Department of Chemistry,
Saurashtra University,
Bhavanagar-2,
May 17th, 1979.

G. C. KAMDAR.
H. H. PATEL.
A. R. PARIKH.

1. Dorah, J. and Shonle, H. A., *J. Org. Chem.*, 1938, 3, 193.
2. Surrey, A. R., *J. Am. Chem. Soc.*, 1949, **71**, 3354.
3. Kinagawa, J. and Nagase, K., *Japanese Patent*, 8542; *Chem. Abst.*, 1965, **63**, 5653h.
4. Reddelien, G. *Ber.*, 1920, 1913, 2712–7, 2718–23, 345–54.
5. Hantzsch, A., *Ber.*, 1901, **34**, 822.
6. Brown, F. C., *Chem. Rev.*, 1961, **61**, 463.
7. Hiremuth, S. P., Purohit, M. G., and Sirsi, M., *Karnatak Uni. J. of Sci.*, 1974, 19, 208.

NUCLEIC ACIDS IN THE DEVELOPING EMBRYO OF *PHILOSAMIA RICINI* (LEPIDOPTERA)

Introduction

CONSIDERABLE attention has been given to nucleic acid metabolism in relation to hormone action[1] and to quantitative changes occurring during metamorphosis of insects[2–4]. Surprisingly, however, except for a few studies[5,6] not much attention seems to have been paid to nucleic acid changes occurring during insect embryogenesis.

With a view to gaining some additional information regarding histogenesis and differentiation of larval tissues during embryonic developpment of *P. ricini*, the present study was undertaken.

Materials and Methods

Philosamia ricini eggs, procured from the Silk Rearing Institute, Eri Seed Supply and Research Station, Ranchi (Bihar), were reared in the laboratory as described earlier[7]. Each moth laid about 100–150 eggs, each about 1·0–1·5 mm wide and weighing about 1·7 mg. Three lots of 2,000 eggs were picked at random for nucleic acid and protein estimations.

Eggs, cleaned with water, ethanol, air-dried and chilled were homogenised to 10% (w/v) tissues concentration with ice-cold distilled water (25 ml) and strained through cheese cloth to remove the debris. To 24 ml of the homogenate 8 ml trichloroacetic acid (30% w/v) were added, refrigerated for 30 min and centrifuged. Residue contained the nucleic acids which were fractionated according to Schmidt and Thannhauser[8].

RNA and DNA were assayed colorimetrically by employing orcinol and diphenylamine reagents according to Mejbaum[9] and Dische[10] respectively. Reagent blanks and standards of RNA and DNA solutions were also run simultaneously. Protein was assaye by Lowry's method[11] employing bovine serum albumin as the standard protein.

Results and Discussion

P. ricini maintains more or less a constant water content (85%) all through embryogenesis and the weight loss of egg is about 20% during this period.

During embryogenesis the energy, required for development and growth of the embryo, is derived from the stored endogenous sources—glycogen of the yolk and lipids[12,13]. Thus, weight loss occurs in the egg but since the rate of utilisation of these reserves is not uniform, the weight loss also correspondingly varies. As represented in Table I, rapid loss of weight was recorded on days 2, 3 and 5 suggesting that during early stages of egg development, when blastokinesis, gastrulation and tissue differentiation occur, high demands for energy are made. Simultaneously, RNA and DNA contents get enhanced manifold, the increase in RNA content being more than DNA all along egg development (Table I). Similar results have been reported in *B. mori*[6] and *Oncopeltus fasciatus*[14] eggs. However, in *Melanoplus differentialis*[5] RNA stands much higher than DNA at the beginning of egg development while during post-diapause period the latter exceeds the former. The authors suggested that RNA perhaps supplies the materials necessary for DNA synthesis. This phenomenon, however, was not observed either in this insect or in others[6,14].

DNA synthesis is associated with cell division and during the growth of an organism, the DNA content has been considered as an estimate of cell number[15]. In the present investigation DNA/Dry weight (DW) and DNA/Protein ratics steadily increased during the initial period of egg development till day 5 with a slight decreasing trend near about hatching (Table II). This indicates that during early period of embryo development vigorous DNA synthesis occurs due to rapid cell multiplication and continuous increase in cell number.

RNA content can be considered as an index of the capacity of an organism for protein synthesis and hence the RNA/DNA ratio is an index for protein synthesis capacity per cell[15]. During embryogenesis of this insect RNA/DW and RNA/Protein ratios steadily increase till day 3 whereafter they remain more or less unaltered (Table II). However, Protein/DW after recording a significant depletion on day 1 gradually increases with intermittent fluctuations till on the eve of hatching, whereafter it significantly declines (Table II). Total free amino acids have been observed to increase gradually all through embryonic development of this insect[14].

The significant depletion of protein with no simultaneous increase in total free amino acids[14] on day 1

TABLE I

Changes in dry weight, water content, ribonucleic acid, deoxyribonucleic acid and protein content during embryonic development of P. ricini

Embryonic Development in days	Dry weight (DW) of egg (μg)	Water content (%)	DNA (μg/egg)	RNA (μg/egg)	RNA/DNA	Protein (μg/egg)
0	262±11	84±3	0·31±0·03	0·73±0·05	2·4±0·3	167± 8
1	261±13	84±4	0·31±0·03	0·68±0·04	2·2±0 2	86± 7
2	228±18	85±3	0·82±0·05	3·60±0·2	4·4±0·3	102± 5
3	216±10	86±4	1·10±0 06	5·20±0·3	4·7±0·2	120± 6
4	215± 7	86±3	1·44±0 08	5·27±0·3	3·6±0·3	119± 7
5	210±10	86±5	1·49±0·07	4·70±0·2	3·1±0·1	102± 5
6	209± 8	86±4	1·02±0·05	5·16±0·3	5·0±0·4	129±10
7	208± 9	86±3	1·29±0·06	4·41±0·3	3·4±0·2	101± 7

TABLE II

DNA/DW, DNA/Protein, RNA/DW and RNA/Protein and Protein/DW ratios during embryonic development of P. ricini

Embryonic development in days	DNA/DW (10^{-3})	DNA/Protein (10^{-3})	RNA/DW (10^{-3})	RNA/Protein (10^{-3})	Protein/DW
0	1·2±0·08	2±0·1	2·8±0·2	4±0·2	0·64±0·05
1	1·2±0·09	4±0·2	2·6±0·2	8±0·5	0·33±0·04
2	3·6±0·21	8±0·5	15·8±1·3	35±2·6	0·45±0·03
3	5·1±0·25	9±0·5	24·1±1·8	43±1·8	0·55±0·05
4	6·7±0·30	12±0·7	24·5±1·5	44±2·3	0·55±0·03
5	7·1±0·29	14±1·0	22·4±1·6	46±3·5	0·50±0·04
6	4·9±0·35	8±0·5	24·7±1·4	40±2·0	0·63±0·05
7	6·2±0·34	13±0·8	21·2±1·7	44±2·5	0·50±0·03

suggests the breakdown of pre-existing reserve yolk proteins into peptides which gradually get hydrolysed to free amino acids and are utilised for the synthesis of organ specific proteins. The concurrent gradual increase, both in the total free amino acids[16] and proteins from day 1 onwards adds weight to this assumption.

Decline in Protein/DW ratio at the tail end of embryogenesis could be attributed to the transformation of soluble proteins to the insoluble larval cuticular proteins which get differentiated at the termination of embryogenesis. Being insoluble, these are retained in the tissue debris and this could explain the observed decline of proteins. Similar reports on *B. mori* eggs lend support to the above view[17].

The initial decline of RNA/DNA could be due to the synthesis of DNA, preparative to mitosis. However, it increases on days 2 and 3 whereafter it registers the highest peak on day 6 just prior to hatching. These peaks are more or less congruent with those of proteins and possibly indicate high synthetic activity.

Interestingly, in the developing embryo of *P. ricini*, all the parameters studied (DNA/DW, DNA/Protein, RNA/DW, RNA/Protein, Protein/DW) increase all

through embryogenesis suggesting high mitotic and synthetic activity during this period.

Department of Biochemistry,
The University,
Allahabad 211 002,
June 11, 1979.

RADHA PANT.
SUMAN KUMAR.

1. Wyatt, G. R., "Insect hormones," *Biochemical Action of Hormones*, (Ed. Litwack, G.), Acad. Press, New York, 1972, **2**, 385.
2. Pant, R. and Agarwal, H. C., *Biochem. J.*, 1965, **96**, 824.
3. Chinzei, Y. and Tojo, S., *J. Insect Physiol.*, 1972, **18**, 1683.
4. Ring, R. A., *Ibid.*, 1973, **19**, 481.
5. Lu, K. H. and Bodine, J. H., *Physiol. Zool.*, 1953, **26**, 242.
6. Chino, H., *Embryol.*, 1956, **3**, 167.
7. Pant, R. and Lacy, P. S., *Indian J. Biochem.*, 1968, **5**, 13.
8. Schmidt, G. and Thannhauser, S. J., *J. Biol. Chem.*, 1945, **161**, 83.
9. Mejbaum, W., *Hoppe-seyler's Z. Physiol. Chem.*, 1939, **258**, 117.
10. Dische, Z. and Schwarz, K., *Mikrochim. Acta*, 1937, **2**, 13.
11. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
12. Pant, R. and Nautiyal, G. C., *Proc. Ind. Acad. Sci.*, 1974, **80**, 121.
13. Pant, R. and Lacy, P. S., *University of Allahabad Studies*, 1970, **2**, 223.
14. Forrest, H. S., Harris, S. E. and Morton, L. J., *J. Insect Physiol.*, 1967, **13**, 359.
15. Lang, C. A., Lau, H. Y. and Jefferson, D. J., *Biochem. J.*, 1965, **95**, 372.
16. Pant, R. and Sharma, S. C., *Indian J. Exp. Biol.*, 1968, **6**, 110.
17. Urbani, E. and Bellini, L., *Ricerca. Scient.*, 1959, **29**, 1725.

EFFECT OF SOME FUROFURANOID LIGNANS ON THREE SPECIES OF SEEDS

THE effect of various compounds of plant origin on germination and growth of seeds of cultivated plants is of considerable interest, owing to their possible role in promoting or preventing growth. Lignans were reported to have growth retarding effect. The monoepoxylignoide (MEL) as reported by Levie *et al.*[1] and acanthotoxin as reported by Ray *et al.*[2], showed growth inhibitory effect on lettuce seeds. The present study was undertaken with an aim to screen the compounds that inhibit germination in non-dormant peanut seeds. Peanut (*Arachis hypogaea*, L.) and cucumber (*Cucumis sativa*, L.) containing lipids and rice (*Oryza sativa*, L.) containing carbohydrates as major source of stored food were chosen for experimentation. The results of three furofuranoid lignans, *viz.*, sesamin (I), fargesin (II), and eudesmin (III), at 50, 100 and 200 ppm concentration, on three non-dormant cultivars of peanut (Azorozo, M.H. 2, and T.M.V. 2) and one cultivar each of cucumber (Cv. Guntur Local) and rice (Cv. TET-30010) are presented in this communication.

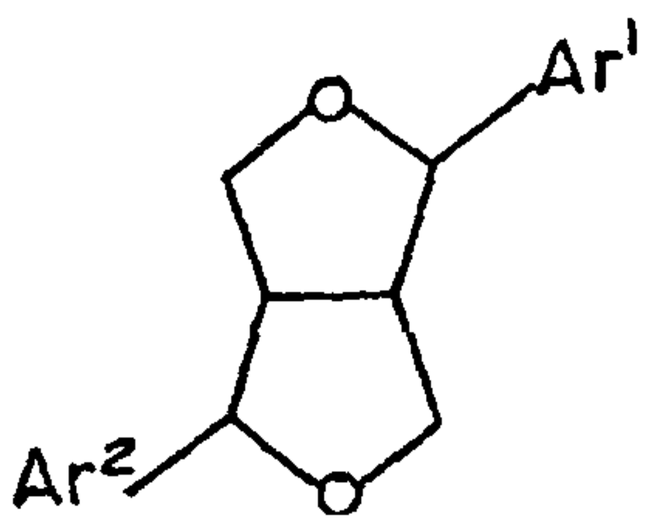

FIG. 1

I, $Ar^1 = Ar^2$ = Piperonyl
II, Ar^1 = Veratryl; Ar^2 = Piperonyl
III, $Ar^1 = Ar^2$ = Veratryl.

As the compounds are not soluble in water, each was dissolved in a minimum amount of EtOH and 1 ml of Tween-80. The volume was made up to the desired concentration with water. All the manipulations were carried under sterile conditions[3]. Seeds were prewashed with NaOCl (3%) followed by HCl (0·1 N) and water. Eight seeds of peanut or eighteen seeds of cucumber/rice were placed in each Petri plate, lined with filter paper, and 10 ml of test solution were added. Controls were maintained with equal volume of EtOH and Tween-80, without the test compound, to ensure uniformity. In addition, 1 ml of penicillin (250 units/ml)-mycostatin (100 units/ml) was pipetted out into each Petri plate to prevent microbial contamination. All samples were taken in triplicate. The Petri plates were kept in dark at 30° C ± 1° C, except during observations made in diffused light. Percentage of germination was recorded for every 24 hr.

Effect of Lignans on Germination

The effect of fargesin and sesamin in peanut and cucumber is considerably more when compared to controls in reducing the percentage of germination, whereas in rice only fargesin showed some effect (Table I). In all the three peanut cultivars tested with fargesin and sesamin, the sign of germination (emergence of radicle) was not observed until day 3. The cotyledons in peanut and cucumber were unopened even at day 8. Distortion of root-stem transition zone, absence of root hairs and root laterals were some of the morphological features observed. Sesamin treated seeds of rice, though did not show inhibition during germination, showed retarded growth of the