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1. Vinogradov, A. P., *The Elementary Chemical Composition of Marine Organisms*, Sears Foundation for Marine Research, Memoir II, New Haven : Yale University, 1953, p. 647.
2. Patel, B. *et al.*, "Uptake of radionuclides by some marine shell-fish of commercial importance," A. E.E.T./H.P./P.M.–4, 1966.
5. Doshi, G. R., *Curr. Sci.*, 1965, 34, (16), 478. *Physiology*, 1966, 13 (1, 2), 158.
4. Sreekumaran, C. *et al.*, *J. Mar. biol. Ass., India*, 1968, 10 (1), 152.
5. Doshi, G. R., *Curr. Sci.*, 1965, 34 (16), 478.
6. Viswanathan, R. *et al.*, *J. Ind. Chem. Soc.*, 1965, 42 (1), 35.
7. Lowman, F. G., *Radioecology*, Reinhold Sciences, New York, 1963, p. 145.
8. Schwarz, K. and Mertz, W., *Archs. Biochem. Biophys.*, 1957, 72, 515.
9. Underwood, E. J., *Trace Elements in Human and Animal Nutrition*, Academic Press, New York and London, 1962.
10. Adelstein, S. J. and Valee, B. L., *Mineral Metabolism*, (Comar, C. L. and Bronner, F,. eds.), Academic Press, New York and London, 1962, 2 B.
11. Bhatt, Y. M. *et al.*, *Proc. Nat. Sci. India*, 1968, 34 (6), 283.
12. Viswanathan, R. and Shah, S. M., *Indian Science Congress Session*, Chemistry Section, 1964, p. 51.
13. Patel, B. *et al.*, *IAEA/SM-158/13*, Session IV, Seattle, Washington, 1972.
14. Matkar, V. M. and Pillai, K. C., *Proc. of Symposium on Indian Ocean*, Mar. Biol. Ass. India, Cochin, 1971.
15. Goldberg, E. D., "*Minor Elements in Sea Water, Chemical Oceanography*," Academic Press, New York and London, 1965, Vol. 1.
16. Brooks, R. R. and Runsby, M. G., *Limnol. and Ocean.*, 1965, 10. 521.
17. Carlisle, D. B., *Proc. Roy. Soc.*, 1968, Sec. B.
18. Fukai, R. and Broquet, D., *Bull. Inst. Oceanogr.*, 1965, 65, 1336.
19. Goldberg, E. D., "Biogeochemistry of trace metals", In *Treatise on Marine Ecology and Palaeoecology*, Geological Society of America, Memoir 67, 1957, 1, 345.
20. Schubert, J., *Interaction of Metals with Small Molecules*, in relation to metal protein complexes, In Gurd, F.R.N. (Ed.), *Chemical Specificity in Biological Interactions*, Academic Press, New York, 1954.

STUDIES ON ACCUMULATION OF CITRATES IN INSECTS

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ABSTRACT

Citrate accumulation — termed as one of the systematic biochemical characteristics of insects — has been studied in four different insects. During metamorphosis, no significant rise or fall of citrate concentration has been noted and having once attained to the lowest level, it continues till adult emergence. During periods when the insect changes from embryo to larva, larva to pupa and from pupa to adult, marked utilization of citrate has been observed. This is assumed to occur either due to a reduced rate of citrate synthesis or due to an increased rate of utilization thereof on account of the sudden change from one phase to another.

Nevertheless, it is noteworthy that each insect hitherto investigated depicts its own pattern of citrate variation during development, although some similarities do exist in some.

INTRODUCTION

HIGH concentration of citrates in insects termed as one of their "Systematic biochemical characteristics" by Levenbook and Hollis[1] was first recorded by Tsuji[2] who estimated it to be 48·5 mmol in *Bombyx mori* blood. Since then and until recently the only citrate analysis published in insects has been that of Levenbook[3]. He examined the larval haemolymph of *Gastrophilus intestinalis* and reported the value to be 45 mg/100 ml. Patterson[4] estimated the blood and tissue homogenate citrates of *Rhodnius prolixus* and *Tenebrio molitor* to be 44 mg and 97 mg/100 ml of haemolymph respectively. Patterson's studies on citrates and on α-amino nitrogen concentration carried out on the same samples of tissue homogenates of insects revealed that the citrate concentrations were maximum at the time of lowest activity and that at a time when least oxygen is utilized the *Tenebrio molitor* pupa accumulated citrate maximally, when the overall oxidative metabolism is increased the citrate concentration falls correspondingly. Patterson[5] also noticed that the accumulation of citrate in the pupal tissue was inversely proportional to the rate of oxygen utilization by a live pupa. This induced him to consider that the reactions of the tricarboxylic acid cycle—now well established for insect tissues by Sacktor[6] and by Rees[7]—of which citrate

is an intermediate, functioned at variable degrees of efficiency during metamorphosis.

Levenbook's findings[8] in *Phormia, Prodenia* and in a number of other insect species of six orders revealed wide variations in citrate titres from 0·33 mmol for the nine-day old adult *Phormia* to 32·1 mmol in the fifth instar *Bombyx mori*. The haemolymph citrate during the larval stages were consistently higher than that of the corresponding adults. According to this author, the variation among either larval or adult stages is much less than what was observed and that even such a comparison is not quite valid since there is some indication that citrate concentration may be related to chronological age in addition to the stage of development.

In what follows, the previous studies made in this laboratory on the accumulation of citrate in the Lepidopteran insects *Ceporanerissa phryne*[9] and *Philosamia ricini*[10-11] and the Dipteran *Musca domestica*[12] have been extended to the tissues of *Musca nebulo, Sarcophaga ruficornis, Periplaneta americana* during larval-pupal development, and of *Antheraea mylitta* during embryogenesis.

Materials and Methods

Musca nebulo.—Pure strains of the Dipteran *Musca nebulo* Fabr. were obtained from the Entomology Department, Haffkine Institute, Bombay. Flies were reared in a temperature controlled room (26° C ± 4°) in wooden cages with wire-netted sides and larvae and pupae in wide-mouthed bottles of 8 g capacity. Humidity was not controlled.

Flies were fed on an aqueous slurry of Lactogen (Glaxo Labs.) mixed with sucrose in the proportion of 3 : 1. To facilitate detachment of eggs on oviposition a cotton wad soaked in the above slurry and wrapped in a piece of nylon cloth was placed in a petri dish. A piece of sponge in another petri dish soaked in sucrose solution (10%, w/v) was also placed in the cage. This provided greater accessibility to the insects and prevented them from getting drowned. Cannibalism was avoided by daily removal of carcasses of dead flies if any. Diet and sugar solution were regularly changed daily.

The oviposited nylon cloth was gently brushed in a beaker and washed thoroughly with distilled water several times and finally sterilized with sodium hypochlorite solution (0·1%, w/v) for 20 min.

About 100 eggs were transferred by means of a pipette to wide-mouthed bottles containing the sterilized synthetic diet. Bottles were plugged with cotton and incubated at 28° C ± 2° for five days. On or about the sixth day of incubation when the larvae started crawling towards the cotton plug in search of dry place to pupate, they were transferred after thoroughly washing with warm distilled water (33–37° C) to a dry glass jar containing small bits of filter-paper. The larvae generally pupated within 24 hours after transfer. The larval period consisted of six days whereas the pupal period lasted four days.

The pupae were spread on filter-papers and kept in petri dishes labelled with dates of pupation. They were then transferred to wooden cages draped in black cloth with a small opening at the top fitted with a milk bottle. The flies being positively phototropic could be easily transferred through this opening when required for experimental purposes.

Sarcophaga ruficornis is a larviparous Dipteran and is of considerable importance. It is a pest on domestic and other animals and is responsible for a high mortality rate in them. It is also a menace to the meat industry. In man it often causes myiasis of the ear, nose, eyes and intestines.

The insects employed in the investigation were from a stock reared in this laboratory for an year by the method of Orr[13]. The larvae were starved for seven hours prior to sacrifice. No assays were made on the minute zero-day larvae.

Periplaneta americana were reared at room temperature on a synthetic diet consisting of dry bread, Wesson's salts, vitamin mixture (Abdec drops), torula yeast powder and cholesterol (100 mg/100 g diet). Water was supplied in cotton plugged test-tubes mixed with milk (1 : 1, v/v).

Antheraea mylitta (Tassar silkworm) cocoons were procured from Central Tassar Silk Institute, Ranchi, Bihar. After emergence, when the female adults started laying, the fertilized eggs were collected, pooled and labelled with the date of oviposition. From this daily 300 eggs were homogenized with ice-cold trichloroacetic acid (5 ml, 5%, w/v) in a Potter-Elvehjem all-glass homogenizer. The homogenate was allowed to stand for 10 min at 0° C and then centrifuged. The supernatant gave a protein-free 5% trichloroacetic acid extract.

Citrate was estimated by the colorimetric method of Weil-Malherbe and Bone[14] using vanadate as a specific oxidizing agent.

Assays were made in duplicate on two individual samples of homogenates (10%, w/v) prepared from 100 insects each (*Musca nebulo* and *Sarcophaga ruficornis*) and more or less at the same time of the day.

In *Periplaneta americana* two lots of 10 pooled oothecae each were homogenized and assayed

whereas for nymphal and adult assays two lots of 25 insects each were employed.

Results and Discussion

Variations in citrate concentrations in *Musca nebulo* Fabr., *Periplaneta americana*, *Sarcophaga ruficornis* during larval-pupal development and in *Antherea mylitta* during embryogenesis have been represented respectively in Figs. 1, 2, 3 and 4.

In *Musca nebulo* (Fig. 1) on the first day during larval period the initial high citrate titre increased to the maximum level on day 2. After this there was a steady decrease till day 5 with a slight rise on day 6 after which on pupation once again there was an increase in the zero day pupa.

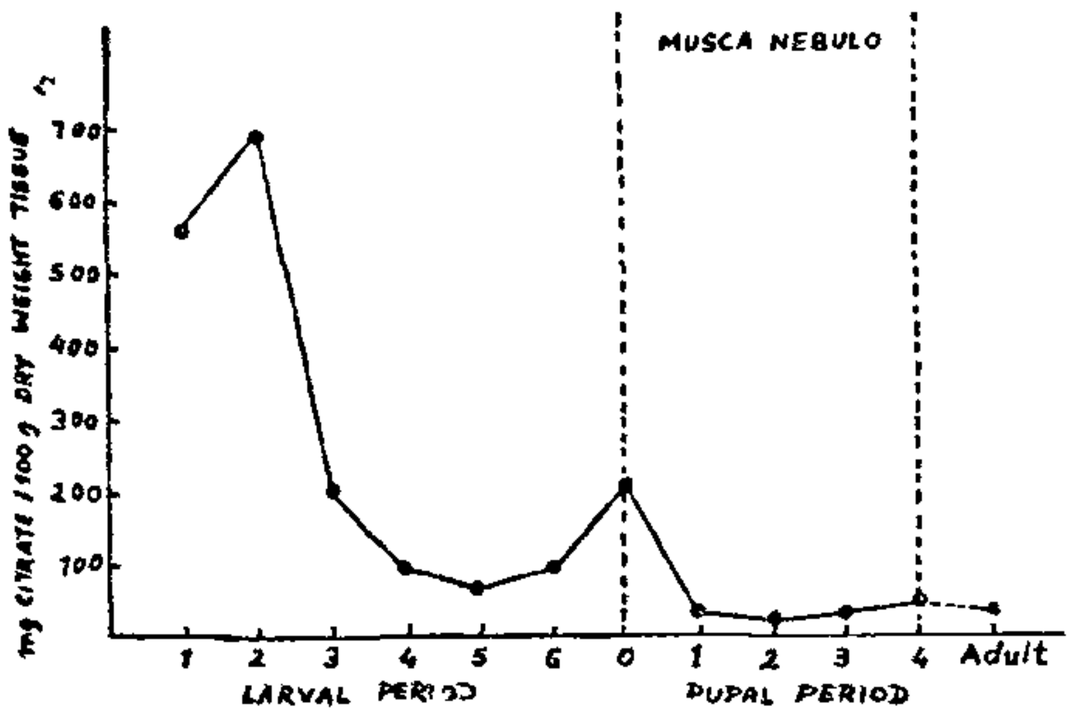

FIG. 1. Variation in citrate in *Musca nebulo* during larval-pupal development.

During pupal development, with the minimum citrate titre on day 1 (27·2 mg/100 g dry tissue) the level remains more or less constant with a slight increase on day 4 on the day of fly-emergence (45·1 mg/100 g dry wt.). It is interesting to note the U-shape pattern of variation through day 3 of larval stage to pupation.

In *Periplaneta americana* (Fig. 2) as in *Musca domestica*[12] and in *Musca nebulo* citrate, concentration manifested its highest level at oothecal stage (430·8 mg/100 g dry tissues). However, on adult emergence it fell through by about 73%. As in the case of the Dipterans no significant difference in citrate concentration was observed among the sexes.

In *Sarcophaga ruficornis* (Fig. 3) on the other hand, during larval period the citrate titre has been observed to be more than fourfold higher in comparison with the lowest pupal stage concentration on day 4. The slight fall in the four-day old larva appears to be associated with 'sleep' period before pupation when advanced histolysis also occurs. Thereafter the level remained more or less between 0·15–0·35% dry wt. tissue. From the peak citrate level in the third day larva, the overall decrease in the concentration in the four-day pupa has been noted to be 766 mg/100 g dry wt. tissue. The pattern of variation although in agreement with

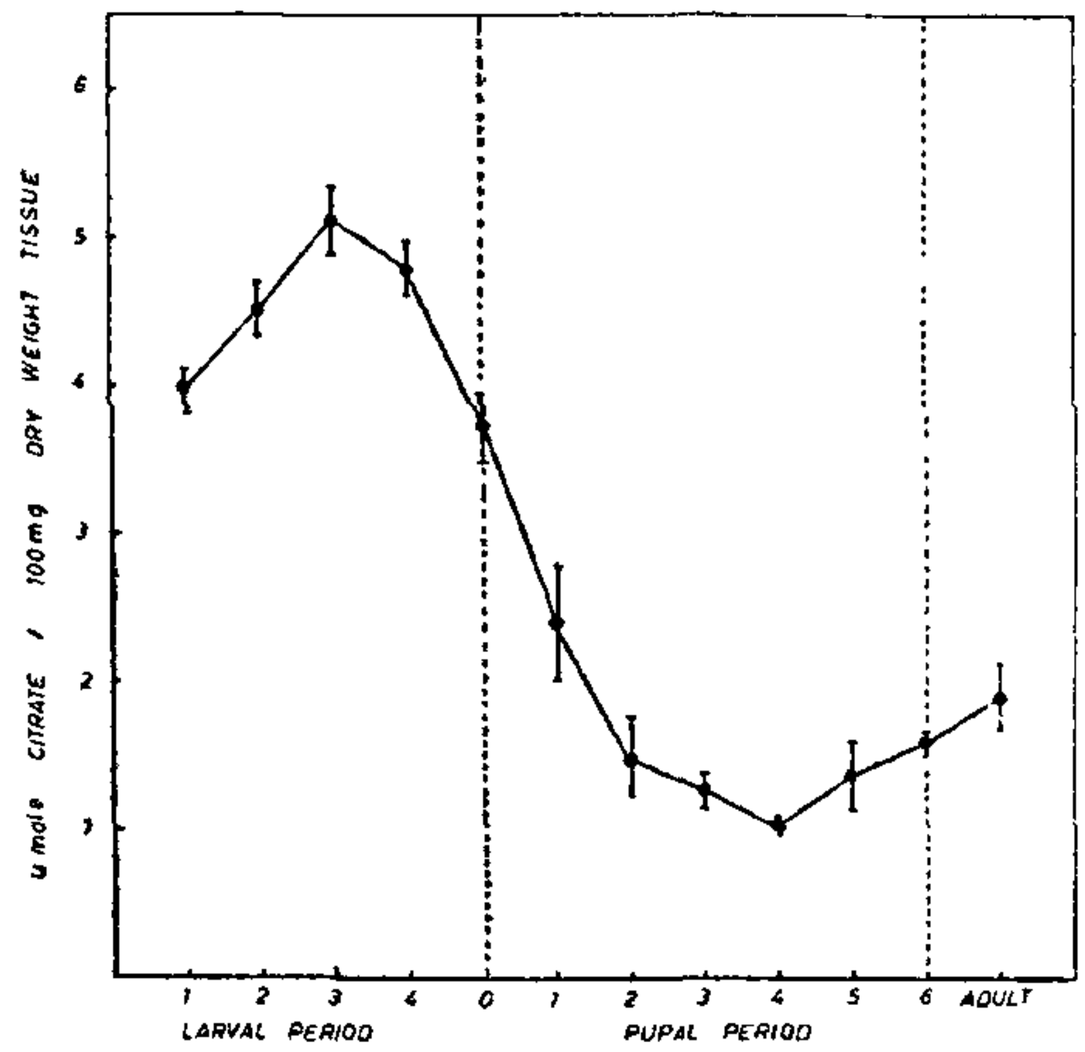

FIG. 3. Variation in citrate in *Sarcophaga ruficornis* during larval-pupal development.

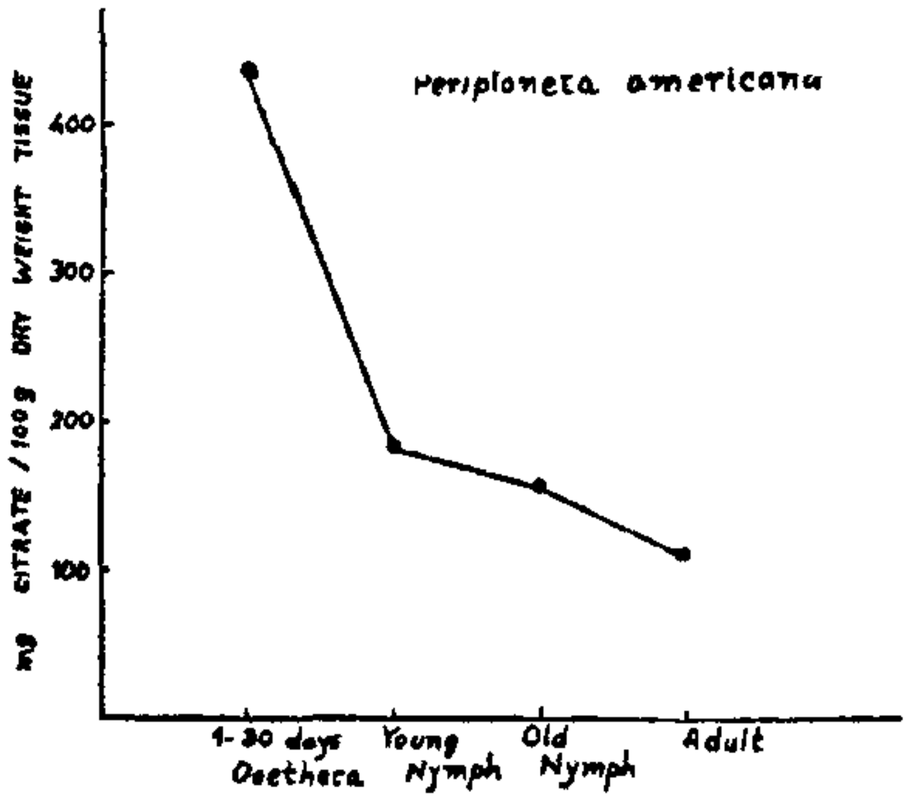

FIG. 2. Variation in citrate in *Periplaneta americana* during metamorphosis.

FIG. 4. Variation in citrate in *Antheraea mylitta* during embryogenesis.

those observed in *Musca domestica, Musca nebulo* and *Ceporanerissa phryne*[9] during early metamorphosis differs from that obtained for *Philosamia ricini*[11] where the variation follows a U-shape pattern during the early stage of metamorphosis.

Previous studies made on *Philosamia ricini* during embryogenesis[10] revealed that the citrate concentration with a very low initial level steadily increased upto the third larval instar stage apparently depicting citrate concentration to be directly proportional to the stage of development.

The present observations on the other hand, in Antheraea mylitta (Fig. 4) during embryogenesis shows that from a low initial concentration the citrate level increases through days 1 and 2 and then suddenly drops on day 3 to the initial level. Thereafter it again resumes rising steadily till hatching of the first instar larva.

During embryonic development fat is the chief reserve source which provides energy for growth and development. According to Farkas[15] fat yields about two-thirds of the total energy while glycogen and protein provide the remaining one-third. As embryonic development proceeds, increased quantities of fat and carbohydrates are utilized and possibly this results in the accumulation of citrate since citric acid is one of the main intermediate metabolic products of carbohydrate and fat metabolism.

Notwithstanding this, it is interesting to note the sudden drop in citrate concentration on day 3 of embryonic development to the initial zero-day level which is peculiar to this insect.

Visualizing the patterns of variation of tissue citrate titre during metamorphosis as well as during embryogenesis one can conclude that every insect, hitherto investigated, evinces a different pattern of variation. Nevertheless, the phenomenon of citrate utilization during early metamorphosis period seems to be common to all insects examined except for *Tenebrio molitor* investigated by Patterson[4] who observed accumulation instead. Variation studies made on *Philosamia ricini* fat body during larval-pupal development[16] also lend support to this view.

In the absence of any correlation between dietary and tissue citrate concentration, Levenbook concluded that citrate in insects is of endogenous origin. Consequently, the recurring rise and fall in its level during the various developmental stages could be considered as indications of the rate of citrate synthesis and utilization, in the face of the possibility that citrate concentration may be related to chronological age as well as to the stage of development of the insect. The marked utilization of citrate during every change of phase in the silkworm *Philosamia ricini* as observed in this laboratory is therefore very significant and intriguing. This peculiar phenomenon could either be attributed to the non-production of citrate at these stages of sudden change in the insects from one phase to another or could it be that these periods are enzymically so active that the citrate formed is metabolized at a remarkably rapid rate? Only a thorough study and understanding of the intermediary metabolism and the biological significance of organic acids in insects, coupled with a knowledge of the activity of the different tricarboxylic acid cycle enzymes at these periods, could perhaps supply satisfactory answers to such questions.

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1. Levenbook, L. and Hollis, V. W., *J. Insect. Physiol.*, 1961, 6, 52.
2. Tsuji, C., *Sanji Hokoku*, 1909, 35, 1.
3. Levenbook, L., *Biochem. J.*, 1950, 47, 336.
4. Patterson, D. S. P., *Ibid.*, 1957, 65, 729.
5. —, *Arch. int. Physiol. Biochem.*, 1956, 64, 681.
6. Sacktor, B., *J. Gen. Physiol.*, 1954, 37, 343.
7. Rees, K. R., *Biochem. J.*, 1954, 58, 196.
8. Levenbook, L., *Arch. Biochem. Biophys.*, 1961, 92, 114.
9. Pant, R. and Sharma, S. C., *Ind. J. Exptl. Biol.*, 1967, 5, 181.
10. — and —, *Ibid.*, 1965, 3, 273.
11. — and —, *Ann. Ent. Soc. Am.*, 1967, 60, 170.
12. — and Verma, R. N., *Ind. J. Biochem.*, 1968, 5, 187.
13. Orr, C. W. M., *J. Insect Physiol.*, 1964, 10, 53.
14. Weil-Malherbe, H. and Bone, A. D., *Biochem. J.*, 1949, 45, 377.
15. Farkas, K. Pflüg. *Arch. ges. Physiol.*, 1903, 98, 490.
16. Pant, R. and Morris, I. D., *J. Biochem. (Tokyo)*, 1972, 71, 1.