed in the eggs of *A. crenulata* where the increase in protein content coincided with the stage when the weight of the egg also increased.

Qualitative electrophoretic analysis revealed the presence of 4 protein fractions in *A. crenulata* eggs that were just oviposited. With progressive development of the embryo, newer protein fractions were evident. Since the insect egg is a closed system (Agrell 1964), the newer protein fractions should have arisen through catabolism or through synthesis from already existing free amino acids in the egg. During embryogenesis the concentration of free amino acids at first increase due to rapid breakdown of yolk reserves probably by cethepsin-type enzymes (Kuk-Meiri *et al* 1966). These amino acids are used in the synthesis of proteins in the embryo and the concentration falls as the rate of protein synthesis increases (Chen 1966). Roberts and Smith (1971) observed 26 amino acids in the egg yolk of *Melanoplus sanguinipes*, many of which appeared at different periods during embryonic development. Elliott and Gillott (1979) in their study on *M. sanguinipes* traced the accumulation of proteins from the fat body, where most of yolk proteins are synthesised, via the haemolymph to the oocytes in the ovary. In any case, the developing egg has all the

raw materials required for the synthesis of newer proteins. Certain protein fractions such as the A-band protein, was present only during the early embryonic stages whereas protein fractions D and N were evident during the later embryonic stages Proteins corresponding to the M-band were present almost throughout embryogenesis Further studies on the isolation and identification of the protein fractions would throw more light in enhancing our present knowledge.

A decrease in the weight of the egg during the first 4 days of incubation was observed after which there is a drastic increase in weight until the 10th day on incubation. The weight of the egg stabilised to a constant level from the 12th day on incubation till hatching. This fluctuation in the weight of the egg may be due to the uptake of water. Rothstein (1952) observed the water content to increase enormously in the egg during embryogenesis, the uptake of water being mainly from the environment as the metabolically formed water amounts to only a smaller fractions. Eggs o *A. crenulata* being laid in moist soil has ample scope for absorbing the requisite water from the surrounding environment.

Although embryo lipid increased during development, lipid as a proportion o embryo weight decreases rapidly from oviposition to about 26% of incubation time and decreases less rapidly thereafter. This change in rate occurs when dry weight protein and carbohydrate begin to increase (Stay and Coop 1973), a feature also observed in the present study. Allais *et al* (1964) observed the decrease in lipid content during embryonic development to be due to the catabolism of glycerides and they also demonstrated lipids to be the major energy source for embryogenesis in *Locusta migratoria*. In view of the fact that there has been a comparative reduction in the total lipid content of *A. crenulata* egg, it could be possible that lipids are the source of energy for the developing embryo from the 8th day of incubation. Also some of the lipids may be broken down for synthesis. However, Bhatt and Krishna (1982) observed an increase in lipid content during egg development of the rice moth *Corcyra cephalonica* which they attributed to be due to phospholipid synthesis during embryo growth.

Changes in the carbohydrate content during embryogenesis of *A. crenulata* is a feature also observed in several grasshopper species (Randall and Derr 1965 Quickenden 1970). Quickenden (1970) opined universal occurrence of trehalose in insect eggs and Bhatt and Krishna (1982) recorded the concentrations of glycoger and trehalose to increase during embryonic development upto 48 h beyond which the level of glycogen alone showed a slight fall. They presumed the initial rise in the proportion of both the saccharides to be a sequel to the utilization of alternate sources of energy during embryogenesis while the reduction in amount of glycoger during the advanced egg stage to be due to its mobilisation in the metabolic cycle for energy supply to the growing embryo. In the present study the decrease in carbo hydrate content was observed only in the later stage i.e. just prior to hatching indicating that these also may be utilized as a probable energy source.

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