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

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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), opinion differ in regard to the haemocyte types that they produce. 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, 24 hr old V instar larvae of *P. demoleus* maintained in the laboratory culture were used. 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 placed on their side in a wax dissecting dish. The *P. demoleus* 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.

For histological study, a few of water-narcotized V instar larvae were dissected in insect Ringer 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 undifferentiated stem cells, some transitional forms (PR-PL intermediates) and certain categories of mature haemocytes; prohaemocytes (PRs), plasmocytes (PLs) and oenocytoids (OE) 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, plasmocytes (PL) and one oenocyte (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 V8 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
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 experiments 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 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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