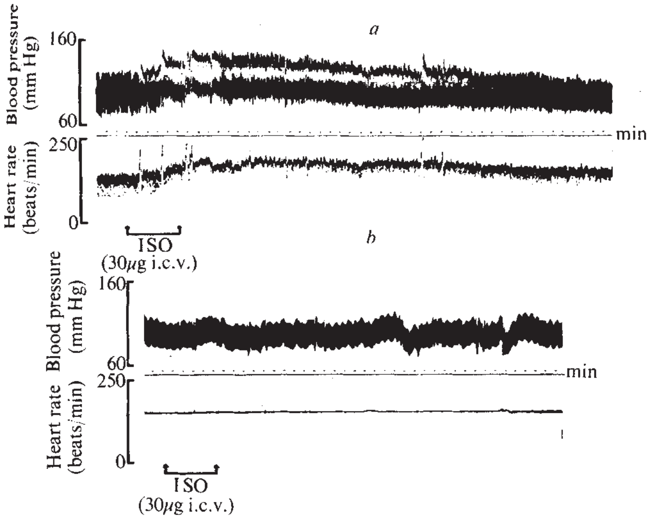

Fig. 2 Conscious cat (2.3 kg). *a*, Increase in arterial blood pressure and heart rate in response to intracerebroventricular infusion of isoprenaline (30 μg). *b*, Abolition of response 90 min after autonomic ganglion blockade with i.v. administered pempidine (5 mg kg^{-1}).

The effects of centrally administered isoprenaline were abolished after adrenergic neurone blockade with bethanidine (5 mg kg^{-1}) administered i.v., suggesting the involvement of the peripheral sympathetic nervous system in both hypertension and tachycardia.

The increase in mean arterial blood pressure by centrally administered isoprenaline was due mainly to an increased systolic rather than diastolic pressure, and this, together with the marked tachycardia, suggests a major cardiac component to the hypertensive response. This was supported by the finding that the effects of isoprenaline injected into the brain were markedly increased after abolition of vagal cardiac inhibitory activity with i.v. administered atropine (250 μg kg^{-1}).

The effects of centrally administered isoprenaline were abolished by pretreatment with intracerebroventricularly administered propranolol at a dose (500 or 600 μg) which did not affect the peripheral responses to i.v. administered isoprenaline. These doses of propranolol given centrally alone caused a marked fall in both arterial blood pressure and heart rate. Thus, in five cats given 500 μg propranolol centrally the average fall in mean blood pressure was 22.2 s.e. ± 3.2 mm Hg and the fall in heart rate 32.5 s.e. ± 4.0 beats/min. In six other cats receiving the 600 μg dose of propranolol the average falls in blood pressure and heart rate were respectively 25.4 s.e. ± 3.4 mm Hg and 39.8 s.e. ± 3.7 beats/min. Gagnon and Melville[8] reported a fall in mean blood pressure of only 5 mm Hg in chloralose anaesthetized cats after the central administration of 30 μg of propranolol.

The differences observed between the cardiovascular effects of centrally administered isoprenaline in our experiments and in those of other workers may possibly be accounted for by either species differences[4,9] or by the use of anaesthetics in those experiments using cats[8,10].

The presence of α- and β-adrenoceptors in the brain exerting antagonistic effects on blood pressure and heart rate may explain the clinically useful antihypertensive action of propranolol as well as the relative ineffectiveness of the α-adrenergic receptor blocking agents in relieving hypertension[11].

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[1] McCubbin, J. W., Kaneko, Y., and Page, I. H., *Circulat. Res.*, **8**, 849 (1960).
[2] Share, N. N., and Melville, K. I., *J. Pharmacol. Exp. Ther.*, **141**, 15 (1963).
[3] Smookler, H. H., Severs, W. B., Kinnard, W. J., and Buckley, J. P., *J. Pharmacol. Exp. Ther.*, **153**, 485 (1966).
[4] Toda, N., Matsuda, Y., and Shimamoto, K., *Int. J. Neuropharmacol.*, **8**, 451 (1969).
[5] Heise, A., and Kroneberg, G., *Eur. J. Pharmacol.*, **17**, 315 (1972).
[6] Schmitt, H., Schmitt, H., and Fénard, S., *Eur. J. Pharmacol.*, **14**, 98 (1971).
[7] Day, M. D., and Whiting, R. L., *J. Pharm. Pharmacol.*, **24**, 263 (1972).
[8] Gagnon, D. J., and Melville, K. I., *Int. J. Neuropharmacol.*, **6**, 245 (1967).
[9] Bhargava, K. P., Mishra, N., and Tangri, K. K., *Brit. J. Pharmacol.*, **45**, 596 (1972).
[10] Schmitt, H., and Fénard, S., *Arch. Int. Pharmacodyn. Ther.*, **190**, 229 (1971).
[11] Beilin, L. J., and Juel-Jensen, B. E., *Lancet*, i, 979 (1972).

Maintenance of Boundaries between Developing Organs in Insects

CLONAL analysis of developing insect embryos has shown that the origin of an organ (such as the leg imaginal disk of *Drosophila*[1]) can be traced to a small group of primordial cells. This subset of cells, which is not a clone, becomes partitioned from the cells around it. Subsequently, cells within it divide and generate a coherent body of leg cells to which the surrounding cells probably never contribute. Although clonal analysis cannot reveal whether the cells are individually determined as leg cells (such a conclusion conventionally depending on transplantation experiments) it is clear that an early expression of the "legness" of the group is the formation of a permanent growth boundary between it and its neighbours.

I have been studying segmentation in the abdomen of *Oncopeltus* embryos and larvae using clonal analysis. I report some observations on the maintenance of boundaries between the growing segments.

X-irradiation of *Oncopeltus* eggs or larvae results in the formation of variously coloured clones of epidermal cells at a high frequency. The genetic basis of this is still obscure. The low frequency of clones in unirradiated controls, and the clear relationships between clone size, frequency, and time of irradiation, indicate that the clone is initiated by the X-rays. The eggs were collected every 2 h and after various intervals batches were irradiated with 100 r (220 kV at 15 mA, 1 mm aluminium filter, 5 cm). The insects were maintained throughout at 29 ± 0.5° C. Fifth-stage larvae were examined between 24 and 48 h after ecdysis, slides made of the clones, and the cell number either counted directly or estimated from the area:

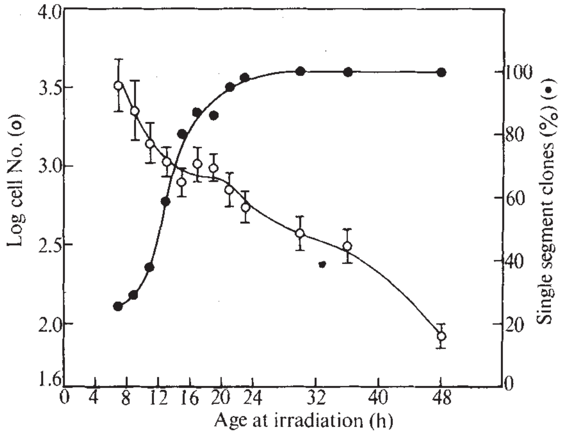

Fig. 1 Mean cell number in clones (○) and percentage of clones restricted to one segment quadrant (●) following irradiation at different times. Vertical lines demarcate ± 2 s.e.m.

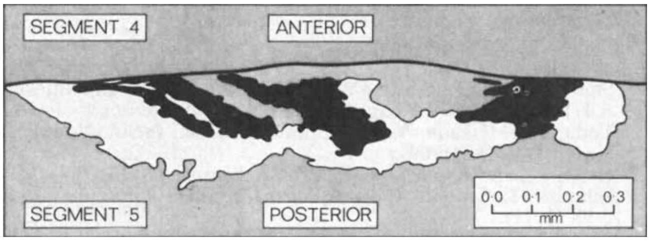

Fig. 2 Camera lucida drawing of twin-spot clone (irradiation at 11 ± 1 h) consisting of regions of dark orange cells (shown black) and white cells. These two cell types are clearly distinguishable from the wild-type colour (shaded). Note how the clone stops at the segment border (thick line).

the main results are shown in Fig. 1. It is clear that the segments grow autonomously from a very early stage, because clones initiated by irradiation after 24 h of egg development are always confined to a single segment quadrant (each segment can be considered as four quadrants; dorsal left and right, and ventral left and right). There is a very rapid increase in the proportion of single-segment clones between 11 and 24 h. This could only in small part be due to the slight decrease in average clone size that occurs over the same period. Some other factor must be segregating the lineage of the different segment quadrants. The shape of the clones themselves also points to the establishment of special boundary conditions between the segments; the clones, which generally have an uneven boundary elsewhere, have a remarkably straight edge at the segment border (Fig. 2). (The segment border described here is the transverse one, separating different segments. Clones do not form such straight edges along the midline. Clones reaching the lateral boundary separating dorsal from ventral are straight-edged.)

Following irradiation at 13 ± 1 h the proportion of clones including cells in more than one segment drops to about 50% of its former level, and the average size of clones is about 10% of the total area of the segment quadrant. From this observation and with a number of plausible assumptions[1], it follows that there are about ten cells in each nascent segment quadrant.

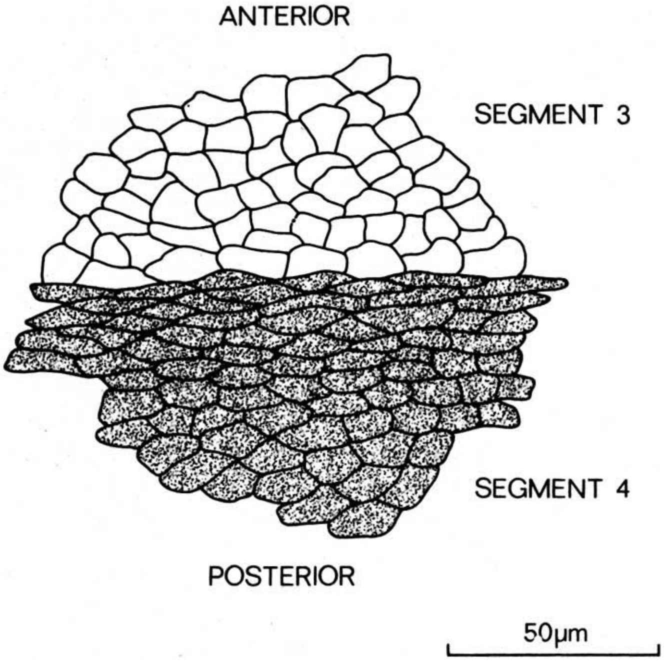

Fig. 3 Camera lucida drawing of cell boundaries (where they contact the cuticle) near the segment margin. The anterior cells of segment 4 are orange-red (shaded); the posterior cells of segment 3 are white. Note the elongated shape of the anterior margin cells.

Examination of the clones in growing larvae has shown how these growth boundaries are maintained. Although the epidermis consists of a simple monolayer of cells there is some discontinuity at the segment margin. Here the most anterior cells in the segment are slightly spindle-shaped, their long axes being oriented at right angles to the anteroposterior axis. There is a gradual change of cell shape as the anterior margin cells merge into the isodiametric cells of their own segment, while there is an abrupt transition to the typical cells of the next (more anterior) segment (Fig. 3). Fourth and fifth-stage larvae were examined when the epidermal cells were dividing and the orientation of mitoses recorded. The most anterior cells of the segment divide predominantly with the cleavage plane oriented parallel to the anteroposterior axis (80% of all mitoses within 30° of this axis) whereas the cells in all other parts of the segment, including the posterior boundary cells, divide at more or less random orientation. The anisometric cells of the anterior margin must slide a little past each other, not disturbing the straight edge to the clone boundaries at the margin, to account for the frequent streaking out of those clones near to or including the anterior margin (Fig. 2).

I suggest that a similar change in cell shape and the subsequent preferred orientation of mitoses may be involved in the subdivision of other developing organs. In *Drosophila* wing, for example, the dorsal and ventral half each have independent cell lineages after the first instar[2]. It is noteworthy that the clones streak out near the border at the wing margin[2-4]. This suggests that a preferred orientation of cleavage planes (at right angles to the wing margin) may maintain the effective growth boundary between the two wing surfaces.

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[1] Bryant, P. J., and Schneiderman, H. A., *Develop. Biol.*, **20**, 263 (1969).
[2] Bryant, P. J., *Develop. Biol.*, **22**, 389 (1970).
[3] Garcia-Bellido, A., and Merriam, J. R., *Develop. Biol.*, **24**, 61 (1971).
[4] Ripoll, P., *Roux Arch. EntwMech. Org.*, **169**, 200 (1972).

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