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Is a Urea Cycle Present in Insects?

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1. The presence of appreciable activity of the urea-cycle enzymes in the tissues of *Sarcophaga ruficornis*, a carnivorous dipteran insect, all through its life-cycle appears significant in view of their total absence barring arginase (L-arginine ureohydrolase, EC 3.5.3.1) in the phytophagous lepidopteran eri silkworm *Philosamia ricini* at any stage of development. Further, the variation of all these enzymes all through its development suggests the possibility of the operation of the Krebs–Henseleit urea cycle in this carnivorous insect. 2. The almost parallel behaviour of arginase and ornithine δ -transaminase (L-ornithine–2-oxo acid aminotransferase, EC 2.6.1.13) in both the insects suggests another important role of the former in proline biosynthesis in insects. 3. High proteolytic activity accompanied with significant protein depletion and simultaneous increase in arginine is suggestive of the degradation of proteins and peptides.

The detection of arginase practically in all insects, and of arginine, citrulline and ornithine in many, has been made at all stages of development (Garcia *et al.* 1956; Kilby & Neville, 1957; Szarkowska & Porembska, 1959; Hayashi, 1961; Porembska & Mochnacka, 1964; Reddy & Campbell, 1969*a*). However, the nutritional and enzymic studies hitherto made on them (House, 1965; Porembska & Mochnacka, 1964; Reddy & Campbell, 1967; Inokuchi *et al.*, 1969) have not given any conclusive and unequivocal evidence regarding the operation of a Krebs–Henseleit urea cycle, and it still remains a disputed topic.

Powles *et al.* (1972) studied the urea-cycle enzymes in the vegetable bug *Nezara viridula* and observed that, of the enzymes of the ornithine cycle, arginase and ornithine transcarbamoylase were active but there was no evidence for the arginine synthetase system. Further, carbamoyl phosphate synthetase was very low in activity and detectable only by the use of radioactive substrates. On the basis of these findings the authors concluded that urea in this insect was derived solely from excess dietary arginine via the action of arginase.

Since previous investigations have mostly been on phytophagous insects, it was considered pertinent to make a comparative study of the urea-cycle enzymes in a carnivorous dipteran *Sarcophaga ruficornis* and a phytophagous lepidopteran *Philosamia ricini* during their entire life cycle.

In view of the possible role of arginase in the conversion of arginine into proline by a route involving pyrrolinecarboxylate reductase, ornithine δ -transaminase activity and proline and arginine concentrations were also studied in the tissues of both the insects during development.

In addition, the rates of protein accumulation and degradation during development of the two insects were also studied.

An account of the present study was given by R. Pant at the International Biochemical Congress in Hamburg, German Federal Republic, in July 1976.

Experimental

Materials

Chemicals. All chemicals were of analytical-reagent grade. ATP, dilithium carbamoyl phosphate, *o*-aminobenzaldehyde, L-arginine hydrochloride, L-ornithine hydrochloride, sodium α -oxoglutarate, pyridoxal 5'-phosphate, barium argininosuccinate and pure arginase were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.

Insects. *S. ruficornis* and *P. ricini* were reared in the laboratory as described previously (Pant & Agrawal, 1965). Briefly, *S. ruficornis* flies were kept in wire-net cages [0.91 m $\times$ 0.91 m $\times$ 0.91 m (36 in $\times$ 36 in $\times$ 36 in)] at $28 \pm 2^\circ\text{C}$. Humidity was partially controlled by keeping damp pads of cotton in a Petri dish. Flies were fed on fresh water, sucrose and fresh goat kidney to induce egg laying. Larvae were reared on fresh goat kidney kept under hurricane-lamp chimneys covered at the top with nylon cloth. The larvae stopped feeding after 4 days and migrated to the damp sawdust that surrounded the Petri dish; they were collected the next day. This 'white pupa' has been termed the 'day-0' pupa. The period from 'white pupa' to fly emergence was 9 days.

Methods

The 6h-starved larvae and pupae and adults were randomly selected from colonies of known age, weighed, chilled and homogenized as described by Brown & Cohen (1959).

All the urea-cycle enzymes, namely ornithine transcarbamoylase (EC 2.1.3.3.), argininosuccinate synthetase (EC 6.3.4.5), argininosuccinase (EC 4.3.2.1) and arginase (EC 3.5.3.1), were assayed by Brown & Cohen's (1959) methods. Urea or citrulline formed during enzymic reactions were determined by Archibald's (1945) method and expressed in $\mu\text{mol}/\text{mg}$ of protein. Practical difficulties (non-availability of ornithine transcarbamoylase) prevented assays of carbamoyl phosphate synthetase.

Ornithine δ -transaminase (EC 2.6.1.13) was assayed by the method of Reddy & Cambell (1969b). The amount of pyrroline-5-carboxylate formed was calculated from the molar extinction coefficient given by Strecker (1965).

Proteolytic activity was assayed by the method of Matsushita & Iwami (1967). Proteins were determined by the method of Lowry *et al.* (1951).

Proline and arginine were determined by the method of Lee & Takahashi (1966) as modified by Pant & Lal (1970).

Experiments were repeated three times; we maintained as far as possible identical conditions and used 25 insects in each experiment. Although results varied quantitatively (7–15%), the variation patterns of all enzymes and metabolites were nearly the same. Mean values are presented with indicators of the range of variation.

Results and Discussion

The urea-cycle enzymes ornithine transcarbamoylase, argininosuccinate synthetase, argininosuccinase and arginase as shown in Figs. 1, 2 and 3 exhibit significant activity in the larvae and adults of *S. ruficornis*, and the quiescent pupae maintain a very low but constant activity all through metamorphosis. Especially in view of the finding that all these enzymes except arginase were immeasurably low in activity all through development in the lepidopteran *P. ricini*, we are inclined to assume the operation of the Krebs–Henseleit urea cycle at least in this dipteran carnivorous insect, although confirmation *in vivo* (e.g. with the use of [^{14}C]ornithine) is desirable.

In mammalian liver, arginase functions principally in urea biosynthesis involving cyclic interconversion of ornithine and arginine. Ornithine δ -transaminase is also present as a liver mitochondrial enzyme in mammals, participating in the conversion of ornithine into glutamate and aspartate via glutamate γ -semi-

aldehyde (Katunuma *et al.*, 1965). In mammalian liver cells that have lost the ability to synthesize arginine (Eliasson & Strecker, 1966) and in micro-organisms that can synthesize arginine but do not form urea as an end product (Laishley & Bernlohr, 1968), arginase functions mainly as a catabolic enzyme.

Arginase is widely distributed in insects, which are generally uricotelic. Despite this, the nutritional requirement of arginine by several species (House, 1965) indicates that insects have a limited capacity to synthesize arginine.

Arginase and ornithine δ -transaminase activities in both the insects run parallel during development (Figs. 3 and 5), the course of variation being somewhat similar to that of the proline and arginine concentrations (Figs. 4 and 6). This suggests that, during the development of *P. ricini*, arginase may function largely in the conversion of arginine via ornithine into proline, though, in addition, in *S. ruficornis* it appears to participate in urea biosynthesis. Reddy & Campbell (1969b) demonstrated the presence of both these

Fig. 1. Variation in ornithine transcarbamoylase activity during development of *S. ruficornis*
For details see the text.

Fig. 2. Variation in argininosuccinate synthetase (O) and argininosuccinase (●) activities during development of *S. ruficornis*
For details see the text.

Fig. 3. Variation in arginase (○) and ornithine δ -transaminase (●) activities during development of *S. ruficornis*. For details see the text.

Fig. 4. Concentrations of arginine (○) and proline (●) during development of *S. ruficornis*. For details see the text.

Fig. 5. Variation in arginase (○) and ornithine δ -transaminase (●) activities during development of *P. ricini*. For details see the text.

enzymes in the pupae of *Hyalophora gloveri* and *Antheraea polyphemus* and ascribed to them a role in the conversion of arginine into proline. A significant increase in the activity of both these enzymes with a simultaneous increase in arginine and proline during transformation of larva $\rightarrow$ pupa $\rightarrow$ adult is note-

Fig. 6. Concentrations of arginine (●) and proline (○) during development of *P. ricini*. For details see the text.

Fig. 7. Variation in proteolytic activity (●) and protein content (○) during development of *S. ruficornis*. For details see the text.

Fig. 8. Variation in proteolytic activity (●) and protein concentration (○) during development of *P. ricini*. For details see the text.

worthy (Figs. 3, 4, 5 and 6). During these periods of metamorphosis the increase in arginine concentration with concurrent high arginase activity is rather intriguing. Previously published investigations have not identified the source of the arginine. The present investigation suggests that it is the proteins and peptides that release arginine as a degradation product

during vigorous hydrolysis. This conclusion is evinced from the somewhat similar variation of proteolytic activity (Figs. 7 and 8) and arginine concentration (Figs. 4 and 6). Both concentrate maximally at pupal transformation and at emergence. The accumulation of a high concentration of protein during larval-pupal transformation despite high proteolytic activity indicates the conversion of larval cuticular and other insoluble proteins into the soluble form. This speculation is lent support by Agrell (1951).

The increase in proline may also be related to the synthesis of cuticular structures. This is evinced by the detection of a high concentration of peptide-bound proline in water and urea extracts of larval and pupal cuticle and in free form in other insect tissues (Hackman & Goldberg, 1958; Srivastava, 1971; Pant & Sharma, 1974; Price, 1961; Corrigan & Kearns, 1963). Proline has also been ascribed another function as an energy reservoir in insect flight muscle, where it is rapidly utilized during flight (Bursell, 1963; Sacktor & Childress, 1967).

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