CLXIII. TYROSINASE IN MACROLEPIDOPTERA.

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I. DISTRIBUTION OF TYROSINASE.

THE work of a number of investigators has shown the presence of tyrosinase in various species of Macrolepidoptera, but it is not clear from the literature whether the occurrence of tyrosinase is exceptional or general. A survey has therefore been made of some widely representative species, comprising four Rhopalocera (butterflies) and 25 Heterocera (moths). Since most tyrosinase is usually found in the larval form of an insect, this was the form chosen for the investigation. The tyrosinase is found in the body fluid.

Method.

The larvae were chloroformed and dried on filter-paper. The skin was then snipped with scissors and the juice squeezed out by gentle massage. The juice was clear, except for a few parasitised larvae. It was added at once to 3 ml. phosphate buffer p_H 6.5 by collection in a fine-pointed teat pipette which was washed out with the buffer solution. Of medium-sized larvae about six specimens were used; of large larvae, fewer were necessary; of small larvae, a greater number.

Of this preparation, 0.5 ml. was added to 2.5 ml. each of the following test solutions: (1) phosphate buffer p_H 6.5 for comparison; (2) saturated solution of tyrosine in the same buffer; (3) 0.2 % *p*-cresol; (4) and (5) 0.2 % guaiacol; (6) 0.2 % catechol. To each was added 0.5 ml. water, except to (5), to which 0.5 ml. 0.05 % hydrogen peroxide was added instead. Coloration of tyrosine, *p*-cresol and catechol indicates the presence of tyrosinase; of guaiacol without hydrogen peroxide indicates laccase; of guaiacol with hydrogen peroxide indicates peroxidase.

Species tested. *Pieris brassicae*, *Pieris rapae*, *Melitaea aurinia*, *Augiades sylvanus*, *Chaerocampa elperor*, *Dicranura vinula*, *Orgyia antiqua*, *Arctia caia*, *Porthesia similis*, *Stilpnotia salicis*, *Lasiocampa quercus*, *Cosmotriche potatoria*, *Saturnia pavonia*, *Hipocrita jacobaeae*, *Triphaena pronuba*, *Barathra brassicae*, *Mamestra glauca*, *Diloba caeruleocephala*, *Miselia oxyacanthae*, *Xanthia lutea*, *Cucullia verbasci*, *Cheimatobia brumata*, *Abraxas grossulariata*, *Gonodontis bidentata*, *Crocallis elinguaris*, *Hybernica marginaria*, *Hybernica defoliaria*, *Phigalia pedaria*.

Results.

There was no peroxidase in any of the juices tested and only a feeble laccase in some; but in every instance action on catechol appeared quickly, on *p*-cresol in a little longer time and on tyrosine after the lapse of either a few minutes or a few hours: tyrosinase was present in the juice of every one of the various kinds of larvae tested. (Exception: in one case the material available was insufficient to colour tyrosine itself.)

Several of the juices were placed on litmus paper and all tested were found to be alkaline. Some of the juices were noticed to darken very rapidly in air even in phosphate buffer p_H 6.5.

Conclusion.

The presence of tyrosinase is quite general in the larvae of Macrolepidoptera, usually free from admixture with other similar oxidising enzymes.

Note. In sawfly larvae (*Trichiocampus viminalis*) the amount of tyrosinase was of a much smaller order, although the dead bodies darkened rapidly. The juice of 14 of these larvae overnight coloured only catechol, and *p*-cresol slightly. When 120 were used, action on catechol, *p*-cresol and tyrosine was obtained as rapidly as is usually obtained with about a dozen similar-sized larvae of the other types tested. Similar results were obtained with specimens from Euston and from Manchester.

II. TYROSINASE IN *PIERIS* PUPAE.

Pieris brassicae (Large White) and *P. rapae* (Small White butterfly) are both double-brooded, and the black markings on the wings are much less distinct in the spring broods than in the summer broods.

The following experiments were carried out to determine whether there was any relationship between length of time in the pupal form, tyrosinase content of the pupae and markings of the emerged insect.

Method.

Pupae of both species were kept in a cool cellar for the winter. Similar pupae were incubated at 20°. From time to time tyrosinase activity was estimated as follows. The pupa was weighed and ground up in a small mortar with 1 ml. water. A further 2 ml. water were added, and the mixture was transferred to a centrifuge-tube. The mortar was washed out with a further 3 ml. water. The mixture was hand-centrifuged for one minute, the liquid was decanted and added to (1) 2 ml. phosphate buffer p_H 6.5; (2) 2 ml. 0.2 % *p*-cresol solution in the same buffer; (3) 2 ml. saturated tyrosine solution in the same buffer. The time required for coloration to appear was noted. The usual precautions were taken to ensure freedom of the reagents from extraneous iron and *o*-dihydric phenols.

Results.					
<i>P. rapae</i>			<i>P. brassicae</i>		
Weight g.	<i>p</i> -Cresol	Tyrosine	Weight g.	<i>p</i> -Cresol	Tyrosine
<i>Larvae slung up about to pupate.</i>					
0.2702	Nearly 1 min.	10 mins.	0.4392	At once	2 mins.
0.2228	1 min.	10 mins.	0.3168	At once	2 mins.
0.2118	2 mins.	18 mins.	0.2715	Almost at once	8 mins.
<i>Pupae 24-48 hours old.</i>					
0.2288	6 hrs.	Overnight	0.3336	3 mins.	25 mins.
0.2300	3 hrs.	Overnight	0.4034	20 mins.	1½ hrs.
0.2040	3 hrs.	Overnight			
<i>Pupae 5 months old.</i>					
Summer temp.	0.1036	3½ hrs.	5 hrs.	0.3332	1½ hrs.
Winter temp.	0.1935	4 hrs.	5½ hrs.	0.3474	1 hr.
					4 hrs.

Intermediate results were similar.

The tyrosinase content of individual pupae varied considerably, but it is evident that there was a very marked drop on pupation, and after that no actual disappearance of tyrosinase even after 5 months. Exposure of the pupae to chloroform vapour before extraction did not make the tyrosinase content any more regular (compare Graubard on *Drosophila* [1933]). The ratio of rate of coloration of *p*-cresol to that of tyrosine was extremely variable. On two occasions, after coloration of tyrosine had proceeded to some extent, the colour faded away, while *p*-cresol continued to colour. From this it would appear possible that the 5:6-quinone of dihydroindole-2-carboxylic acid [Raper, 1927] may be able to function as a hydrogen acceptor and carrier. There was no regular difference between the tyrosinase contents of *P. rapae* pupae of different colours—green, grey or blackish.

After 2 months *P. rapae* at 20° began to emerge (mid-December). During the next few weeks altogether eight specimens emerged. In markings they were all intermediate between typical spring and summer broods. At this point an accidental interference with the temperature of the incubator resulted in the death of most of the *P. brassicae*. The survivors were used for estimations. The pupae in the cellar were brought to the laboratory in mid-April. During the next 3 weeks eight specimens of *P. rapae* emerged with typical spring markings, and two *P. brassicae*. The remainder of the pupae died.

Conclusion.

The difference in intensity of markings of spring and summer broods of *P. rapae*, and probably *P. brassicae*, is not due to any difference in tyrosinase content, but to some other factor, depending on the temperature at which the pupae are kept. This conclusion is in agreement with the findings of Onslow [1916] that the markings are due to the action of tyrosinase on chromogen present in restricted areas of wing surface.

REFERENCES.

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