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Haemopoietic organs and effect of their ablation on total haemocyte count in lemon-butterfly, *Papilio demoleus* L.

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The haemopoietic organs in Vth instar larvae of *P.demoleus* are in the form of thin transparent cellular sheets, closely wrapped around the base of each wing-pad. Three cell types viz; prohaemocytes, plasmatocytes and oenocytoids appear to be derived from these organs and their ablation caused a reduction in cell number which, in turn, revealed that the haemocytes in general are derived both from the haemopoietic organs as well as from the circulating blood cells.

The haemopoietic organs (HPOs) are found either attached or closely associated with the imaginal wing-pads of all lepidopterous insects. While researchers generally agree that they give rise to haemocytes (as this view has been experimentally strengthened also[1-6]), opinion differ in regard to the haemocyte types that they produce[3,7,8]. The present study has, therefore, been carried out in the lemon-butterfly, *Papilio demoleus* with a view to examine the types of haemocytes produced by these organs and the effect of their ablation on total haemocyte count (THC).

For HPO- ablation, 24hr old Vth instar larvae of *P.demoleus* maintained in the laboratory culture were used[9]. They were water-narcotized for 30-45 min and placed on their side in a wax dissecting dish. The cuticle was incised over the positions of the wing-pads in the meso-and meta-thorax and the wing-pads whose bases are closely wrapped with HPOs were pulled out, one by one, by a fine pair of forceps. But in the larvae which served as sham-operated controls, only the cuticle of respective segments was incised, wing-pads were not removed. In both the cases, wounds were sprinkled with antibiotic-phenylthiourea (1:1w/w) mixture to disinfect and prevent haemolymph melanization. The operation was then repeated on other side also. The operated larvae were then transferred to a refrigerator and left for 24 hr to immobilise them and prevent excessive bleeding. The wing-pad ablated larvae and their controls were heat-fixed and the THC was determined at designated intervals after surgery. The methods of haemolymph collection, calculation of haemocyte number, preparation of blood smears and their staining were similar to those applied earlier[10-12].

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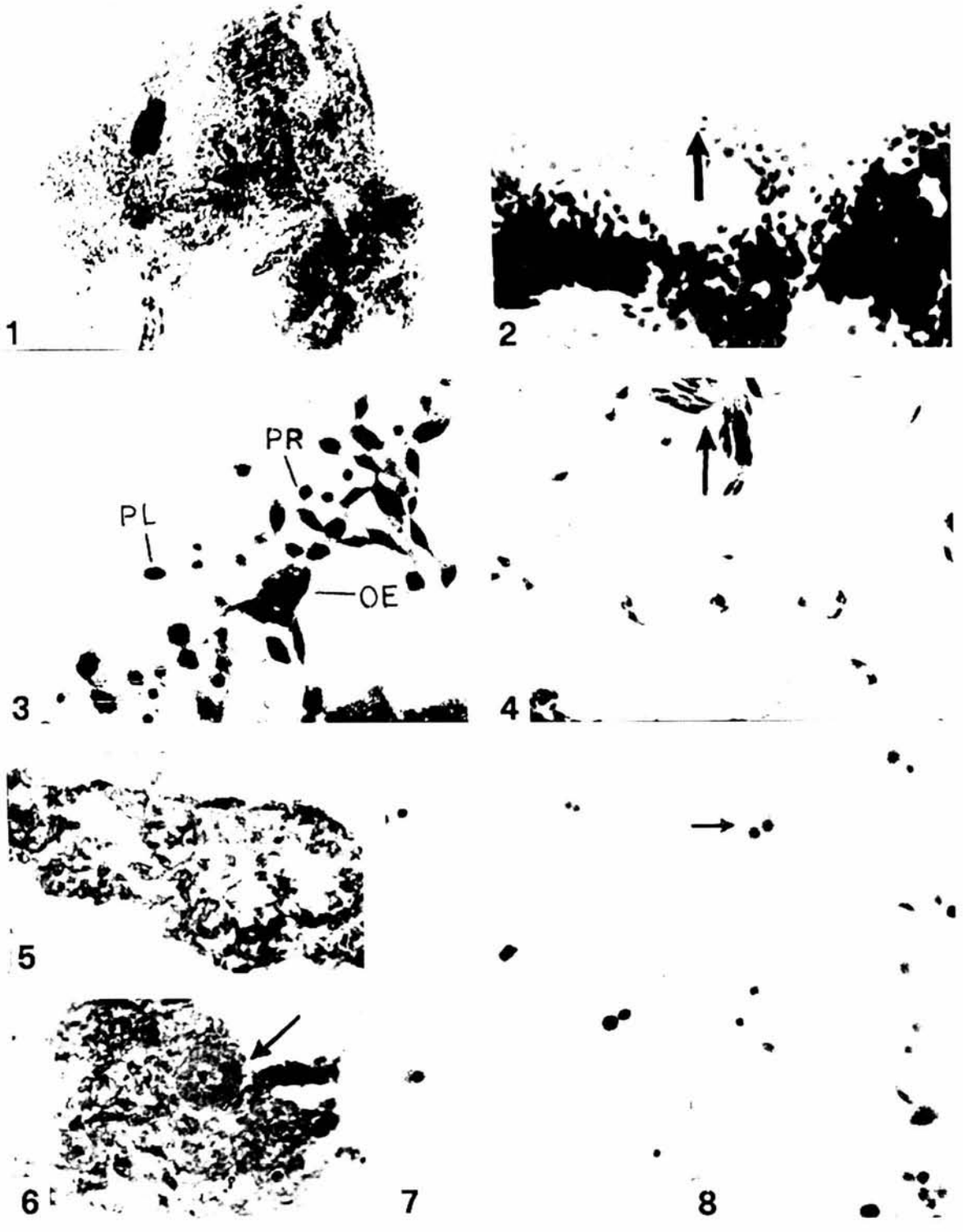
For histological study, a few of water-narcotized Vth instar larvae were dissected in insect Ringer[13], their wing-pads along with HPOs were taken out and fixed in Bouins fluid. Sections (7 μm thick) were cut and stained in haematoxylin and eosin. The experimental data were subjected to statistical analysis by using Student's '*t*' test.

Haemopoietic organs in *P.demoleus* are located one on each imaginal wing-pad as in many other lepidopterans. They are in the form of thin transparent cellular sheets (Fig.1) which are closely wrapped around the bases of the wing-pads and disintegrated early in the pupal stage. Squash preparations of HPOs (Figs 2-4) show the presence of groups of undifferentiated stem cells, some transitional forms (PR-PL intermediates) and certain categories of mature haemocytes; prohaemocytes (PRs), plasmatocytes (PLs) and oenocytoids (OEs) identical to those found in circulating haemolymph. This indicates that these categories of cells found in the circulation are infact derived from the HPOs. Nests of spindle shaped cells (PLs) can be seen in close bundles about to separate off from the HPOs (Fig.4). Mitotic figures are also seen in them (Fig.2). In sections, some undifferentiated and some fully differentiated cells of certain categories (Fig.5) including OE can be observed (Fig.6). Thus, 3 categories of cells; PRs, PLs and OEs have been encountered in the HPOs of present insect.

Effect of HPO-ablation on THC—The results of this experiment, summarised in Table1 showed a drastic drop in the THC in wing-pad (HPO) ablated insects compared to sham-operated controls at 24 hr after surgery. However, the count increased gradually and reached its maximum close to that of the sham-operated controls at 120 hr post- surgery but a drop



Figs 1-8—(1) Whole mount of haemopoietic organ (HPO) showing its shape. ×150. (2) Squash preparation of HPO showing a dense cluster of detaching cells. Note a cell with mitotic figure (arrow). ×600. (3) Squash preparation of HPO showing prohaemocytes (PR) as a predominant cell type, plasmatocytes (PL) and one oenocytoids (OE). ×900. (4) Squash preparation of HPO showing nests of PLs (arrow) still not separated. ×600. (5) Section of HPO showing differentiating haemocyte categories.×600. (6) A portion of HPO showing a fully differentiated OE (arrow).×600. (7) Blood smear showing low cell density and absence of PLs in HPO-ablated V^{th} instar larva. ×600 and (8) Blood smear showing an increase in granulocytes (GR) number in HPO-ablated larva 72 hr post-surgery and also note a cell in mitosis (arrow). ×600

Table 1—Effect of HPO-ablation on THC in *P.demoleus*

[Values are mean ± SE from 10 animals]

Hours after surgery	Number of larvae	THC/mm3 of blood	
		HPO-ablated	Sham-operated controls
24	20	3010 ± 16.58*	8295 ± 18.08
48	15	3580 ± 18.43*	10850 ± 25.09
96	15	4840 ± 21.79*	12290 ± 21.49
120 (Prepupa)	20	11720 ± 25.88**	14950 ± 23.25
144 (Pupa)	10	2074 ± 14.76***	2325 ± 12.72

P values : *< 0.001; **< 0.01; *** Not Significant

in cell numbers of both the experimental and control insects was observed soon after pupation (144 hr). Differences occurred also in population of the cell types. While PRs, PLs and OEs were markedly reduced (Fig.7) indicating a positive role of HPOs in the production of these haemocyte types; the granulocytes (GRs) rose in number at 72 hr post- surgery comparing to that of sham-operated controls (Fig.8). The THC in sham-operated controls however, remained higher than that of the normal controls (not shown in Table1).

Hinks and Arnold[3] on the basis of the shape of HPOs divided lepidopteran organs into 3 categories, (i) lobed cellular sheets, (ii) loose aggregation of cellular spheroids and (iii) irregular lobed ring. The HPOs of *P.demoleus* fall in the first of these categories.

As regards origin of haemocytes, these cells arise from either of the following (i) the densely packed haemocyte bodies[14], (ii) the phagocytic organs as in *Locusta* and *Gryllus*[15-16], (iii) the pre-existing circulating haemocytes alone[17-19], (iv) both HPOs and circulating haemocytes[3], (v) spherulocytes inclusion[20] and (vi) the haemocytes nuclei [21]. But due to lack of critical evidences, the last two views could be ignored. In the present study, both the histological and experimental evidences reveal that haemocytes arise both from the HPOs and circulating cells in the haemolymph. In both squash preparations and histological sections, the HPOs are seen to contain cells (PRs, PLs and OEs) identical to those found in the circulating haemolymph. For experimental evidences, HPOs ablation was carried out which showed a drastic drop in the THC indicating that the HPOs are indeed involved in the production of haemocytes in this insect. This is in agreement with the findings of other workers[1-4,22,23].

The fact that the THC starts recovering sometimes after surgery, indicates formation of new haemocytes. This can happen only by mitosis in the pre-existing circulating haemocytes since the HPOs are absent. An observation incidental to HPO-ablation experiment is that the THC in sham-operated controls is not only higher than that of experimentals but also higher than that of normal (unoperated) controls. The former situation could be due to the lack of HPOs and the latter to the effect of injury which not only induces mitosis in the existing haemocytes[24] but also stimulates a greater release of cell by HPOs, hence such an increase in THC is not evident in the case of HPO-ablated insects.

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